Evaluation of Biological Potential and Phytochemical Screening of some Novel Plants from Gilgit-Baltistan

By

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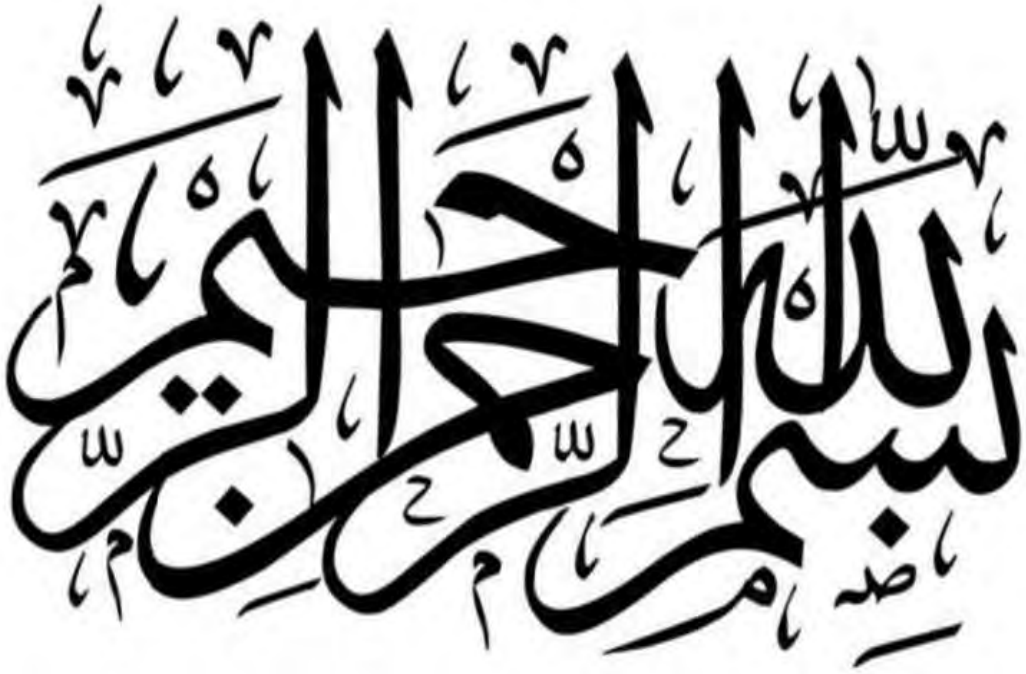
بسم الله الرحمن الرحيم

DEDICATED

TO

HOLY PROPHET

HAZRAT MUHAMMAD

(S.A.W.W)

& HIS HOLY PROGENY(A.S)

Special appreciation to

My respected, great and ever-loving Parents (Mr. and Mrs. Syed Anjum Ali Tirmazi) to whom I belong &

Who made all this possible through their endless support, encouragement and prayers.

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In the Name of **Allah**, the Merciful, the Beneficent, all praise for the Lord of all worlds who empowered me to complete my formidable work satisfactorily. Prayers and peace be upon our **Prophet Hazrat Muhammad (S.A.W.W) and his family** who are blessing for the entire universe.

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Dr. Syeda Anber Zahra

APPROVAL CERTIFICATE

This is to certify that the dissertation entitled "**Evaluation of Biological Potential and Phytochemical Screening of some Novel Plants from Gilgit-Baltistan**" submitted by Ms. **Syeda Anber Zahra** is accepted in its present form by the Department of Plant Sciences, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan, as satisfying the dissertation requirement for the degree of Ph.D. in Plant Sciences (Botany).

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I Ms. **Syeda Anber Zahra** hereby state that my Ph.D. thesis titled "**Evaluation of Biological Potential and Phytochemical Screening of some Novel Plants from Gilgit-Baltistan**" is my own work and has not been submitted previously by me for taking any degree from Department of Plant Sciences, Quaid-i-Azam University or anywhere else in the country/world. At any time if my statement is found to be incorrect even after my Graduate the university has the right to withdraw my Ph.D. degree.

S. Anber

Syeda Anber Zahra

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List of Abbreviations

S. No.	Title	Abbreviation
1	*Echinops niveus* Wall. ex Wall.	EN
2	*Echinops niveus* Methanol	ENM
3	*Echinops niveus* Ethanol	ENE
4	*Echinops niveus* Chloroform	ENC
5	*Echinops niveus* Ethyl-acetate	ENA
6	*Echinops niveus* n-Hexane	ENH
7	*Echinops niveus* Aqueous	ENQ
8	*Iris lactea* Pall.	IL
9	*Iris lactea* Methanol	ILM
10	*Iris lactea* Ethanol	IL E
11	*Iris lactea* Chloroform	IL C
12	*Iris lactea* Ethyl-acetate	IL A
13	*Iris lactea* n-Hexane	IL H
14	*Iris lactea* Aqueous	IL Q
15	*Lactuca orientalis* (Boiss.) Boiss.	LO
16	*Lactuca orientalis* seed Methanol	LOSM
17	*Lactuca orientalis* seed Ethanol	LOSC
18	*Lactuca orientalis* seed Chloroform	LOSE
19	*Lactuca orientalis* seed Ethyl-acetate	LOSEA
20	*Lactuca orientalis* seed n-Hexane	LOSH
21	*Lactuca orientalis* seed Aqueous	LOSAq
22	*Polygonum affine* D. Don	PAf
23	*Polygonum affine* Methanol	PAfM
24	*Polygonum affine* Ethanol	PAfE
25	*Polygonum affine* Chloroform	PAfC
26	*Polygonum affine* Ethyl-acetate	PAfA

27	*Polygonum affine* n-Hexane	PAfH
28	*Polygonum affine* Aqueous	PAfQ
29	*Rhodiola imbricata* Edgew	RI
30	*Rhodiola imbricata* Methanol	RIM
31	*Rhodiola imbricata* Ethanol	RIE
32	*Rhodiola imbricata* Chloroform	RIC
33	*Rhodiola imbricata* Ethyl-acetate	RIA
34	*Rhodiola imbricata* n-Hexane	RIH
35	*Rhodiola imbricata* Aqueous	RIQ
36	*Salix planifolia* Pursh	SP
37	*Salix planifolia* Methanol	SPM
38	*Salix planifolia* Ethanol	SPE
39	*Salix planifolia* Chloroform	SPC
40	*Salix planifolia* Ethyl-acetate	SPA
41	*Salix planifolia* n-Hexane	SPH
42	*Salix planifolia* Aqueous	SPQ
43	*Saxifraga flagellaris* Willd.	SF
43	*Saxifraga flagellaris* Methanol	SFM
44	*Saxifraga flagellaris Ethanol*	SHE
45	*Saxifraga flagellaris* Chloroform	SHC
46	*Saxifraga flagellaris* Ethyl-acetate	SHA
47	*Saxifraga flagellaris* n-Hexane	SHH
48	*Saxifraga flagellaris* Aqueous	SHQ
49	*Sophora alopecuroides* L.	SA
50	*Sophora alopecuroides* seed Methanol	SASM
51	*Sophora alopecuroides* seed Ethanol	SASE
52	*Sophora alopecuroides* seed Chloroform	SASC
53	*Sophora alopecuroides* seed Ethyl-acetate	SASEA
54	*Sophora alopecuroides* seed n-Hexane	SASH
55	*Sophora alopecuroides* seed Aqueous	SASAq
56	Milliliter	mL
57	Gram	g

58	Milligram	Mg
59	Percentage	%
60	Microliter	µL
61	Dimethyl sulfoxide	DMSO
62	Nanometer	Nm
63	2,2-diphenylpicrylhydrazyl	DPPH
64	Milliliter	mL
65	Meter	M
66	Colony Forming Units per miliiliter	CFU/mL
67	Absent	A
68	Microgram	Mg
69	Microgram per milliliter	µg/mL
70	Hours	Hrs
71	Celsius	°C
72	Protein Kinase Inhibition	PKI
73	Zone of inhibition	ZOI
74	Standard deviation	SD
75	Inhibitory Concentration	IC_{50}
78	Lethal Dose	LD_{50}
79	Centimeter	Cm
80	Kilometer	Km
81	Larger then	<
82	Strongly present	+++
83	Moderatory present	++
84	Weakly present	+
85	Absent	-
86	Hydro-oxyal group	OH
87	Smaller then	>
88	Gallic acid	GAE
89	Quercetin Equivalents	QE
90	Total Phenolic contents	TPC
91	Total Flavonoid contents	TFC

92	Total Antioxidant Capacity	TAC
93	Total Reducing Power	TRC
94	Ascorbic acid	AAE
95	Minimum Inhibitory Concentration	MIC
96	no activity/ Nil	NI
97	Alpha-Amylase Inhibition	AAI
98	Brine Shrimp Cytotoxicity Test	BSCT
99	Minimum	min
100	Maximum	Max
101	Standard Error	SE
102	Present	P
103	Light Microscopy	LM
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Abstract

The current study was focused on medicinal plants used as raw material for the synthesis of drugs. In this project, eight medicinal plants were collected from Gilgit-Baltistan, Northern areas of Pakistan and tested for *in-vitro* phytochemical, antioxidant, antimicrobial, cytotoxic, anticancer and antidiabetic potential. The selected medicinal plants: *Echinops niveus* Wall. ex Wall*., Iris lactea* Pall., *Lactuca orientalis* (Boiss.) Boiss*., Polygonum affine* D. Don., *Rhodiola imbricata* Edgew., *Salix planifolia* Pursh, *Saxifraga flagellaris* Willd and *Sophora alopecuroides* L used in the present study are novel. *Salix planifolia* Pursh was reported for the first time from Pakistan. *Salix planifolia* Pursh *, Iris lactea* Pall. and *Saxifraga flagellaris* Willd were checked for their biological potential for the first time. Scanning Electron Microscopy was used as an identification tool and reported for the first time for seeds of *L. orientalis* and *S. alopecuroides*. Extracts were prepared using six different solvents. Total phenolic and total flavonoid contents were determined by Folin-Ciocalteu and Spectrophotometer UV method. Antioxidant potential was measured by DPPH, phosphomolybdenum and total reducing power assay. Antibacterial and antifungal activities were assessed by disc diffusion method. Moreover, *Leishmania tropica* Kwh_{23} promastigotes strain was used for the assessment of anti-leishmanial activity. For anticancer and cytotoxicity evalution, PC-3 cell line, Brine shrimp and Protein Kinase Inhibition assays were used. Alpha-amylase Inhibition assay was used for the estimation of antidiabetic potential. Among all extracts methanol seeds extract of *L. orientalis* showed highest phenolic contents (95.76 mg GAE/g). The methanol extract of *S. flagellaris* had the highest concentration of flavonoid contents (85.69 mg QE/g). *S. alopecuroides* seed methanol extract revealed the best DPPH scavenging potential (82 % at 250 mg/mL), best total antioxidant capacity (90.60 mg AAE/g) and total reducing power (94.44 mg AAE/g). Significant antibacterial and antifungal activities in polar extracts. Moreover, best cytotoxicity (LD_{50} 37.54 µg/mL) and anticancer activity (85 %) were found for *S. flagellaris* n-hexane extract and inhibition in PC3 cells. Furthermore *R.imbricata* ethanolic extract showed highest protein kinase inhibition (27 mm). *L.orientalis* methanolic extract has shown (78.8 %) alpha-amylase inhibition potential. It was seen that the selected plants are rich reservoir of medicinally important active biochemicals and may be used further for future drug development.

CHAPTER: 1

INTRODUCTION

1.1 General Overview of Medicinal Plants

Plants have been used as food, medicines, fragrances, clothing, flavors, fertilizers and construction material since prehistoric time. Medicinal plants are source of new drugs and most of them are used in modern drug synthesis (Nankaya et al., 2020). Medicinal plants have huge diversity globally, about 391,000 plants species reported to science, out of which only 18,000 (5 %) plant species were reported for having pharmacological potential. According to an estimation, more than 250,000 flowering plants species are ethnopharmacological used (Sile et al., 2020). According to World Health Organization, in developing countries more than 3.5 billion people relay on medicinal plants for their primary health care (Khan et al., 2019). As an important natural source, the medicinal plants are used for the welfare of mankind and for the treatment of many ailments. Plants are dominant among all other alternative medicinal system such as Homeopathy, Native American medicinces, Naturopathy, Sidha, traditional Chinese medicines, Eastern medicines and Ayurvedic. The natural preparations from plants are regarded as safe, effective and economically good source as compared to synthetic drugs (Azam et al., 2019). Now a days by using different methods such as extraction or isolation of bio-compounds from particular medicinal plant are used to make different drugs that are effective against a large number of diseases (Ruwali and Negi, 2019).

In the last 20-25 years the use of herbal drugs has increased tremendously (Ahmed et al., 2019). According to transcribed knowledge, the use of plants as therapeutic agents is as old as 4000 - 5000 B.C., Chinese people used first time herbal preparations for medical purposes (Baptista et al., 2018). Since prehistoric time, all the medicinal therapies were based on herbs until nineteenth century, when the synthetic drugs were formalized (Khan et al., 2019). Drugs obtained from medicinal plants are low in cost and have less side effects as compared to synthetic drugs (Mishra et al., 2018). For the treatment of different ailments, local communities have long history based on medicinal plants (Abdalla and Zidorn, 2020). Medicinal plants have richest constituents that can be used for drug development. The plants continuously offer the new sources of medicines to mankind.

1.2 Importance of Phytochemical Screening

Medicinal plants are one of the greatest blessings of nature. Different types of plants are present globally with diverse medicinal capability to treat many ailments. Medicinal plants have competency due to the presence of many different chemical compounds, known as phytochemicals (Suryavanshi et al., 2019). Phytochemicals are non-nutritive bioactive compounds extracted from different plant parts and produced during metabolic process in plants. Usually these bioactive compounds have a major role in plant defense system and primed plants against environmental and physiological stress. Yahaya et al. (2020) has reported that phytochemicals have antibacterial properties. These phytochemicals provide shield against pathogens (bacteria and fungi) within the plant body. Medicinal plants can produce vast variety of secondary metabolites that have strong therapeutic potential and have power to fight against varied diseases caused by oxidative stress (Santos et al., 2017). Different types of secondary metabolites in plant body have valuable role in food industries (Thakuria et al., 2018). These biochemical components are used as nutrients and herbal drugs. Phenolics and flavonoids have been known for their antioxidant, antimicrobial, anti-inflammatory, cardioprotective, skin protection against UV radiation, immune system enhancement and as an important constituent of medicinal and pharmaceutical applications (Tungmunnithum et al., 2018).

Plants are the apparent source of phytochemicals having captivating biological actions. Phytochemical investigation of medicinal plants is the first step for the assessment of novel plant based products that are used in the synthesis of herbal medicines. Plant metabolites have many health benefits, they have anti-diabetic, anti-inflammatory, antimicrobial, antihypertensive and anticancerous properties (Kasote et al., 2015). Usually, these bioactive constituents are the major source of many different structural preparations and properties. Mosleh et al. (2020) mentioned in their recent report that well known secondary metabolites are flavonoids, phenols, tannins, terpenoids cyanogenic glycosides, glycosides, steroids and saponins. Tannins and terpenoids were reported to have anti-inflammatory and analgesic activities. Glycosides, conferring to many reports were used to treat high blood pressure (Maes et al., 2020). In

pharmaceutical industries, the saponins are used owing to its cytotoxic potential (Mohite and Shingare, 2020). All-inclusive, the medicinal properties of plants have been examined because of their effective role in economic feasibility, pharmacological activities and have low toxicity (Waseem et al., 2020).

1.3 Plants as a Source of Natural Antioxidants

Antioxidants are secondary metabolites that are capable of lessen or stop the process of oxidation. Antioxidants enhance the immune system and help to prevent damages caused by oxidative stress (Ruwali and Negi, 2019). In human body, the antioxidants act as scavenger to reduce the damages of free radicals by initiating the process of regeneration and cell repairing mechanism (Adebiyi et al., 2017). The external source of antioxidants are required to lessen the damaging effect in human body once the internal defense system are faced with the free radicals. Antioxidants are mostly present in plants, animal and in microorganisms. Vegetables, fruits, cereals, oilseeds and legumes are the major source of plant derived antioxidants (Chandrasekara and Shahidi, 2018).

Plants enclosed huge amount of antioxidants including ascorbic acid, carotenoids, polyphenols and glutathione. Oribayo et al. (2018) described that all these phytochemicals are considered to be more active to reduce the side effects of oxidative stress. Naturally antioxidants are present in food such as in fruits and vegetables, but in small amount therefore to reduce the effect of oxidation, supplementary antioxidants are added to increase life age by terminating the free radicals. Reactive oxygen species are linked with the development of many different diseases in human body such as tissue damage, atherosclerosis, cancer, tissue damage, injury in cells of central nervous system, obesity and cardiovascular diseases. By using antioxidants the detrimental effect of such diseases may be decreased and protect the individual life (Koksal et al., 2017).

1.4 Medicinal Plants as Antimicrobial Agent

Modern medicines innovation has been associated with well-known scientist Sir Alexander Fleming, who discovered penicillin from *Penicillin notatum* (fungal strain) providing the road for the discovery of many modern antibiotic (Saleem and Saeed, 2020). Antibiotic is the most prevailing mediator used to treat many infectious diseases.

However, with time the microorganisms are becoming multi-drug resistance. Mulat et al. (2020) studied the occurrence of multidrug resistance in microbial strains increases the microbial infections. Resistance against antibiotics is one of the most important threat of 21st century. Researchers of the present day are now in search for the new sources of antimicrobial agents that can deal with antibiotic resistance (Mandrone et al., 2019).

From prehistoric time herbs are used as a potential source of traditional medicine. Therefore, scientist are working on plants in order to find out novel compounds that can be used against multi-drug resistant microbes (De Zoysa et al., 2019). Novel chemo-preventive bio-agents have been derived from plants which are essential elements of potentially useful therapeutics (Moussaoui and Alaoui, 2016; Swamy et al., 2016). Plant based products are effective and used to treat many infectious diseases with lesser side effects as compared to synthetic drugs (Ahmed et al., 2019).

1.5 Anti-leishmanial Potential of Medicinal Plants

Leishmaniasis is a neglected tropical disease caused by a parasite and it affect masses of people in Asia, Africa and South America (Hammi et al., 2019). Worldwide it becomes one of the most important health problems and each year about 2 million new cases arise. Dogs, rodents and wild animals are reservoirs of *Leishmania* parasites that are transmitted to animal or human host by the bite with female infected sandfly belongs to the genus Lutzomyia and Phlebotomus. Three types of leishmaniasis are found namely cutaneous, visceral and mucocutaneous. Cutaneous leishmaniasis is the most common type, caused by *L. tropica* and *L. amazonensis* (Paula et al., 2019). According to Silva et al. (2018), chemotherapy for the treatment of leishmaniasis is miltefosine, amphotericin B and antimonials, these drugs are expensive, have serious side effect and multidrug resistance. Plants and their secondary metabolites are therapeutic source of novel antiprotozoal drugs and might be used to treat drug resistance phenomena in protozoan parasites especially in *Leishmania* (Mehwish et al., 2019). Thus, it is important to search for novel drugs against parasites of *Leishmania* from medicinal plants (Moreira et al., 2019).

1.6 Anticancer Potential of Medicinal Plants

Cancer is a very complex genetic ailment occurring due to many causes. Unbalanced growth and uncontrolled cell division are the main signs of cancer. Cancerous cells attack and damage normal cells. Frequently cancer is incurable and irrepressible occurring at heretical and unpredicted stages of a healthy person life (Abdullahi et al., 2018). According to Beeby et al. (2020) cancer becomes the second leading cause of death worldwide. Nature provides best remedy in the form of plants used to treat different diseases including cancer. Plants contain unlimited number of bio-compounds that have cytotoxic potential. Due to vast cytotoxic capacity the researchers of the present day are paying attention towards the plants in order to find out novel drugs, that are used to treat cancer (Ang et al., 2019). Different *in-vitro* assays are used to determine the anticancerous activity of plants and among them the Brine shrimp cytotoxic test (BSCT), Protein Kinase inhibition (PKI) test and anticancer human cell line assays are considered best.

Preliminary cytotoxic assessment of plants are mostly carried out using BSCT. This assay is low-cost, simple and rapid bio-test used to determine bioactivities of plant sample. Brine shrimp are *Artemia nauplii,* microscopic zoological organism and used extensively for studying the toxicity of plant sample. Commonly dried cysts of *A. nauplii* are available, which on providing suitable conditions for hatching change into live microscopic organism and on commercial scale used extensively for studying the toxicity of plant sample (Arumugam et al., 2019). Protein kinases have crucial role in many cellular functions such as cell morphogenesis, cell differentiation, cell survival and death. Any mutation in these kinases leads towards the generation of cancer. Among all these kinases the protein kinase C (PKC) plays an important role in carcinogenesis (Rahman et al., 2018). Yao et al. (2011) depicted that the cancer causing agents altered the expression of PKC gene expression leading to uncontrolled cell division. Many natural compounds from medicinal plants have been identified and used against tumor cells. For cancer treatment many inhibitors of PKC extracted from plants have been checked against different cancer cell lines (Isakov, 2018).

1.7 Medicinal Plants as Antidiabetic agent

Diabetes mellitus is the metabolic disorder caused by either the deficiency in discharge of insulin hormone or the human body cell become resistance to insulin (Justino et al., 2018). Starch is the main energy source for human beings which is digested in several stages by using several amylotic enzymes which includes the Alpha-amylase and Alpha-glycosidase. Alpha-amylase is the vital pancreatic enzyme of salivary and pancreas glands, have a key role in glycogen digestion (Ali, 2016; Gyawali et al., 2020). Inhibitors of alpha-amylase reduce the carbohydrate digestion rate, causing in the reduction in absorption of glucose and lowering the blood glucose levels. Shang et al. (2020) has reported that some inhibitors like miglitol and acarbose which inhibit alpha-amylase but have adverse effects on human health.

Many *in-vivo* researches have cleared the fact that several plants extracts have potential to inhibit the action of digestion enzymes alpha-amylase and alpha-glucosidase (Sunmonu and Lewu, 2019). Therefore, plant based medicines are safe and effective in the treatment of diabetes (Agarwal and Gupta, 2016). Since ancient time, humans have been exploiting plants as a good source of drugs used to treat different diseases including diabetes. Up to 90 % population of developing countries use herbal related things for the treatment of diabetes. Herbal drugs are receiving more importance for the treatment of diabetes, as these herbal drugs has no side effects and are inexpensive as compared to hypoglycemic synthetic agents (Liyanagamage et al., 2020)

1.8 Phytodiversity of Pakistan

Pakistan is blessed with treasure of higher plants (six thousand) species, out of which 600-700 have medicinal values. About three thousand have been b reported from the Northern areas of Pakistan. Alamgeer et al. (2018) reported that only 10 % of total plant species from Pakistan have documented for their medicinal values. Pakistan is regarded to be the major region around the globe in terms of biodiversity and cultural domination about the use of medicinal plants (Shinwari et al., 2017). Three mountain ranges surrounding the Pakistan are Karakorum, Himalaya and Hindu Kush collectively contain round about 25 thousand plant species (10 % of the world reported plant species)

and out of these plants only ten thousand are used for their therapeutic potential. These mountainous areas provide the natural environment which is necessary for the growth of important medicinal plants (Khan and Baig, 2020). Local people 70-80 % that are living in these regions rely on medicinal plants for their basic health care (Aziz et al., 2020). However, slight attention has been required to the quality of ethnobotanical work. There is a great variability in the quality, focus, and content of ethnopharmacological studies, resulting in less suitability in global research communities (Yaseen et al., 2019).

1.9 Medicinal Plants of Gilgit-Baltistan

Gilgit-Baltistan, is the hotspot area for consumption of pharmacologically important plants against different ailments, about three hundreds medicinal plant species were stated. This region has ideal hilly landscape that is more suitable for the growth of highly medicinal plants (Figure 1-4). For many generations the local communities of this area is using these natural resources for their basic provisions of health. Previously many researchers recognized the conventionally used medicinal plant species from different districts of this regions (Salim et al., 2019). Till date there is a vast literature available regarding the uses of medicinal plants of Gilgit-Baltistan. However few studies on various aspects were recorded on Deosai plateau and these all studies are scattered and did not conclude the complete knowledge about the medicinal flora of Deosai plateau (Khan et al., 2018).

Deosai, the most beautiful plateau of the world is located on North Slope of Himalayan ranges. The surrounding of Deosai shows the picture of heaven on earth. The mountain ranges with the altitude of 3400-4300 m, and the area with natural rivers, lakes, glaciers and streams which increases its beauty. During summer, Deosai climate is most favorable for growth of different type of flora and fauna. This region is the main focus of unique biodiversity of economically important species of wild plants such as *Artemisia*, *Angelica*, *Valariana*, *Saussurea*, *Arnebia Colchicum* and *Aconitum* and hundreds of ethnomedicinal plants which are being used in the curing of several diseases (Khan et al., 2015; Khan et al., 2018).

Figure 1: Floristics View of Deosai National Park.

Figure 2: Panoramic View of Study Site (Deosai National Park).

Figure 3: Floristics View of Sakardu Valley.

Figure 4: Panoramic View of Nagar Valley (Gilgit).

1.10 Background Justification of the Present Project

Synthetic drugs are very effective and used for the treatment of many ailments. However, all these drugs have harmful effects on human health, because of their toxicity and also certain microorganisms developed resistance against these drugs. Therefore, the trend is shifted towards the use of herbal therapy (Ang et al., 2019). Scientists are paying huge intrest to do researches in order to use medicinal plant for specific herbal drug discovery. Human from the day of his creation used plants from their surrounding for their basic health requirement. The plants are the major source of primary and secondary metabolites. The bioactive compounds have played major role in drug discoveries against several diseases (Mirzaee et al., 2017; Chanda and Ramachandra, 2019). The bioactive compounds such as phenolics, flavonoids, saponins, alkaloids and steroids from plants have antioxidative role and used to lessen the effects of reactive oxygen species and prevent the human body against the damaging effects of oxidation (Bezerra et al., 2019; Navarro et al., 2019).

Microorganisms have developed multidrug resistance against synthetic drugs researchers are trying to find out new anti-microbial components from plants that are more effective against certain group of microorganisms (Majeed et al., 2019; Mondal et al., 2019). For cancer therapy, scientists are in search for the development of safe and clinically active chemotherapeutic agents (Hegazy et al., 2019).

Gilgit-Baltistan is the diversified area with a large number of highly medicinal plants. There is great need to explore and evaluate the medicinal plants from that area with special attention on traditional knowledge and therapeutic potential of selected medicinal plants by using different bio-assays to highlight the significance of plant species. Many researches on different aspects were held in Gilgit-Baltistan, Pakistan, but their studies have not provided somehow complete information about medicinal plants of Gilgit Baltistan, especially Deosai plateau (Khan et al., 2018). Detailed information of selected medicinal plants used in the present study is mentioned in Table 1.

Table 1: Botanical Names, Voucher No. Family, Cultivation Status, Ethnomedicinal Uses, Reported Phytochemicals and World-Wide Distribution of Collected plants.

Botanical name, Voucher No.	Common name	Family	Cultivation Status	Ethnomedicinal uses	Phytochemicals	Distribution in World
***Echinops niveus* Wall. ex Wall. SAZ5**	Globe thistles	Asteraceae	Wild	Respiratory diseases, diseases caused by different bacteria, Inflammation, aphrodisiac, to fasten expulsion of placenta, removal of kidney stones (Bitew and Hymete, 2019)	Flavonoids (Sytar et al., 2015)	Pakistan, India, Nepal, Croatia (https://www.gbif.org/occurrence/search?offset=40&q=Echinops%20niveus&occurrence_status=present)
***Iris lactea* Pall. SAZ2**	Milky iris	Iridaceae	Wild	Anticancer drug, diuretic, laxative, fever, jaundice, menorrhagia, carbuncles, heat pain, nausea, vomiting, urination, sore throats, mouthwashes and toothpastes	Flavonoid (Hoang et al., 2020)	China, Korea, Mongolia, Japan (https://www.gbif.org/occurrence/search?offset=40&q=Iris%20lactea&occurrence_status=present)

				adjuvants (Hoang et al., 2020)		
***Lactuca orientalis* (Boiss.) Boiss. SAZ3**	lettuce	Asteraceae	Wild	Various infectious and Alzheimer diseases (Zahra et al., 2021)	Saponins, Flavonoids, phenolic (Zahra et al., 2021)	Pakistan, Israel, Afghanistan, Turkey, Iran (https://www.gbif.org/occurrence/search?occurrence_status=present&q=Lactuca%20orientalis)
***Polygonum affine* D. Don SAZ4**	Fleece flower, or knotweed	Polygonaceae	Wild	Dysentery and haemorrhoids (Sharma et al., 2020)	Yet not reported	Pakistan, India, Nepal, Germany, Netherland, Austria (https://www.gbif.org/occurrence/search?offset=20&q=Polygonum%20affine&occurrence status present)
***Rhodiola imbricata* Edgew. SAZ8**	Rose root	Crassulaceae	Wild	Increasing energy, stamina, strength and mental capacity (Rattan et al., 2020)	Rosavin, Polyphenols (Bhardwaj et al., 2018)	India, Nepal, China, Tajikistan, South Africa (https://www.gbif.org/occurrence/search?occurrence status present&qRhodiola%20imbricata)
***Salix planifolia* Pursh SAZ7**	Planeleaf willow	Salicaceae	Wild	Fevers, cold and joint pains (Carello et al., 2018)	Yet not reported	Canada, United States of America (https://www.gbif.org/occurrence/searc

						h?offset80&qSalix%20planifolia&occurrence status)
***Saxifraga flagellaris* Willd. SAZ1**	Spider plant	Saxifragaceae	Wild	Urinary calculi (known as kidney or bladder stones) (Rehman et al., 2019)	Yet not reported	Canada, Norway, United States of America, Greenland, India (https://www.gbif.org/occurrence/search?offset20&qSaxifraga%20flagellaris&occurrence status present)
***Sophora alopecuroides* L.** **SAZ6**	Bitter bean	Fabaceae	Wild	Bacillary dysentery, enteritis, hepatitis, other infectious diseases, anti-tumor (Ma et al., 2018)	Alkaloids, steroids, flavonoids, polysaccharides (Wang et al., 2020)	Pakistan, Korea, India, Iran (https://www.gbif.org/occurrence/search?occurrence status present&qSophora%20alopecuroides)

1.11 Overview of Targeted Medicinal Plants

Echinops niveus Wall. ex Wall. belongs to family Asteraceae, native to Pakistan, India, Nepal and Croatia (https://www. gbif.org/occurrence /search? Offset =40 & q= Echinops%20niveus&occurrence status=present). The aerial part of *E. niveus* is used to treat different respiratory diseases, infections caused by different bacteria, inflammation, aphrodisiac, to fasten expulsion of placenta and removal of kidney stones (Bitew and Hymete, 2019). The phytochemicals already reported in *E. niveus* are flavonoids (Sytar et al., 2015).

Iris lactea Pall. is a renowned medicinal plant of family Iridaceae. Common name of *I. lactea* is Milky iris, widely distributed in China, Korea, Mongolia and Japan (https:// www.gbif.org/occurrence/search?offset=40&q=Iris%20lactea&occurrencestatuspresent). *I. lactea* is ethnomedicinally used to treat various diseases such as cancer, diuretic, laxative, fever, jaundice, menorrhagia, carbuncles, heat pain, nausea, vomiting, urination, sore throats, like mouthwashes and toothpastes adjuvants (Hoang et al., 2020). The plant aerial parts were used to cure different diseases toothaches, inflammation, cardiac stimulant, diarrhea, cough, asthma, CNS disorders, hepato-protective activity, anti malaria, antidiabetic, antimicrobial, antioxidant, appetizer and aphrodisiac (Makkar et al., 2009).

Lactuca orientalis (Boiss.) Boiss. of family Asteraceae is distributed in Pakistan, Israel, Afghanistan, Turkey and Iran (https://www.gbif. org/occurrence/search? Occurrence status =present&q=Lactuca%20orientalis). Seeds of this plant are used to treat various microbial infectious. Alkaloids, flavonoids, saponins, phenolics, tannins, terpenoids, glycosides, pentacyclic triterpenes and anthraquinones are present in this medicinally important plant (Zahra et al. 2021).

Polygonum affine D. Don belongs to family Polygonaceae is commonly known as name fleece flower or knotweed. This species is mainly distributed in Pakistan, India, Nepal, Germany, Netherland and Austria (https://www'gbif. org/occurren ce/searc h?offset =20&q=Polygonum%20affine&occurrence status present). *Polygonum affine* has been reported for the treatment of dysentery and haemorrhoids in the previous literature

(Sharma et al., 2020). There is very less information available in literatute regarding to its therapeutic potential.

Rhodiola imbricata Edgew. is an perennial herb of family Crassulaceae, commonly known as Rose root. Mostly distributed in India, Nepal, China, Tajikistan and South Africa (https://www.gbif.org/occurrence/search?occurrence status present& q Rhodiola % 20imbricata). *R.imbricata* herb is reported to be used for the treatment of stamina, strength, mental capacity and also increasing energy (Rattan et al., 2020).

Salix planifolia Pursh. belongs to Salicaceae, common name is Planeleaf willow. Mostly distributed in Canada and United States of America (https://www.gbif.org/ occurrence/search?offset80&qSalix%20planifolia&occurrencestatus). Ethnomedicinally, the herb is reported for the treatment of diseases caused by bacteria, fevers, cold and joint pains (Carello et al., 2018).

Saxifraga flagellaris Willd. is a well known medicinal plant of family Saxifragaceae, commonly known as Spider plant. Naturally distributed *in* Canada, Norway, United States of America, Greenland India and Pakistan (https ://www.gbif.org/ occurrence/ search? offset20 &q Saxifraga %20 flagellaris&occurrence status present). Ethnomedicinally this herb is being used in Urinary calculi known as kidney or bladder stones (Rehman et al., 2019).

Sophora alopecuroides L. belongs to family Fabaceae, widely distributed in Pakistan, Korea, India and Iran (https://www.gbif org/occurrence/search occurrenc status present&q Sophora % 20 alopecuroides). Ethnomedicinally the herb is useful in Bacillary dysentery, enteritis, hepatitis and tumor (Ma et al., 2018). Reported phytochemicals in *S. alopecuroides* are alkaloids, steroids, flavonoid and polysaccharides (Wang et al., 2020).

Objectives of the Study

The study was designed to evaluate the phytochemical and therapeutic potential of selected medicinal plants. Following were the key objectives of the present study.

- Preliminary phytochemical screening (Qualitative and Quantitative) for the presence or absence of different classes of chemical compounds and estimation of total phenolic and total flavonoid contents
- Screening and characterization of phytochemicals
- Evaluation of *in-vitro* antimicrobial potential (antibacterial, antifungal and anti-leishmanial activities) of studied plant extracts
- *In-vitro* evaluation of antioxidative potential by different assays
- Assessment of cytotoxic and anticancer potential of selected plants
- Investigation of anatomical and morphological features of seeds of selected medicinal plants
- Evaluation of *in-vitro* antidiabetic potential of studied plants extracts

CHAPTER 2

MATERIAL AND METHODS

2.1 Collection and Identification of Plant Material

Medicinal plants were collected from Gilgit-Baltistan (Hunza, Nagar, Manthal, Satpara lake, Skardu city and Deosai Plateau) Northern areas of Pakistan in July-August 2019 and collection sites cover an area of 72,971 km^2 (Figure 5-9). The ethnomedicinal information was collected from indigenous community and traditional healers. Plant samples were pressed and dried by using blotting papers. After plant collection, samples were identified by senior plant taxonomist Prof. Dr. Mir Ajab Khan. The dried poisoned plant specimens were mounted on Herbarium sheets and submitted into PBMB laboratory at QAU Islamabad, Pakistan. Details of selected medicinal plants and their collection site along with their voucher number are given in Table 2.

Table 2: List of Selected Plants, their Collection Sites and Voucher Numbers.

Sr.#.	Selected Plants	Collection Sites	Voucher Numbers
1.	*Echinops niveus* Wall. ex Wall.	Gilgit (Hunza)	SAZ5
2.	*Iris lactea* Pall.	Gilgit (Nagar)	SAZ2
3.	*Lactuca orientalis* (Boiss.) Boiss.	Skardu (Manthal)	SAZ3
4.	*Polygonum affine* D. Don	Deosai	SAZ4
5.	*Rhodiola imbricata* Edgew.	Deosai	SAZ8
6.	*Salix planifolia* Pursh	Deosai	SAZ7
7.	*Saxifraga flagellaris* Willd.	Skardu (Satpara lake on the way to Deosai)	SAZ1
8.	*Sophora alopecuroides L.*	Skardu	SAZ6

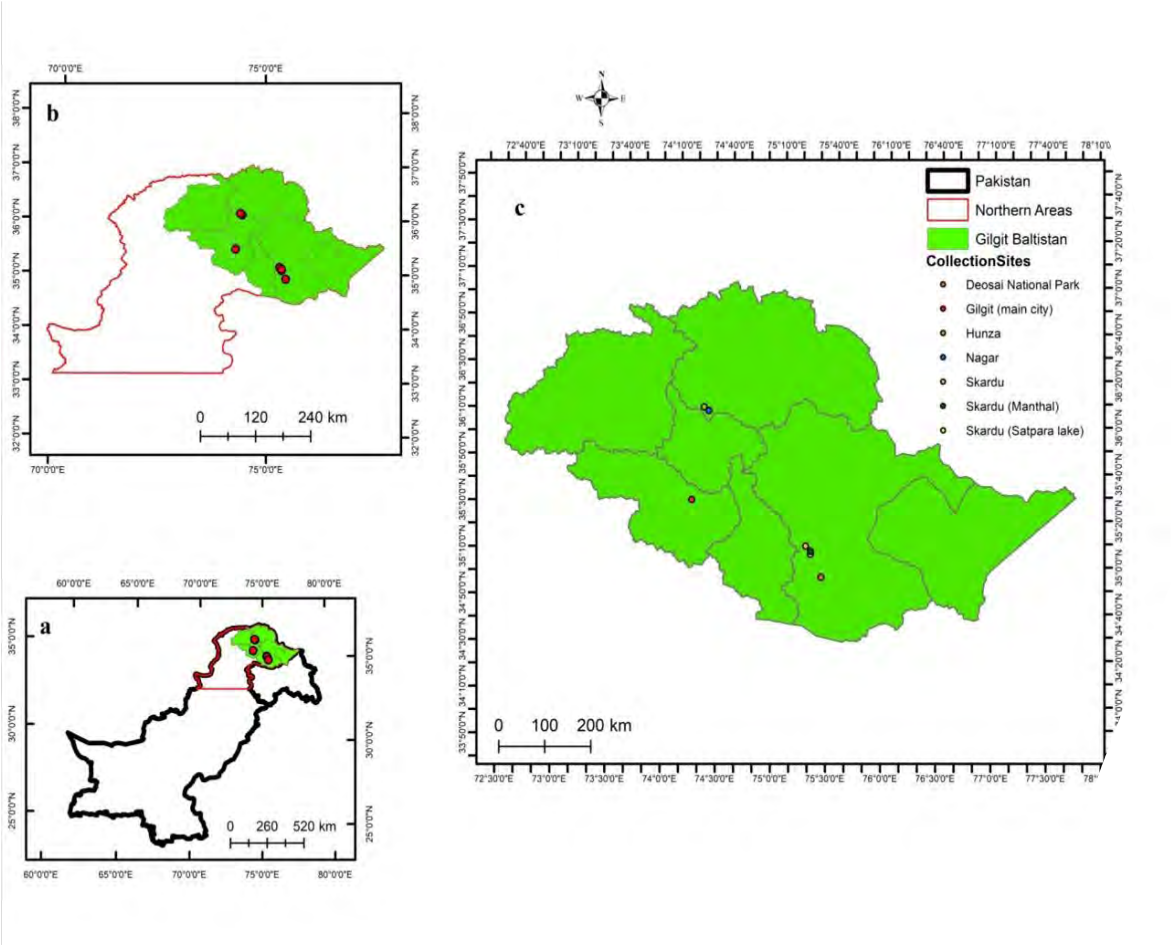

Figure 5: Geographical Representation of Collection Sites (MAP).

Figure 6: Plant Collection during Field Survey at Gilgit (Hunza).

Figure 7: Plant Collection during Field Survey at Gilgit (Nagar).

Figure 8: Plant Collection during Field Visit to Deosai National Park.

Figure 9: Plant Collection during Field Visit to Deosai (Kala Pani Lake).

2.2 Preparation of Crude Extracts

The collected plant samples were washed under tab water for the removal of dust, debris and at room temperature the plant were dried (Figure 10-11). The dried plant samples were grinded to fine powder (1 mm diameter) and for further pharmacological evaluation the plant powders were kept in airtight glass containers. Grinded plant powders (30 g) were soaked in 300 mL of each solvent methanol, ethanol, chloroform, ethyl acetate n-hexane and distilled water for 7 days at room temperature. Six solvents were used for plant extraction, polarity scheme starting from polar solvents and moving towards the non polar solvents was followed. Whatman No: 1 filtering papers were used for filtration and with the help of rotary evaporator extracts were collected (Figure 12-13). Extracts were further labelled as given in Table 3 and stored at 4 °C for further analysis.

2.3 Preliminary Phytochemicals Screening of the Plants Extracts

Phytochemical screening tests were performed to establish the profile of plants extracts for relative occurrence of secondary metabolites. Various tests were performed using standard procedures such as Alkaline detection assay (flavonoids), Mayer's assay (alkaloids), Salkowski assay (glycosides), Millon's reagent test (amino acids), Ferric chloride test (phenols), Gelatin test (tannins), Libermann's test (steroids and terpenoids), and Foam test for saponins (Harborne and Mabry, 2013; Ruwali and Pateliya, 2019)

2.4 Quantitative Phytochemicals Screening of the Plants Extracts

2.4.1 Determination of Total Phenolic Contents (TPC)

Folin–Ciocalteu method with minor changes was used for TPC analysis (Khalil et al., 2018). For this purpose, plant samples (20 µL) and Folin Ciocalteu reagent (90 µL) were mixed in 96-well plate and incubated for 5 minutes and 6 % sodium carbonate (90 µL) was added. Pure Dimethyl sulfoxide (DMSO) was used as a negative and Gallic acid as a positive control. Finally, 96-well plate containing reaction mixture was placed in microplate reader and absorbance was measured at 630 nm.

2.4.2 Determination of Total Flavonoid Contents (TFC)

TFC of selected plants extracts were measured using aluminum chloride method (Chang et al., 2002). Extracts (20 µL), 10 % aluminum chloride (10 µL), 1 M potassium

Figure 10: Drying of Collected Plants at Room Temperature.

Figure 11: Drying of Collected Plants at Room Temperature.

Figure 12: Filteration of Plants Extracts.

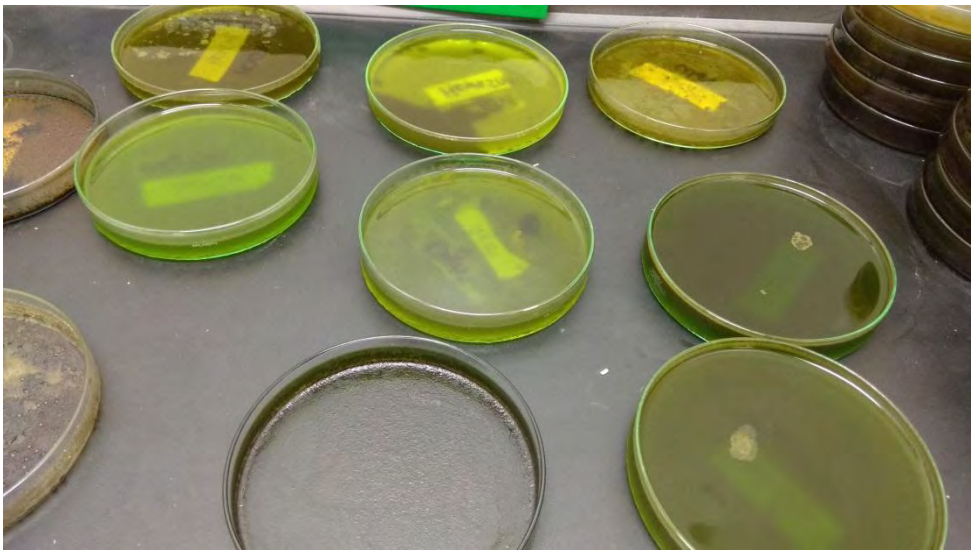

Figure 13: Plants Extracts Evaporation in Fume Hood.

Table 3: Plants Extracts used in the Present Study.

Sr. No.	Plant Names	Part used	Solvent used	Abbreviation
1.	*Echinops niveus* Wall. ex Wall.	Whole plant	Methanol	ENM
			Ethanol	ENE
			Chloroform	ENC
			Ethyl acetate	ENA
			n-Hexane	ENH
			Water	ENQ
2.	*Iris lactea* Pall.	Whole plant	Methanol	ILM
			Ethanol	ILE
			Chloroform	ILC
			Ethyl acetate	ILA
			n-Hexane	ILH
			Water	ILQ
3.	*Lactuca orientalis* (Boiss.) Boiss.	Seeds	Methanol	LOSM
			Ethanol	LOSE
			Chloroform	LOSC
			Ethyl acetate	LOSEA
			n-Hexane	LOSF
			Water	LOSAq
4.	*Polygonum affine* D. Don.	Whole plant	Methanol	SPM
			Ethanol	SPE
			Chloroform	SPC
			Ethyl acetate	SPA
			n-Hexane	SPH
			Water	SPQ
5.	*Rhodiola imbricata* Edgew.	Whole plant	Methanol	RIM
			Ethanol	RIC
			Chloroform	RIE
			Ethyl acetate	RIA
			n-Hexane	RIH
			Water	RIQ
6.	*Salix planifolia* Pursh.	Whole plant	Methanol	SPM
			Ethanol	SPE
			Chloroform	SPC
			Ethyl acetate	SPA
			n-Hexane	SPH
			Water	SPQ
7.	*Saxifraga flagellaris* Willd.	Whole plant	Methanol	SFM
			Ethanol	SFE
			Chloroform	SFC
			Ethyl acetate	SFA
			n-Hexane	SFH
			Water	SFQ

8.	*Sophora alopecuroides L.*	Seeds	Methanol	SASM
			Ethanol	SASE
			Chloroform	SASC
			Ethyl acetate	SASEA
			n-Hexane	SASH
			Water	SASAq

acetate (10 µL), and distilled water (160 µL) were mixed in wells of 96-well plate and was incubated for 30 minutes. Quercetin and pure DMSO were used as a positive and negative control respectively. Finally, absorbance of reaction mixture was measured at 405 nm using microplate reader.

2.5 Antioxidant Assays

2.5.1 DPPH Radical Scavenging Assay

Antioxidant capacity of plant samples were analyzed by proposed method of Phull et al. (2016) with few modifications. Test samples (20 uL) and DPPH solution (180 uL) were poured into 96-well plate. Ascorbic acid (AA) was used as standard for measurement of antioxidant potential. The reaction mixtures were incubated for 30 minutes at room temperature and absorbance at 517 nm was checked using microplate reader (Bioteck). Percentage scavenging potentials of the samples were calculated using the formula below;

PS 1 (OD of test sample/ OD of control) 100

Where "PS" is percentage scavenging and OD is optical density.

2.5.2 Total Antioxidant Capacity (TAC)

Initially, sodium phosphate (28 mM) ammonium molybdate (4 mM) and sulfuric acid were used to make reagent solution. The samples solution (100 µL) and reagent solutions (90 µL) were mixed and incubated at 95 °C for 90 minutes. Further reaction solutions were cooled and optical density was measured at 695 nm using microplate reader. Ascorbic acid was used as positive control (Phull et al., 2016).

2.5.3 Total Reducing Power Assay

Plants extracts (100 µL) were mixed with 10 % trichloroacetic acid (250 µL) and 0.2 M Phosphate buffer (200 µL) and for 10 minutes at 3000 rpm centrifugation was done. After that ferric chloride (50 µL) was mixed with supernatant (150 µL) of reaction mixture in 96-well plate (Siddhuraju et al., 2002; Umamaheswari and Chatterjee, 2008). Ascorbic acid was used as a positive control and finally microplate reader was used to measure absorbance at 630 nm to determine their total reducing power potential.

2.6 Antimicrobial Activity

2.6.1 Antibacterial Activity

Antibacterial potential of different plants extracts were checked against five pathogenic bacterial strains. Gram positive bacterial strains such as *Staphylococcus aureus* (ATCC 6538) and *Bacillus subtilus* (ATCC 19659) and gram negative bacterial strains; *Pseudomonas aeruginosa* (ATCC 90271), *Escherichia coli* (ATCC 33456) and *Klebsiella pneumonia* (ATCC 1705) were used. Bacterial isolates on nutrient broth media were sub-cultured at 37 °C and were incubated for 18 hrs. Further, bacterial cultures were standardized by regulating OD to 0.5 (1×10^8 CFU/mL). Antibacterial assay was carried out using disc diffusion method at different concentrations of plants extracts ranging from 100, 33.33, 11.11, 3.7 µg/mL and minimum inhibitory concentration (MIC) values were measured.

2.6.2 Antifungal Activity

The antifungal potentials of plants extracts were evaluated by disc diffusion method. For this purpose, five fungal strains; *Candida albicans* (FCBP 478), *Fusarium solani* (FCBP 0291), *Aspergillus flavus* (FCBP 0064), *Aspergillus niger* (FCBP 0918) and *Mucor racemosus* (FCBP 0300) were sub-cultured in SDA media at 25°C for 24 hrs. After treatment with plants extracts, fungal plates were placed in incubator for 24 hrs and ZOI were measured. Clotrimazole was used as positive and DMSO as a negative control. Further, extracts were screened at different concentrations (3.7, 11.11, 33.33 and 100 µg/mL) of extracts to determine their dose dependent response and their MIC values were calculated.

2.7 Alpha-amylase Inhibition (AAI) Assay

Different plant extracts were evaluated by *in-vitro* AAI assay proposed by (Khalil et al., 2018) with little modifications. For this purpose, reaction mixture was prepared using test samples (10 µL), phosphate buffered saline (15 µL), alpha-amylase enzyme (25 µL), starch solution (40 µL) and the whole mixture was incubated at 50 °C for 30 minutes. Further, 1 M HCl (20 µL) and iodine solution (90 µL) were added. Finally, different concentrations of extracts (6, 12.5, 25, 50, 100, 200 µg/mL) were used to confirm their AAI potential. Acarbose and distilled water were used as positive and

negative control respectively to determine their AAI potential. Percentage Enzyme inhibition of the samples were calculated using the formula below

PEI bsor bance of Sample bsorbance of Negative / bsor bance of Negative 100

Where PEI is Percentage Enzyme inhibition

2.8 Anti-leishmanial Assay

Anti-leishmanial assay of plant extracts were performed against *L. tropica (*KWH_{23} strain) using MTT assay (Ovais et al., 2018). *L. tropica* Parasites were cultured in MI99 media using 10 % fetal bovine serum (FBS). Tested plants extracts (20 µL) were added in 96-well plate with *L. tropica* and incubated for 72 hrs at 24 °C in 5 % CO_2 incubator. DMSO was utilized as a negative control and Amphotericin B as positive control. Finally, 96-well plate was placed in spectrophotometer and readings were taken at 540 nm.

2.9 Brine Shrimp Cytotoxicity Assay

Lethality potential of brine shrimps at different concentrations of plants extracts were evaluated using previously reported protocol (Madjos and Luceno, 2019). *Artemia salina* eggs (Ocean Star, USA) were kept in sea water for hatching (3.8 % sea salt enhanced with 6 mg/mL yeast at pH 7) for 24 hrs. Different concentrations of plants extracts ranging from 1000, 500, 250, 62.5 and 31.25 µL were poured in the vials and final volume was raised up to 5 mL with sea salt solution. After 24 hrs, ten brine shrimps were transferred in each vial and were incubated at 32 °C for 24 hrs and living shrimps were counted in each vial (Mehwish et al., 2019). Percentage mortality of *Artemia salina* and LD_{50} values of plants extracts were calculated.

2.10 Protein Kinase Inhibition Assay (PKI)

This assay was carried out under sterilized conditions using *Streptomyces* 85E strain by following optimized protocol of Yao et al. (2011). For this purpose ISP4 media was prepared and inoculated with *Streptomyces* 85E strain. Autoclaved filter discs (6 mm) were loaded with 250 µg of plants extracts (10 µL) and placed on cultured plates and then incubation was done for 72 hrs at 30 °C. Surfactin was used as a standard. Clear zone indicates cell toxicity of samples whereas appearance of bald zone characterizes growth inhibition of hyphae.

2.11 Anticancer Assay

The plants extracts which show highest cytotoxicity potential were further investigated using PC-3 cancer cell line using previously optimized protocol (Mosmann, 1983). For this purpose, confluent HUH-7 cancer cells were propagated in 96-well plate and were sub-cultured in Dulbecco's modified eagle media (DMEM) added with 5 % FBS, and 1 % Pen-Strep. Further, seeded cells were incubated at 37 °C in 5 % CO_2 incubator. MTT assay was performed in the Eliza plate using various concentrations of test sample (100, 33.33, 11.11 µg/mL) of extracts for 48 hrs. Each well of 96-well plate was loaded with 30 mL of MTT solution and was further incubated for 3 hrs. The formazan produced by living cells was monitored at 570 nm using Elisa plate-reader.

2.12 Scanning Electron Microscopy (SEM) of Seeds

Micro-morphological features of seeds such as size and shape were measured with the help of Light Microscopy (Meiji MT4300H). Comprehensive variations in micro-morphological characters such as seed coat surface were studied with the help of SEM. For this purpose, seeds were dipped in solution of 70 % ethyl alcohol (3 minutes) for debris removal. For visualization the seeds were placed on stubs of SEM (Model JEOL JSM- 5910) that were coated with gold–palladium particles. Seed characters such as margins, shapes, surface, and arrangements of periclinal cell wall were observed and photographed (polaroid films p/n665) (Munir et al., 2019).

2.13 FTIR Spectra Analysis

FTIR analysis (Perkin Elmer-Spectrum 65 was carried out using previously proposed method by Meenambal et al. (2012) to determine the different functional groups present in plant samples. For this purpose, powdered plant material was subjected to a pressure of about 5×10^6 Pa in an evacuated dye and spectra were recorded at 4000-400 cm^{-1} frequency.

2.14 Statistical Analysis

Data of present study was analyzed as a mean ± SD. All measurements were performed in triplicate. Correlation analysis was determined by using correlation and regression line in Microsoft Excel Program. Table curve 2D ver. 4 software was used for IC_{50} and LD_{50} calculations. GraphPad Prism5 software was used for graphs. For anatomical studies

CHAPTER: 3

RESULTS & DISCUSSION

RESULTS & DISCUSSION

This chapter comprises of experimental findings of the present research. The selected medicinal plants were*: Echinops niveus* Wall. ex Wall, *Iris lactea* Pall. *Lactuca orientalis* (Boiss.) Boiss. *Polygonum affine* D. Don, *Rhodiola imbricata* Edgew. *Salix planifolia* Pursh, *Saxifraga flagellaris* Willd. *Sophora alopecuroides* L. The studied medicinal plants analyzed for preliminary phytochemical analysis, quantitative phytochemical analysis, antioxidant potential, antimicrobial activity, cytotoxicity assessment, antidiabetic and anticancer activities. This chapter is further divided into following sections.

SECTION 1 : *Echinops niveus* Wall. ex Wall.

SECTION 2 : *Iris lactea* Pall.

SECTION 3 : *Lactuca orientalis* (Boiss.) Boiss.

SECTION 4 : *Polygonum affine* D. Don,

SECTION 5 : *Rhodiola imbricata* Edgew.

SECTION 6: *Salix planifolia* Pursh,

SECTION 7 : *Saxifraga flagellaris* Willd.

SECTION 8 : *Sophora alopecuroides* L.

SECTION 1 : *Echinops niveus* Wall. ex Wall.

3.1 *Echinops niveus* Wall. ex Wall.

Echinops niveus Wall. ex Wall. belongs to family Asteraceae (formerly Compositae). Naturally found in Pakistan, Nepal, China, Bhutan and India (https://www.gbif.org/occurrence/search?taxon_key=7880458). Its synonym is *Echinops niveus* Hend. (http://ww .theplantlist.org/tpl1.1/ search?q=Echinops+niveus+ Wall.+ ex+ Wall). People of rural areas mostly relay on traditional herbal system of medicines due to their believe and less availability of allopathic medicines (Aziz et al., 2018). For thousands of years, different *Echinops* species were applied in traditional medicinal system. The therapeutic effect of *Echinops* are widely due to the presence of polyphenols that exhibit anti-inflammatory, anti-allergic, antiviral, anti-allergic, diuretic, antibacterial, vasodilator actions, antithrombotic, arteriosclerosis, neurodegenerative diseases, cancer, arthritis and anticancer properties (Bibi et al., 2011; Minutolo et al., 2012).

Figure 14: Field Photograph of *Echinops niveus* Wall. ex Wall.

Mainly *Echinops* species are commonly used in traditional medicine to treat contagious ailments and tumors (Seukep et al., 2020). Local people of Hunza valley used areal part of *E. niveus* for the cure of snake bite, diuretic, bacterial, inflammation and allergic responces.

3.1.1 Preliminary Phytochemical Analysis of *E. niveus* Extracts

Globally plants are present with varied medicinal capability to treat many diseases. This capability is due to the presence of diverse biochemical compounds. These bioactive compounds extracted from different plant parts. Due to these extraordinary therapeutic potential of phytochemicals, medicinal plants become the center of research (Suryavanshi et al., 2019). Initial phytochemical screening of *E. niveus* extracts shows the presence of alkaloids, flavonoids, phenols, saponins, tannins, amino acids in all the six ENM, ENE, ENC, ENEA, ENH and ENQ extracts. Glycosides were moderately present in all extracts of EN except ENH. amino acids was missing in ENH and ENQ. Carbohydrates were more concentrated in ENC while absent in ENA and ENM. Steroids were missing in ENE, ENC and ENH extracts (Table 4). Present study showed that EN extracts revealed the existence of phytochemicals alkaloids, flavonoids, amino acids, phenols, glycosides tannins, steroids and saponins which is in harmony with the findings of Liu et al. (2019) provide detail phytochemical analysis of various extracts of *E. grijsii.*

Table 4: Preliminary Phytochemical Analysis of *E. niveus* Extracts.

Plant Extracts	Alkaloids	Flavonoids	Phenols	Carbohydrates	Steroids	Glycosides	Saponins	Tannins	Amino acids
ENM	+++	+++	+++	-	+++	++	+	+++	+
ENE	++	++	++	++	-	+	+	+	+
ENC	++	+++	+++	+++	-	++	++	++	+
ENA	++	++	++	-	+	+	++	+	++
ENH	+	+	+	++	-	-	+	+	-
ENQ	++	++	++	+	++	++	+++	+	-

+++ Strongly present; ++ Moderately present; + Weakly present; -: Absent. ENM: *E. niveus* methanol, ENE: *E. niveus* ethanol, ENC: *E. niveus* chloroform, ENA: *E. niveus* ethyl acetate, ENH: *E. niveus* n-hexane, ENQ: *E. niveus* aqueous extract.

3.1.2 Quantitative Phytochemical Examination of *E. niveus* Extracts

Quantitative phytochemical examination of *E. niveus* has exposed the presence of phenolic and flavonoids content in good amount which have potential against various diseases. Total phenolic contents in various extracts of EN were assessed. The findings of phenolic contents of EN extracts displayed following order ENC>ENM>ENE>ENA> ENQ>ENH and were summarized in Figure 15. ENC had highest phenolic contents (63.14 mg GAE/g), followed by ENM (61.52 mg GAE/g), ENE (52.37 mg GAE/g), ENA (47.56 mg GAE/g), ENQ (38.54 mg GAE/g) and ENH (21.22 mg GAE/g). Similar results were demonstrated by Mohseni et al. (2017) for *E. percicus* extracts showing a reasonable amount of phenolic contents.

Data of total flavonoids obtained from different extracts of *E. niveus* is summarized in Figure 16. Among all ENC has highest flavonoid contents (71.96 mg QE/g), followed by ENM (68.50 mg QE/g), ENE (60.69 mg QE/g), ENA (54.97 mg QE/g), ENQ (51.43 mg QE/g) and ENH extract (46.39 mg QE/g). Other members of *Echinops such as E. echinatus* also possess highest amount of flavonoids (Jamila et al., 2020).

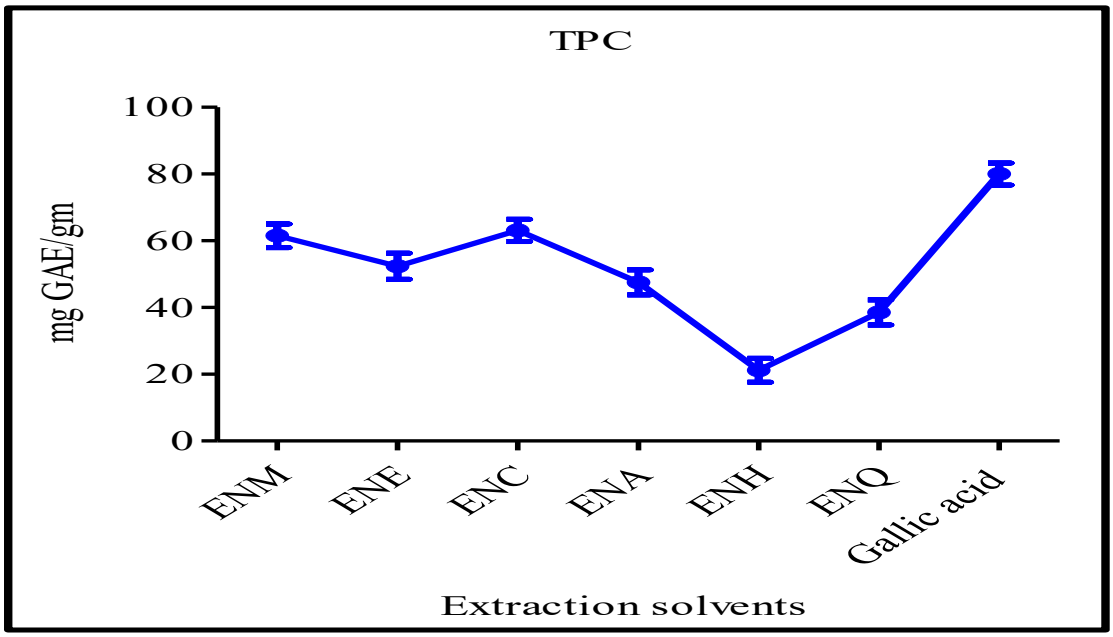

Figure 15: Total phenolic contents of *E. niveus*. ENM: *E. niveus* methanol, ENE: *E. niveus* ethanol, ENC: *E. niveus* chloroform, ENA: *E. niveus* ethyl acetate, ENH: *E. niveus* n-hexane, ENQ: *E. niveus* aqueous.

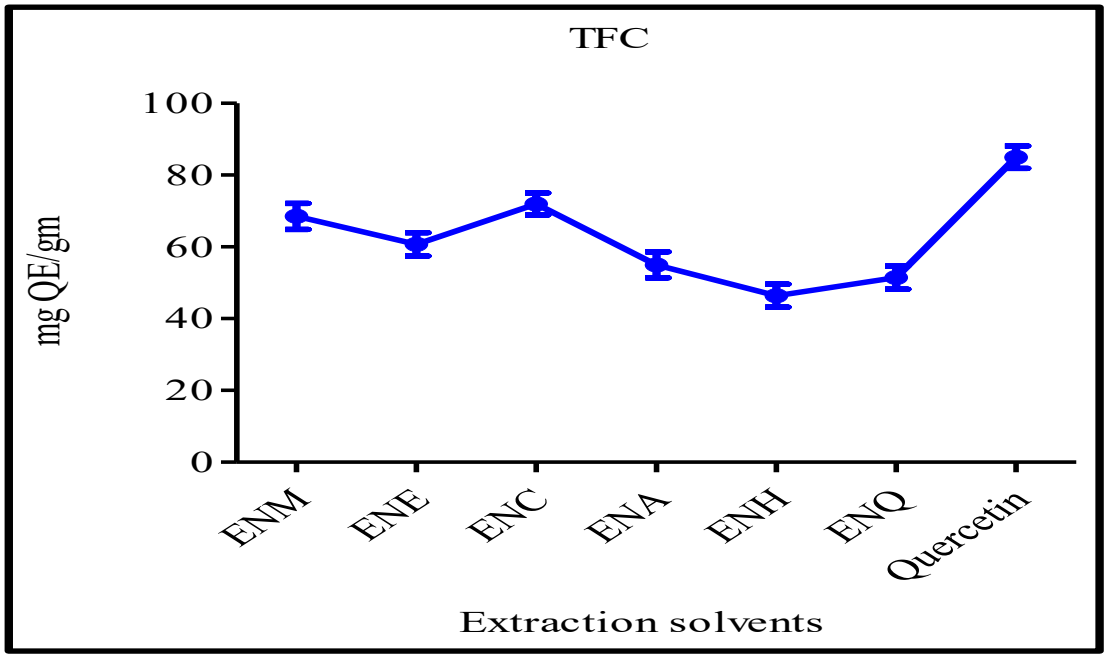

Figure 16: Total flavonoid contents of *E. niveus*. ENM: *E. niveus* methanol, ENE: *E. niveus* ethanol, ENC: *E. niveus* chloroform, ENA: *E. niveus* ethyl acetate, ENH: *E. niveus* n-hexane, ENQ: *E. niveus* aqueous.

3.1.3 FTIR Spectral Analysis (cm-^{1}) of *E. niveus*

FTIR analysis revealed the presence of various functional groups with strong and medium peak intensities. At first sight, C-H stretch was observed in the sample indicating the presence of alkanes. One broad peak is explicitly visible showing the presence of alcohols and phenols in *E. niveus* Some strong and medium peaks were also observed which indicated the existence of C=O stretch, C-H bend, C-H wag, C-N, C-Br and C-Cl stretch and hence confirmed the presence of alkanes, carboxylic acids, aliphatic amines and alkyl halides. FTIR spectra of *E.niveus* is shown in Figure 17. The availability of these different functional groups showed that these biomolecules play significant role in the biological potential of EN-mediated extracts.

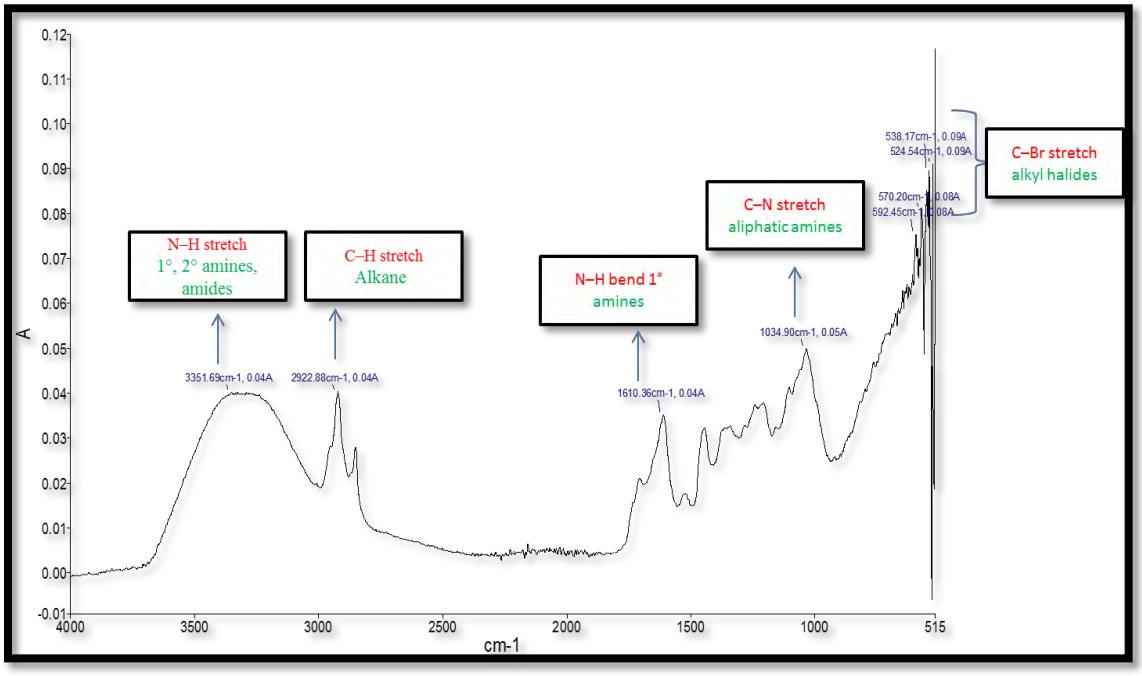

Figure 17: FTIR spectral analysis (cm-1) of *E. niveus*.

3.1.4 Antioxidant Potential of *E. niveus* Extracts

Antioxidant potential shows its dynamic role to over-come the effects of oxidative stress (Alkadi, 2020). From plants a large number of natural products are obtained having reducing ability to scavenge the free radicals (Szymanska et al., 2018). DPPH assay is most widely used antioxidant screening assay (Shakeri et al., 2018). The noticed

order of IC_{50} values for different extracts of *E. niveus* was ENC < ENM < ENE < EN < ENQ < ENH (Figure 18). Among all extracts ENC showed highest IC_{50} value (33.45± 2.7 µg/mL) that were followed by ENM (50.43±2.1 µg/mL), ENE (68.14±2.4 µg/mL), ENA (79.43±2.1 µg/mL), ENQ (94.54 ± 2.7 µg/mL) and ENH (104.76±1.2 µg/mL). Similar results were demonstrated in a study on *E. persicus* (Jamila et al., 2020) in which authors showed slightly polar extracts showed higher IC_{50} value for DPPH assay.

Antioxidant capacity of plant samples was detected by the appearance of phosphomolybdenum (V) green color (Gawad et al., 2019). Highest antioxidant capacity was given by ENC (63.49±1.46mg AAE/g sample) followed by ENM (52.49±2.43 mg AAE/g sample), ENE (50.05±1.24 mg AAE/g sample), ENA (44.29±1.32 mg AAE/g sample), ENQ (39.32±2.43 mg AAE/g sample) and ENH (30.85±1.87 mg AAE/g sample) and was found to decrease in the order of ENC < ENM < ENE < ENA< ENQ <ENH (Figure 19). Our findings are in agreement with the previous study of Hegazy et al. (2019) which described that *E. spinosus* extracts exhibited notable antioxidant capacity.

Reducing power of *E. niveus* was measured by using potassium ferri-cynide reducing method. In this assay the antioxidant potential of plants was accessed by their ability of electron donation and result into the reduction of ferric ions to ferrous ions (Shukla et al., 2019). ENC showed the maximum reducing power with 73.14±1.47 mg AAE/g sample measured at 200 µg/mL of extract followed by ENM (62.156±1.32 mg AAE/g sample), ENE (60.26±2.34 mg AAE/g sample), ENA (58.65±1.76 mg AAE/g sample) ENQ (49.75±1.43 mg AAE/g sample) and ENH (42.97±1.65 mg AAE/g sample) as shown in Figure 19. The result was considerably correlated with TFC and TPC. These findings followed the pattern of ENC < ENM < ENE < ENA < ENQ < ENH at 200 µg/mL. Present study is in agreement with a report (Sarvaiya et al., 2017) in which the reducing power capacity from different extracts of *E. echinatus* were determined.

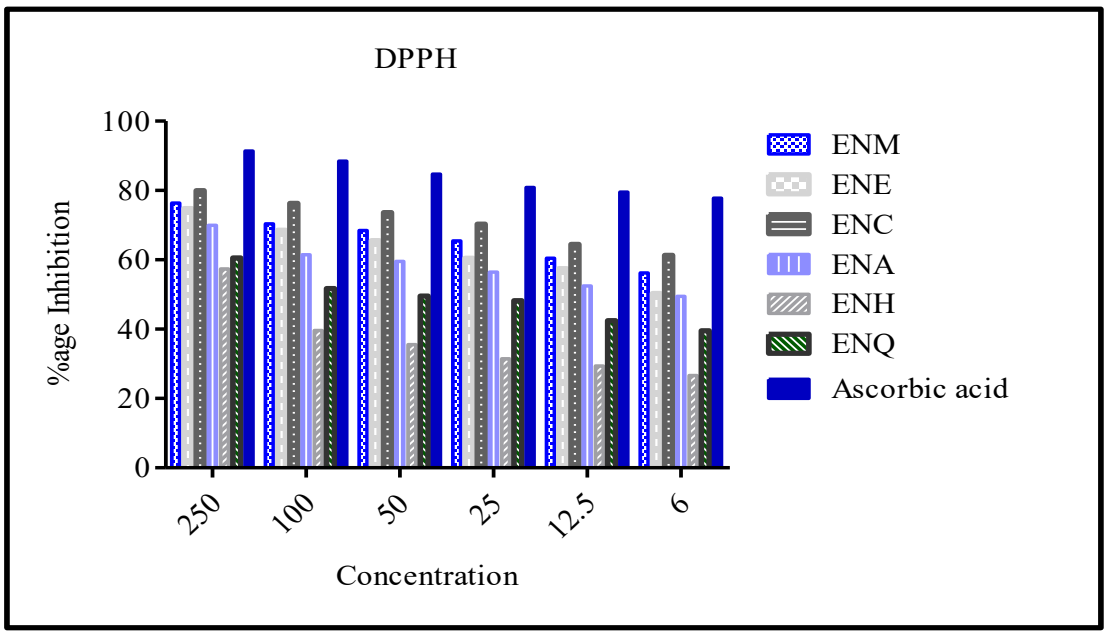

Figure 18: DPPH assay of different extracts of *E. niveus*. ENM: *E. niveus* methanol, ENE: *E. niveus* ethanol, ENC: *E. niveus* chloroform, ENA: *E. niveus* ethyl acetate, ENH: *E. niveus* n-hexane, ENQ: *E. niveus* aqueous.

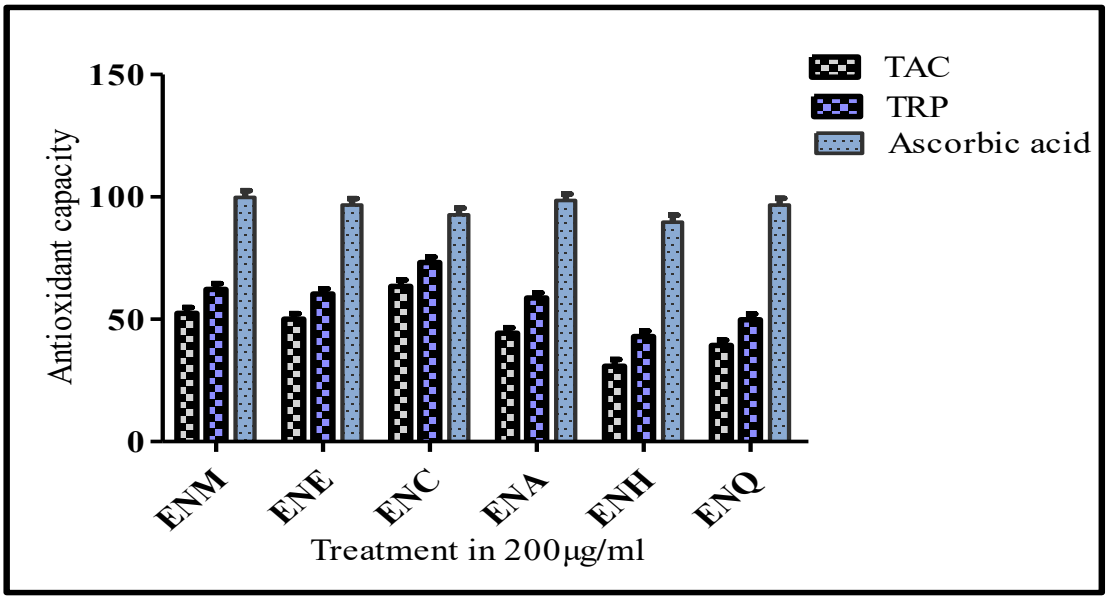

Figure 19: Total antioxidant capacity and total reducing power activity of different extracts of *E. niveus*. ENM: *E. niveus* methanol, ENE: *E. niveus* ethanol, ENC: *E. niveus* chloroform, ENA: *E. niveus* ethyl acetate, ENH: *E. niveus* n-hexane, ENQ: *E. niveus* aqueous.

3.1.5 Antimicrobial Activity of *E. niveus* Extracts

From the ancient times plants were used as a potential source of traditional medicine. So researchers are working on medicinal plants in order to discover new compounds that can be used against multi drug resistance microbes (Zoysa et al., 2019). Antibacterial activity of *E. niveus* was evaluated qualitatively and quantitatively against five bacterial strains. The presence or the absence zone of inhibition (ZOI), ZOI diameters and their MIC values were checked. The preliminary screening results and MIC values of bacterial strains are given in Table 5. ENA shows best anti-bacterial capacity against three tested bacterial strains. The extreme sensitive bacterial strain proven to be *P. aeruginosa* followed by *E. coli* displaying ZOI of 19±1.65 mm and 17±1.16 mm, *B subtilis* showed least activity that exhibits the ZOI of 11±1.94 mm. ENA extract against *S. aureus* and *K. pneumonia* shows no activity. ENQ inhibited growth of only one bacterial strain that was *K. pneumonia* with ZOI 11±1.04 mm. ENA has best antibacterial activity as it showed inhibitory response for three tested bacterial strains indicating the presence of secondary matabolits having antibacterial potential. Present findings were in line with the finding of Jiang et al. (2017) in which the antibacterial property of *E. ritro* were studied.

Fungi is available abundantly in our environment, mostly they are not harmful to health but some of them are unsafe and may cause serious health problems. For eras many of the herbal plants were used to counter fungi, as plants contain antimicrobial agents that are used to treat infections caused by fungi (Abdullah et al., 2019). The antifungal potential of *E. niveus* we`re tested against fungal strains. The recorded data is avalible in Table 6.

ENC and ENM showed noteworthy ZOI whereas ENE, ENA, ENQ and ENH gave moderate antifungal activities. ENC inhabited growth of tested fungal strains,the susceptible fungal strain towards ENC was *C. albicans* (18±1.6 mm) while the less susceptible strain was *F. solani* (6±1.4 mm). ENQ gives minimal antifungal actity against two fungal stains and show insensitivity against *M. racemosus*, *C. albicans* and *A. flavus*. Similar findings were reported for *E. grijsii* regarding its antifungal activity (Liu et al., 2019).

Table 5: Inhibition Zones and MIC of *E. niveus* Extracts against Bacterial Strains.

Micro-organisms	*E. coli* ATCC 33456		*P. aeruginosa* ATCC 90271		*K. pneumonia* ATCC 1705		*B. subtilus* ATCC 19659		*S. aureus* ATCC 6538	
Plant Extracts	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)
ENM	NI		NI		NI		NI		18± 1.24	33.33
ENE	5± 1.04		NI		NI		10± 1.65	100	NI	
ENC	NI		12± 1.24	100	10± 1.27	100	NI		3± 1.98	
ENA	17± 1.16	100	19± 1.65	33.33	NI		11± 1.94	100	NI	
ENH	16± 2.35	33.33	NI		NI		NI		11± 1.18	100
ENQ	NI		NI		11± 1.04	100	NI		NI	

Values (mean ± standard deviation) was obtained through from triplicate analysis. NI no activity, ENM *E. niveus* methanol, ENE *E. niveus* ethanol, ENC *E. niveus* chloroform, ENA *E. niveus* ethyl acetate, ENH *E. niveus* n-hexane, ENQ *E. niveus* aqueous

Table 6: Inhibition Zones and MIC of *E. niveus* Extracts against Fungal Strains.

Fungus strains	*M. racemosus* FCBP 0300		*C. albicans* FCBP 478		*A. niger* FCBP 0918		*F. solani* FCBP 0291		*A. flavus* FCBP 0064	
Plant Extracts	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)
ENM	15±2.5	33.33	14±2.6	100	11±1.5	100	NI		6±1.6	
ENE	NI		13±2.6	100	11±2.4	100	4±2.5		NI	
ENC	11±2.4	100	18±1.6	33.33	17±1.5	33.33	6±1.4		14±2.4	100
ENA	7±1.34		3		NI		11±2.4		NI	
ENH	NI		NI		5±2.6		NI		10±1.4	100
ENQ	NI		NI		5±2.8		17±2.6	33.33	NI	

Values (mean ± standard deviation) was obtained through from triplicate analysis. NI no activity, ENM *E. niveus* methanol, ENE *E. niveus* ethanol, ENC *E. niveus* chloroform, ENA *E. niveus* ethyl acetate, ENH *E. niveus* n-hexane, AHQ *E. niveus* aqueous extract.

Leishmaniosis is a diseased caused by species of *Leishmania*, mainly affect people of developing countries(Tullius et al., 2016). *E. niveus* confirms the noticeable inhibition in growth of *L. tropica* by all six extracts (Figure 20). . *E. niveus* extracts were found percentage inhibition greater than 50 and may be used as a source of anti-leishmanial drug. ENA showed highest percentage inhibition of 64 % followed by ENM with 58.42 %. Amphotericin B as a standard with 92 % and in case of negative control (pure DMSO) no activity was noted. The result shows that the compounds that potent anti-leishmanial activity were more concentrated in moderately polar extracts. Present findings were in exact association with the report of Dutra et al. (2019) which state that polar extracts of *M. fasciculata* showed effective anti-leishmanial activity like *E. niveus* , Similar members of family Asteraceae also possess anti-leishmanial activity (Neto et al., 2019).

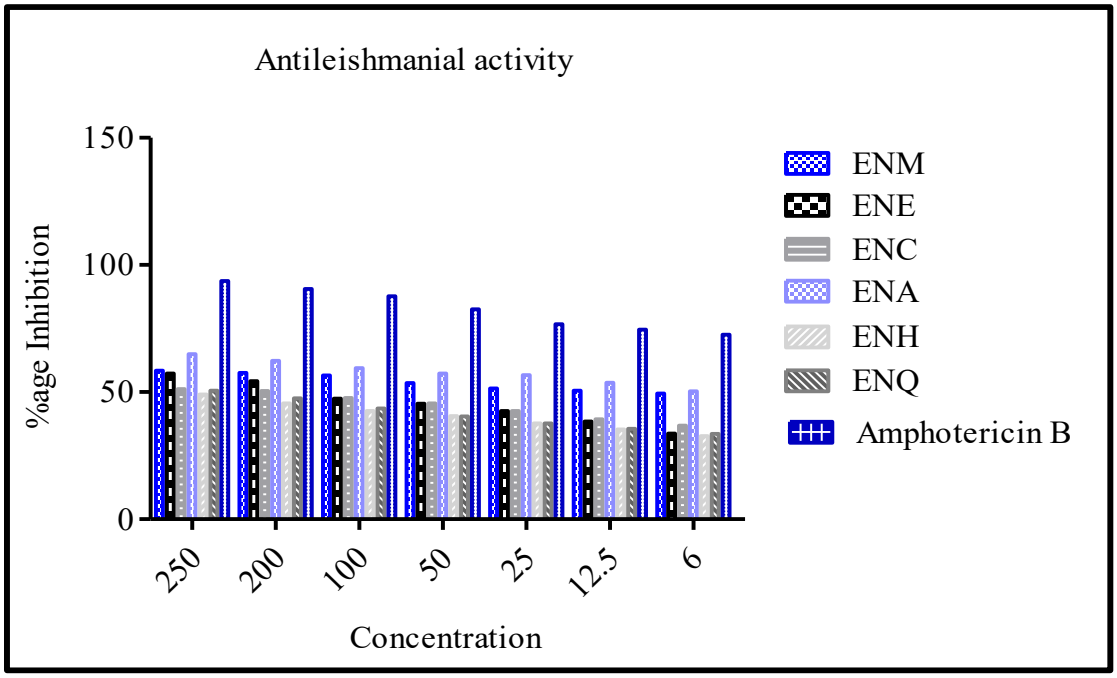

Figure 20: Anti-leishmanial assay of different extracts of *E. niveus*. ENM: *E. niveus* methanol, ENE: *E. niveus* ethanol, ENC: *E. niveus* chloroform, ENA: *E. niveus* ethyl acetate, ENH: *E. niveus* n-hexane, ENQ: *E. niveus* aqueous.

3.1.6 Cytotoxicity and Anticancer Potential of *E. niveus* Extracts

Plants have numerous health benefits, beside this some studies shows that certain plant species are toxic to humans. Bioactive compounds obtain from plants are toxic if they are used in high dosage. Plants that are toxic can be used in the treatment of cancer. The best simple, cost-effective, rapid and reliable technique used for initial screening for toxicity is brine shrimp bioassay. *Artemia nauplii* used extensively for studying the toxicity of plants (Munodawafa et al., 2016). Cytotoxic potential of *E. niveus* extracts were tested against larvae of brine shrimp. Toxicity level was measured in direct relation with the extract concentration when examined by serial dilution methods. *E. niveus* six organic extracts were initially screened for cytotoxic potential, 33.33 % extracts displayed LD_{50} value under 60 µg/mL and were identified to be extremely cytotoxic where as 16.6 % considered to be moderately cytotoxic. 50 % remaining extracts were low cytotoxic with LD_{50} values >200 µg/mL (Table 7).

Doxorubicin, the positive control demonstrated the value of LD_{50} as 7.37 µg/mL. Among all tested extracts, ENQ was found cytotoxic with LD_{50} 56.15 µg/mL demonstrating that the water as a solvent, is highly effective in the extraction of cytotoxic compounds from *E. niveus* as compared to polar and non-polar solvents. Similar with these finding from *E. niveus,* other plant species belonging to family Asteraceae have strong cytotoxic potential (Nino et al., 2006).

Different *in-vitro* assays are used to determine the anticancerous activities of plants and among them Protein Kinase inhibition (PKI) assay is considered best. PKI is well-known class of serine/threonine kinases that are used in the treatment of cancer (Khan et al., 2018). Present days, many scientists are taken interest in protein kinase inhibition activity of plants. Till to date, no evidence about PKI activity of *E. niveus* (ethnomedicinal important herb) was found. The result indicates that EN has ability for inhibition of protein kinases (Table 7). Direct relationship was found among different concentrations of tested plant extracts and PK inhibition activity. The significant bald area with (ZOI 19±1.55 mm) was measured around ENA loaded disc that was followed by ENM (14±2.4 mm) and ENE (11±1.9 mm), Our findings that ethyl-acetate and methanol extract have best PKI potential is in agreement with the reported data related to

PKI potential of ethyl-acetate and methanol extract of *F. ananassa* leaves (Khan et al., 2018).

The cytotoxic potency of the *E. niveus* extract against prostrate cell line (PC3) was demonstrated using MTT assay. The result of ENQ extract has confirmed reduction in metabolic activity of PC3 cells as shown in Figure 21. The reduced metabolic activity has shown that ENQ extract might have strong anticancer potential. The cytotoxicity induced by ENQ extract at lower doses could be due to the plant cytotoxic active functional groups, the present data is in close proximity to the previous studies of antiproliferative potential of *E. lanceolatus* (Seukep et al., 2020).

Table 7: Brine Shrimp Lethality and Protein Kinase Inhibition Assay.

Samples	Brine shrimp lethality (µg/mL)		Protein kinase inhibition (µg/mL)		
	% Mortality	LD_{50} (µg/mL)	Diameter (mm) at 100 µg/disc		MIC
	250		Clear zone	Bald zone	
ENM	66.3±1.43	58.4±2.37	----	14±2.4mm	33.33
ENE	53.2±2.34	67.3±1.28	----	11±1.9mm	100
ENC	39.6±2.75	97.43±2.28	----	10±2.1mm	100
ENA	19.8±1.26	121.65±1.36	----	19±1.7mm	11.11
ENH	44.3±2.47	87.54±1.14	----	----	
ENQ	79.8±2.87	56.15±2.23		10±1.5 mm	100

Values (mean ± standard deviation) was obtained through from triplicate analysis. ----: No activity. ENM: *E. niveus* methanol, ENE: *E. niveus* ethanol, ENC: *E. niveus* chloroform, ENA: *E. niveus* ethyl acetate, ENH: *E. niveus* n-hexane, ENQ: *E. niveus* aqueous.

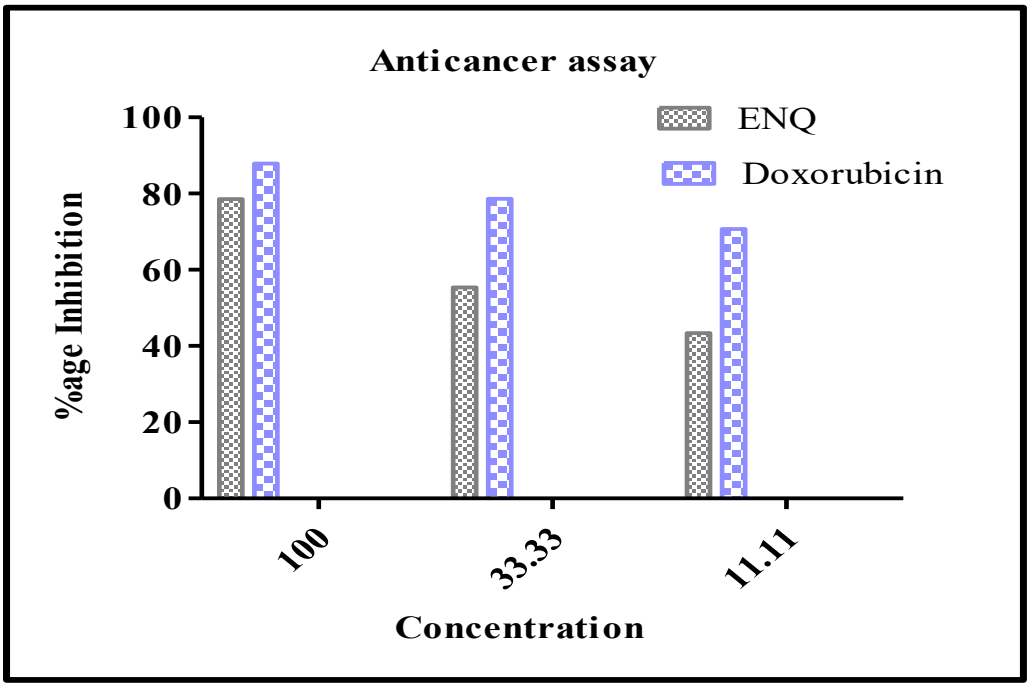

Figure 21: Anticancer activity of *E. niveus* extract against PC-3 Cells.

3.1.7 Antidiabetic Potential of *E. niveus* Extracts

Diabetes is the metabolic disorder and each year it affects more than 300 million persons around the globe.Those compounds that have potential to inhibit the function of carbohydrate hydrolyzing enzymes such as alpha-amylase, can inhibit the digestion of carbohydrates, thus decreasing the blood glucose level (Rodrigues et al., 2017). The available synthetic drugs such as acarbose, voglibose and miglitol are used to target the α-glucosidase and α-amylase, all the drugs have several side effects like gassiness and abdominal stiffness (Salehi et al., 2019).

In order to analyze antidiabetic potential of different extracts of *E. niveus* alpha-amylase Inhibition (AMI) was used. It was seen that ENC shows highest AMI antactivity (51.24±3.54 %) followed by ENA, ENE, ENM, ENQ and ENH (Table 8). The antidiabetic potential was compared with standard drug acarbose which revealed inhibition of 90.9±3.1 %. Present findings are in line with the report of Jamila et al. (2020) in which authors reported the antidiabetic potential of *A. sphathulifolius*.

Table 8: Antidiabetic Potential of *E. niveus* Extracts and Respective IC_{50} Values.

Plant Extracts	%age inhibition of alpha-amylase	IC_{50} µg/mL
ENM	42.47 ±1.36	-----
ENE	45.20 ±2.35	-----
ENC	51.24 ±3.54	112.44
ENA	48.44 ±1.97	-----
ENH	40.27 ±1.46	-----
ENQ	41.76 ±2.54	-----

Values (mean ± standard deviation) was obtained through from triplicate analysis. ----- No activity. ENM: *E. niveus* methanol, ENE: *E. niveus* ethanol, ENC: *E. niveus* chloroform, ENA: *E. niveus* ethyl-acetate, ENH: *E. niveus* n-hexane, ENQ: *E. niveus* aqueous extract.

SECTION 2 : *Iris lactea* Pall.

3.2 *Iris lactea* Pall.

Iris lactea Pall. (IL) is the member of family Iridaceae (Figure 22). Naturally found in Korea, China, Mongolia and Japan (https://www.gbif.org/ occurrence/ search? Offset =20 &q =iris % 20 lactea). *Eremiris lactea* (Pall.) Rodion and *Iris haematophylla* Fisch. ex Link are synonyms of *Iris lactea* Pall.(http://www.Theplantlist.org/tpl1.1/record/kew-322026). *I. lactea* plant is commonly known as white flowered iris or milky iris. The word *"Iris"* comes from Greek that means rainbow. Most of the species from this genus are used as food, forage, medicines and for ornamental purposes. *Iris* species are mostly used to treat diabetes, malaria and gastrointestinal diseases (Crisan and Cantor, 2016). Oils obtained from different species of *Iris* showed antimicrobial activities (Mykhailenko, 2018). *I. lactea* is rhizomatous perennial plant, from central Asia, with pale blue or violet flowers. In Pakistan *I. lactea* grow in Swat, Chitral and Nagar, Gilgit-Baltistan, Northern Pakistan. It has similar pharmacological potential as like other *Iris* species but less explored (Chen et al., 2018).

Figure 22: Field Photograph of *Iris lactea* Pall.

3.2.1 Preliminary Phytochemical Analysis of *I. lactea* Extracts

Initial phytochemical screening of *I. lactea* extracts shows the presence of alkaloids, flavonoids, phenols and saponins in all the six ILM, ILE, ILC, ILA, ILH and ILQ extracts (Table 9). Present results showed that *I. lactea* extracts revealed the existence of phytochemicals alkaloids, flavonoids, amino acids, phenols, glycosides tannins, steroids and saponins which is in accordance with the findings of Bhat (2019) in which authors found different phytochemical compounds in various extracts of *I. kashmirinia*.

Table 9: Preliminary Phytochemical Analysis of *I. lactea* Extracts.

Plant Extracts	Alkaloids	Flavonoids	Phenols	Carbohydrates	Steroids	Glycosides	Saponins	Tannins	Amino acids
ILM	++	+++	+++	++	+++	++	+++	+++	-
ILE	++	++	++	++	++	+++	+++	++	-
ILC	+	++	+++	+++	-	-	+	+	++
ILA	++	+	+	++	-	-	++	-	++
ILH	++	+	+	++	-	-	+	-	+
ILQ	+	++	+	+	++	++	+++	++	-

+++ Strongly present; ++ Moderately present; + Weakly present; - Absent. ILM: *I. lactea* methanol, ILE: *I. lactea* ethanol, ILC: *I. lactea* chloroform, ILA: *I. lactea* ethyl acetate, ILH: *I. lactea* n-hexane, ILQ: *I. lactea* aqueous extract.

3.2.2 Quantitative Phytochemical Analysis of *I. lactea* Extracts

Quantitative phytochemical examination of *I. lactea* has revealed the presence of phenolic and flavonoid contents in higher concentrations that have potential against various diseases. Total phenolic contents in various extracts of IL were evaluated and measured as mg GAE/g of the total dry weight of extracts. The results of phenolic contents of IL various extracts were summarized in Figure 23. Data showed the following order ILA>ILE>ILM>ILQ>ILC>ILH. It was found that the ILA has highest phenolic contents (84.23 mg GAE/g), followed by ILE (75.76 mg GAE/g), ILM (72.38 mg GAE/g), ILQ (69.54 mg GAE/g), ILC (61.32 mg GAE/g) and least in ILH (58.23 mg GAE/g). Similar results were shown in a report on *I. germinica* indicating that *Iris* plant extracts possess significant amount of phenolic contents (Basgedik et al., 2014).

Flavonoids are highly effective as they have power to scavange most of the reactive oxygen species and protect from oxidative stress (Widodo et al., 2019). The total flavonoids assessed from various extracts of *I. lactea* are summarized in Figure 24. It was found that ILA had highest flavonoid contents (81.31 mg QE/g), followed by ILE (70.51 mg QE/g), ILM (68.12 mg QE/g), ILQ (59.87 mg QE/g), ILC (57.97 mg QE/g) and least in ILH extract (54.92 mg QE/g). Other members of *Iris* have also reported highest amount of flavonoids, as Mocan et al. (2018) studied flavonoid contents of *I. schachtii* and also explored strong antioxidant capacity of this plant.

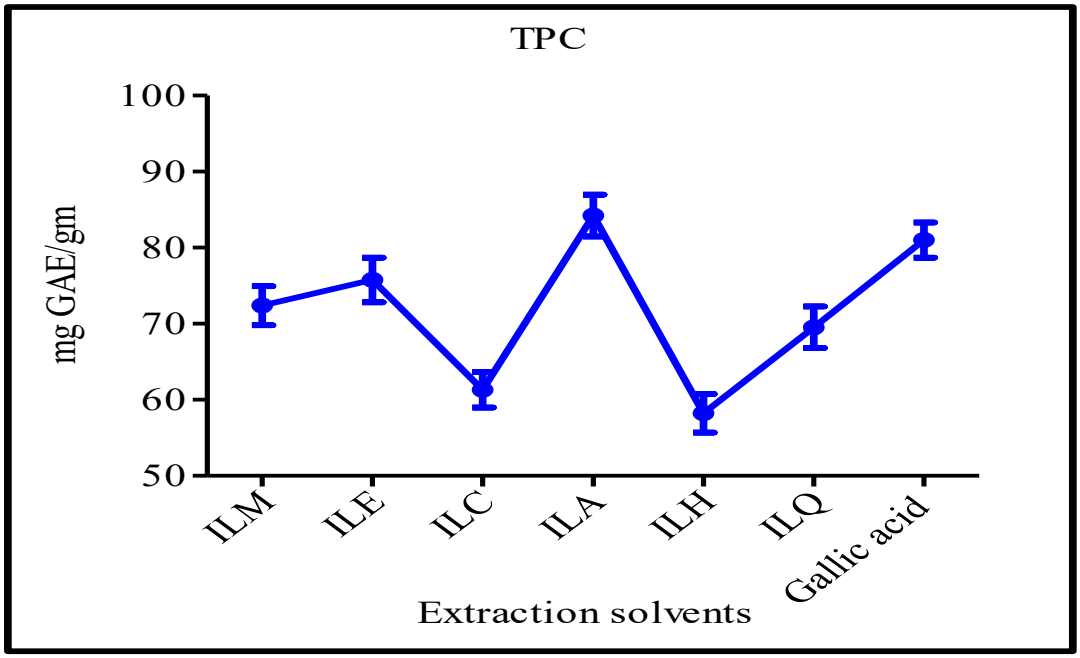

Figure 23: Total phenolic contents of *I. lactea*. ILM: *I. lactea* methanol, ILE: *I. lactea* ethanol, ILC: *I. lactea* chloroform, ILA: *I. lactea* ethyl acetate, ILH: *I. lactea* n-hexane, ILQ: *I. lactea* aqueous extract.

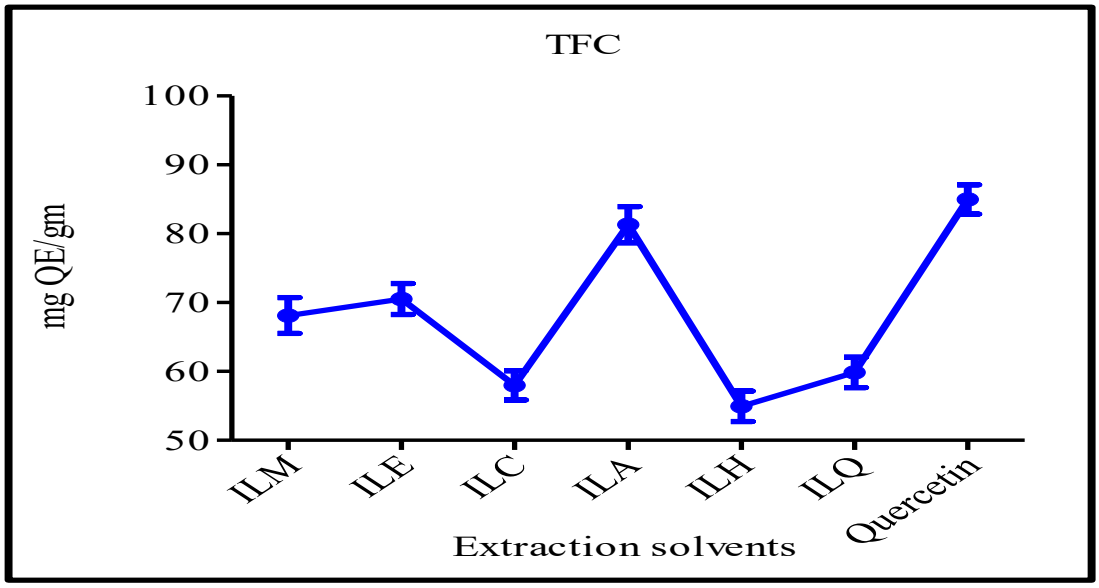

Figure 24: Total flavonoid contents of *I. lactea*. ILM: *I. lactea* methanol, ILE: *I. lactea* ethanol, ILC: *I. lactea* chloroform, ILA: *I. lactea* ethyl acetate, ILH: *I. lactea* n-hexane, ILQ: *I. lactea* aqueous extract.

3.2.3 FTIR Spectral Analysis (cm^{-1}) of *I. lactea*

FTIR analysis revealed the presence of various functional groups with strong and medium peak intensities. At first sight, C-H stretch was observed in the sample indicating the presence of alkanes. One broad peak is explicitly visible showing the presence of alcohols and phenols in *I. lactea* . Some strong and medium peaks were observed which indicated the existence of C=O stretch, C-H bend, C-H wag, C-N, C-Br and C-Cl stretch and hence confirmed the presence of alkanes, carboxylic acids, aliphatic amines and alkyl halides. FTIR spectra of *I. lactea* is shown in Figure 25. The availability of these different functional groups showed that these biomolecules play significant role in the biological potentials of IL-mediated extracts.

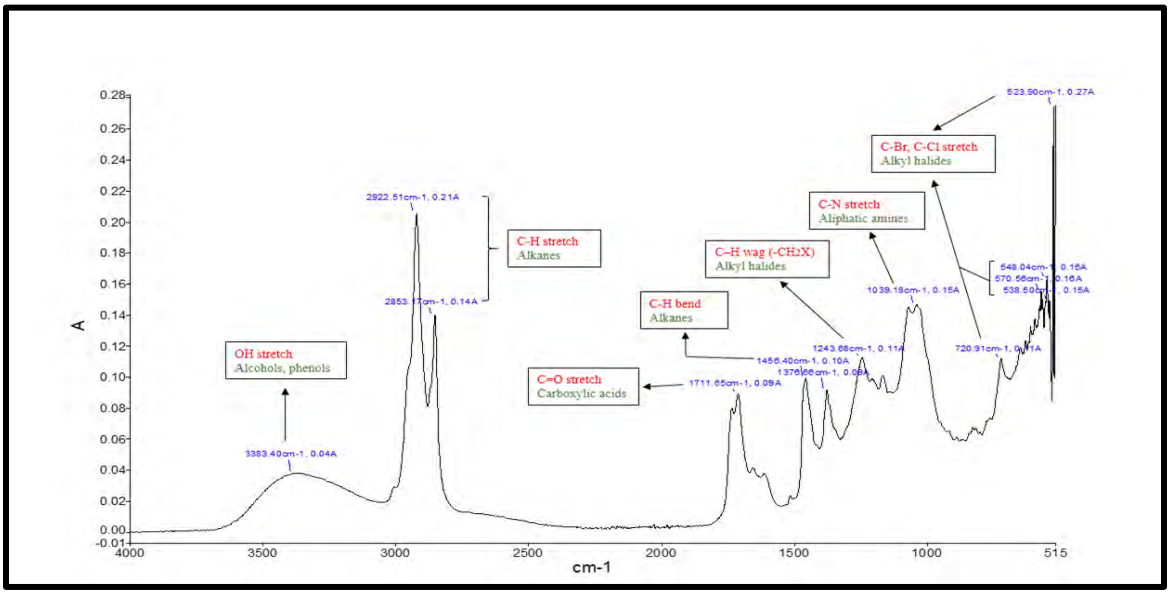

Figure 25: FTIR Spectral Analysis (cm-1) of *I. lactea.*

3.2.4 Antioxidant Potential of *I. lactea* Extracts

The findings of DPPH assay of various extracts of *I. lactea* is shown in Figure 26. Data showed that ILA has best IC_{50} (43.56±2.8 µg/mL) that were followed by ILE (55.12±2.5 µg/mL), ILM (69.1±2.8 µg/mL), ILQ (83.13±2.2 µg/mL), ILC (94.12±2.3 µg/mL) and ILH (1109.76±2.6 µg/mL).

Total antioxidant capacity of various extract of *I. lactea* was measured by phosphomolybdate assay and expressed as the equivalents of standard ascorbic acid at 200 µg/mL of sample (Figure 27). Highest antioxidant capacity was given by ILA (74.53±2.28 mg AAE/g sample) followed by ILE (73.77±2.48 mg AAE/g sample), ILM (70.54±2.54 mg AAE/g sample), ILQ (69.43±2.43 mg AAE/g sample), ILC (64.15±2.34 mg AAE/g sample) and ILH (59.85±2.73 mg AAE /g sample). Our findings are in agreement with the previous study of Kostic et al. (2019) where authors described that three species of genus *Iris* exhibited antioxidant capacity.

ILA showed the maximum reducing power with 83.08±2.75 mg AAE/g sample measured at 200 µg/mL of extract followed by ILE (77.40±2.37 mg AAE/g sample), ILM (75.93±2.53 mg AAE/g sample), ILQ (73.65±2.12 mg AAE/g sample) ILC (70.96±2.32 mg AAE/g sample) and ILH (62.48±1.43 mg AAE/g sample) as shown in Figure 27. The result of this assay was considerably correlated with TFC and TPC. Similar findings were reported by Hacibekiroglu and Kolak (2011) regarding the reducing power and antioxidant capacity of different extracts of *I. suaveolens*

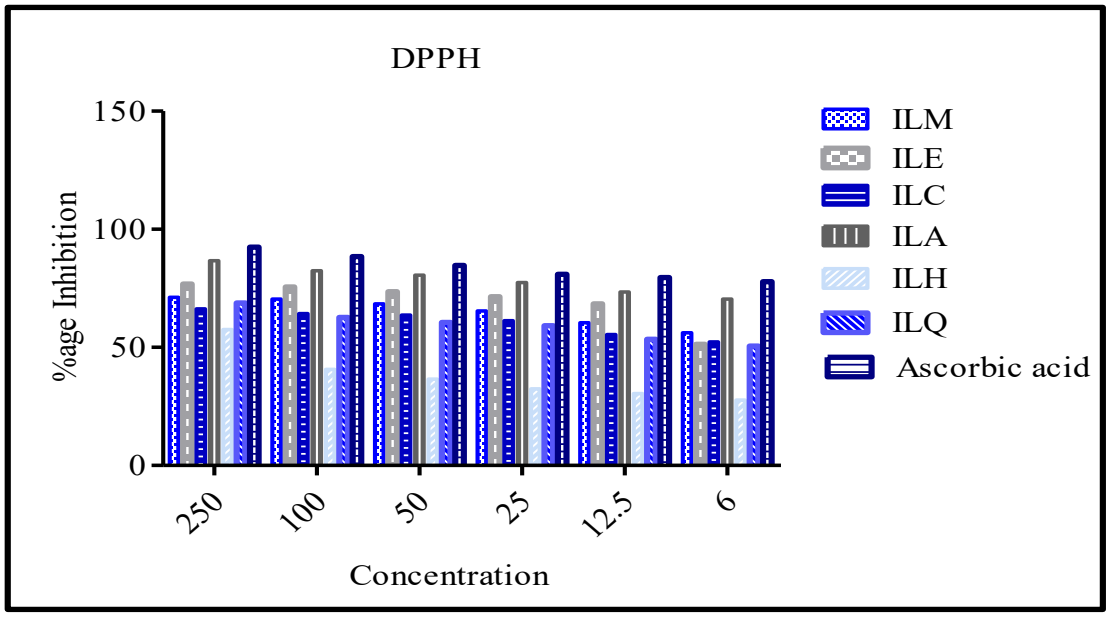

Figure 26: DPPH assay of different extracts of *I. lactea*. ILM: *I. lactea* methanol, ILE: *I. lactea* ethanol, ILC: *I. lactea* chloroform, ILA: *I. lactea* ethyl acetate, ILH: *I. lactea* n-hexane, ILQ: *I. lactea* aqueous extract.

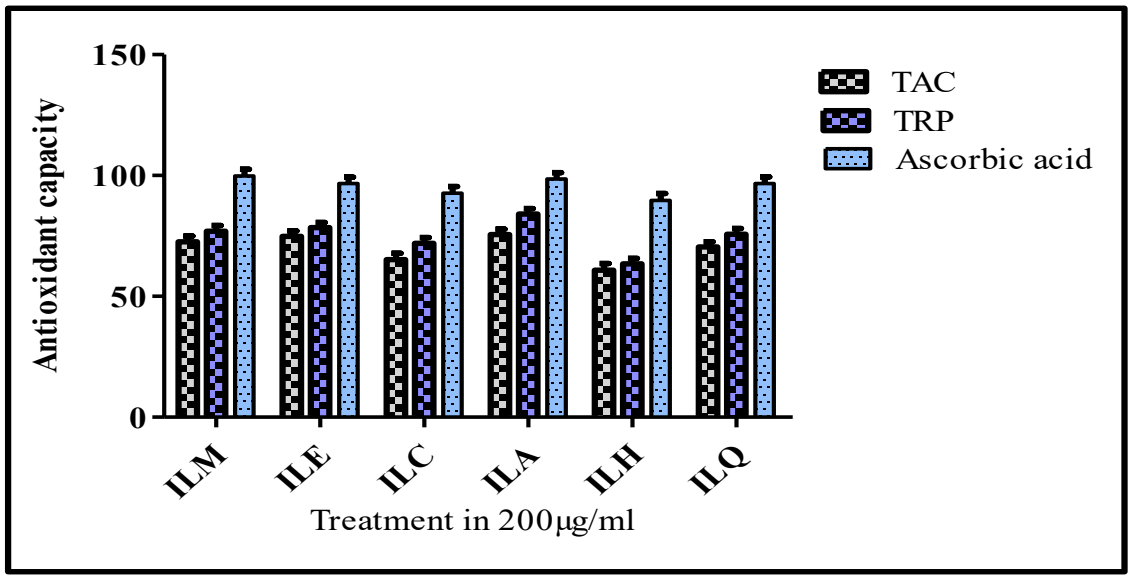

Figure 27: Total antioxidant capacity and total reducing power assay of different extracts of *I. lactea*. ILM: *I. lactea* methanol, ILE: *I. lactea* ethanol, ILC: *I. lactea* chloroform, ILA: *I. lactea* ethyl acetate, ILH: *I. lactea* n-hexane, ILQ: *I. lactea* aqueous extract.

3.2.5 Antimicrobial Potential of *I. lactea* Extracts

Qualitatively and quantitatively antibacterial activity of *I. lactea* was evaluated against five bacterial strains by the presence or the absence of ZOI, ZOI diameters and their MIC values. The preliminary screening results and MIC values of bacterial strains were given in Table 10. The extreme sensitive bacterial strain demonstrated to be *S. aureus* followed by *K. pneumonia* (ZOI 18±2.46 mm and 14±2.15mm). ILA had the best antibacterial activity as it showed inhibitory response against three tested bacterial strain namely *S. aureus*, *K. pneumonia* and *E.coli* which might be due to the presence of those secondary metabolites responsible for antibacterial potency. Present findings were in line with the results of Mykhailenko et al. (2017), in which researchers stated the antibacterial property of *I. hungarica* extracts (prominently in ethyl acetate extracts).

I. lactea extracts have showed significant antifungal properties as shown in Table 11. ILA, ILE and ILM showed significant ZOI whereas ILC, ILQ and ILH gave moderate results. ILA inhibited growth of four fungal strains and most susceptible fungal strain towards ILA was *M. racemosus* (17±1.34 mm). In case of ILM extract, the most sensitive fungal strains was *A. flavus* (17±1.6 mm) while the less sensitive was *A. niger* (11±1.5 mm) while remaining three under study fungal strains gave no response. ILQ showed least anti fungal potential and inhibited only two strains *A. niger* (17±2.6 mm) and *C. albicans* (4±2.8 mm). Present findings are agreement with the finding of Uzair et al. (2016) in which authors reported that the powder of *I. germinica* possess antifungal activity.

Table 10: Inhibition Zones and MIC of *I. lactea* Extracts against Tested Bacterial Strains.

Micro-organisms	*E. coli* ATCC 33456		*P. aeruginosa* ATCC 90271		*K. pneumonia* ATCC 1705		*B. subtilus ATCC* 19659		*S. aureus* ATCC 6538	
Plant Extracts	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/ mL)	ZOI (mm)	MIC (µg/mL)
ILM	10± 1.13	100	NI		NI		10± 1.35	100	NI	
ILE	10± 1.43	100	NI		4± 1.97		12± 1.46	100	NI	
ILC	NI		10± 1.97	100	NI		NI		10± 2.09	100
ILA	11±2.12	100	NI		14± 2.15	100	NI		18±2.46	33.33
ILH	NI		NI		2± 1.12		4±2.43		NI	
ILQ	10±2.43	100	NI		NI		NI		18± 1.24	33.33

Values (mean ± standard deviation) was obtained through from triplicate analysis. NI: no activity, ILM: *I. lactea* methanol, ILE: *I. lactea* ethanol, ILC: *I. lactea* chloroform, ILA: *I. lactea* ethyl acetate, ILH: *I. lactea* n-hexane, ILQ: *I. lactea* aqueous extract.

Table 11: Inhibition Zones and MIC of *I. lactea* Extracts against Tested Fungal Strains.

Fungus strains	*M. racemosus* FCBP 0300		*C. albicans* FCBP 478		*A. niger* FCBP 0918		*F. solani* FCBP 0291		*A. flavus* FCBP 0064	
Plant Extracts	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)
ILM	NI		NI		11±1.5	100	NI		17±1.6	33.33
ILE	12±2.1	100	NI		10±2.4	100	NI		6±2.4	
ILC	NI		NI		NI		NI		16±2.4	33.33
ILA	17±1.34	33.33	8± 2.43		NI		12±2.3	100	11±1.43	100
ILH	NI		5±2.6		NI		10±1.4	100	NI	
ILQ	NI		4±2.8		17±2.6	33.33	NI		NI	

Values (mean ± standard deviation) was obtained through from triplicate analysis. NI: no activity, ILM: *I. lactea* methanol, ILE: *I. lactea* ethanol, ILC: *I. lactea* chloroform, ILA: *I. lactea* ethyl acetate, ILH: *I. lactea* n-hexane, ILQ: *I. lactea* aqueous extract.

Noticeable inhibition in growth of *L. tropica* was displayed by all six extracts (Figure 28). ILA showed highest percentage inhibition with IC_{50} of 68±1.82 µg/mL, followed by ILM (IC_{50} 79±1.32 µg/mL), ILE (IC_{50} 94±1.31 µg/mL), ILC (IC_{50} 114±2.17 µg/mL), ILQ (IC_{50} 131±1.67 µg/mL) and ILH (IC_{50} 204±1.87). Amphotericin B as a standard (IC_{50} 12.3 µg/mL) and pure DMSO (negative control), no activity was noticed. The data indicated that the compounds responsible for anti-leishmanial potential were more concentrated in moderately polar extracts. Rguez et al. (2019) studied that the ethyl-acetate extracts of *C. sempervirens* showed effective anti-leishmanial activity which is similar to our findings.

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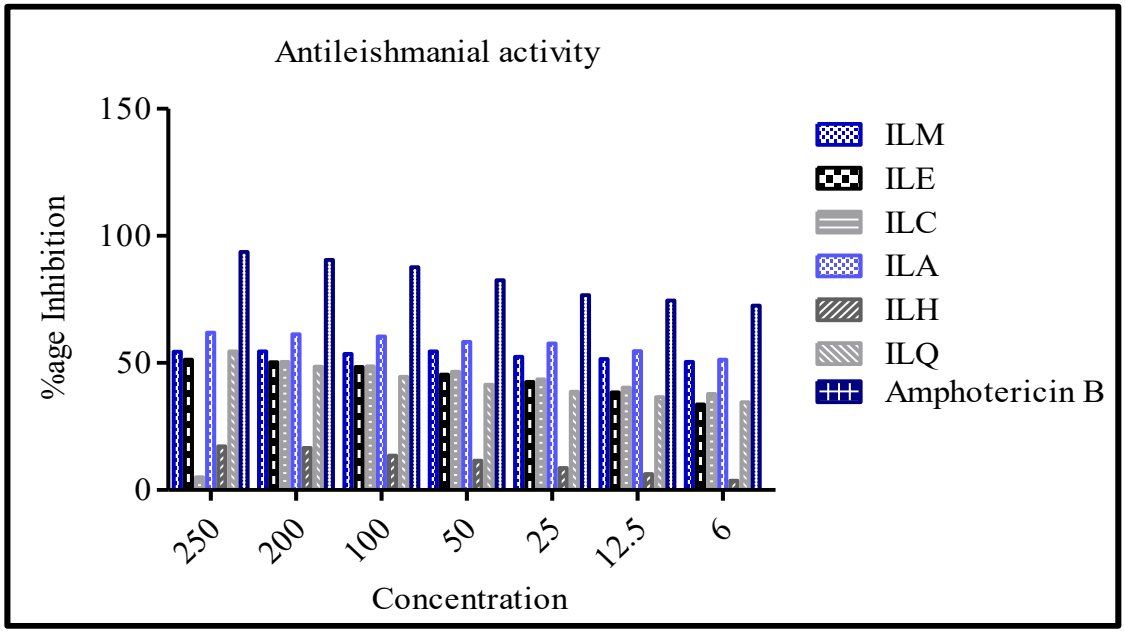

Figure 28: Anti-leishmanial assay of different extracts of *I. lactea*. ILM: *I. lactea* methanol, ILE: *I. lactea* ethanol, ILC: *I. lactea* chloroform, ILA: *I. lactea* ethyl acetate, ILH: *I. lactea* n-hexane, ILQ: *I. lactea* aqueous extract.

3.2.1 Cytotoxicity Assessment and Anticancer Potential of *I. lactea* Extracts

Cytotoxic potential of *I. lactea* extracts against larvae of *A. salina* were shown in Table 12. Extracts were initially screened for assessing cytotoxic potential by BSCT, 33 % extracts displayed LD_{50} value under 60 µg/mL and were identified to be extremely cytotoxic whereas 50 % considered as low cytotoxic while 17 % had shown no cytotoxicity. Doxorubicin was a positive control having LD_{50} 7.36±1.43 µg/mL. Among all tested extracts, ILH was found to have the best cytotoxic potential with LD_{50} 45.54±1.23 µg/mL demonstrating that in case of *I. lactea* non-polar solvents are highly effective in the extraction of cytotoxic compounds as compared to polar solvents.

PKI is a well-known class of serine/threonine kinases that are used in the treatment of cancer (Khan et al., 2018). Till to date, no report about PKI activity of *I. lactea* is present and in the current study it was observed that IL has the ability for inhibition of protein kinases (Table 12). Direct relationship was revealed among different concentrations of tested plant extracts and PK inhibition activity. The significant bald area zone with ZOI 13±2.5 mm and MIC 100 µg/mL was measured around ILE loaded disc that was followed by ILC, ILH and ILQ. However, ILM and ILA have shown no PKI activity. Our findings are in aggrement with Mykhailenko (2018) who reported anticancerous activity of species belonging to genus.

Recently many researchers have paid attention on finding out new sources of herbal drugs used to treat cancer (Sarkar et al., 2019). In the present study, the cytotoxic potency of *I. lactea* extracts against prostrate cell line (PC3) showed that ILH extract has confirmed reduction in metabolic activity of PC3 cells (Figure 29). Reduced metabolic activity of ILH extract might be linked with its strong anticancer potential. The cytotoxicity induced by ILH extract at lower doses could be due to the presence of plant cytotoxic active functional groups. These results verified the findings of Alam et al. (2017), which is a report indicating anticancer potential of a specie of *Iris* (*I. kashmiriana*).

Table 12: Brine Shrimp Lethality and Protein Kinase Inhibition Assay.

Samples	Brine shrimp lethality (µg/mL)		Protein kinase inhibition (µg/mL)		
	% Mortality	LD_{50} (µg/mL)	Diameter (mm) at 100 µg/disc		MIC
	250		Clear zone	Bald zone	
ILM	56.3±2.34	88.4±2.53	----	----	
ILE	53.2±1.32	97.3±1.18	----	13±2.5mm	100
ILC	45.6±1.56	209.43±2.28	----	12±2.1mm	100
ILA	69.8±2.26	56.65±2.43	----	----	
ILH	79.8±1.67	44.54±1.23	----	12±1.8mm	100
ILQ	49.8±2.23	102.15±1.13		11±1.5 mm	100

Values (mean ± standard deviation) were obtained through from triplicate analysis. ----: No activity ILM: *I. lactea* methanol, ILE: *I. lactea* ethanol, ILC: *I. lactea* chloroform, ILA: *I. lactea* ethyl acetate, ILH: *I. lactea* n-hexane, ILQ: *I. lactea* aqueous extract.

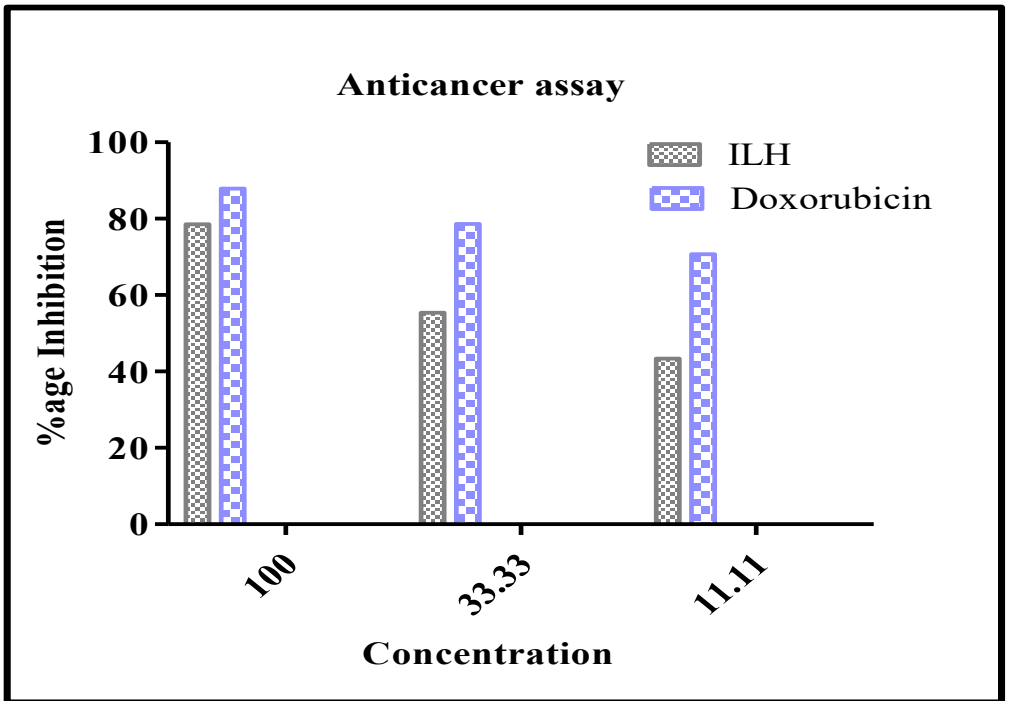

Figure 29: Anticancer Activity of *I. lactea* Extract against PC-3 Cells

3.2.1 Antidiabetic Potential of *I. lactea* Extracts

Plants contain several kind of compounds that have capacity to block the activity of alpha-amylase enzyme (Bonesi et al., 2019; Rani et al., 2020). Various extracts of *I. lactea* were evaluated for AAI assay and data is given in Table 13. The finding of AAI assay showed that ILM has highest AAI activity with the value of 74.27±2.5 % that was followed by ILE, ILA, ILH, ILC and ILQ 73.14±1.95 %, 73.15±2.54 %, 73.12±2.66 % 72.17±1.65 % and 71.74±3.54 % respectively. The antidiabetic potential was compared with standard drug acarbose which revealed inhibition of 89.9±3.1 %. Present results are in line with previous literature based on antidiabetic potential of different species of *Iris* such as *I. loczyi* (Mosihuzzman et al., 2013).

Table 13: Antidiabetic Potential of *I. lactea* Extracts and Respective IC_{50} values.

Plant Extracts	%age inhibition of alpha-amylase	IC_{50} µg/mL
ILM	75.27 ±2.5	52.34
ILE	74.14 ±1.95	55.43
ILC	73.17 ±1.65	58.34
ILA	74.15 ±2.54	55.75
ILH	74.12 ±2.66	55.39
ILQ	72.74 ±3.54	68.76

Values (mean ± standard deviation) was obtained through from triplicate analysis. ILM: *I. lactea* methanol, ILE: *I. lactea* ethanol, ILC: *I. lactea* chloroform, ILA: *I. lactea* ethyl acetate, ILH: *I. lactea* n-hexane, ILQ: *I. lactea* aqueous extract.

SECTION 3 : *Lactuca orientalis* (Boiss.) Boiss.

3.3 *Lactuca orientalis* (Boiss.) Boiss

Lactuca orientalis (Boiss.) Boiss. (Local name split-leaf lettuce) belongs to family Asteraceae (Compositae) and grows as dwarf-shrub in rock mountains. The plant has branched stems, white colored and intricate and the colour of its flowers is yellow. Its flowering time is frequently between July and August and seedling at the end of August and September. This wild edible plant has yellowish to brownish small almond-shape seeds. The whole plant of *L. orientalis* have been used in traditional medicinal system for the treatment of different infectious aliments and Alzheimer disease (Aati et al., 2019). The plant is widely distributed in several regions of the world including the rocky Mediterranean, Irano-Turanian, Northern African, Western Asian, Caucasian, Middle Asian, Indian sub-continental regions and Pakistan (https://www.gbif.org/occurrence/search?offset=80&taxon_key=3140453).

Figure 30: Field Photograph of *Lactuca orientalis* (Boiss.) Boiss.

3.3.1 Seed Scanning of *Lactuca orientalis*

Seeds micro-morphological characters along with seed size and seed shape, were the diagnostic outfits for the proper identification of different plant species (Luqman et al., 2019). Seed colour was yellowish to brown, with length and width of 3.84 mm and 0.93 mm. When visualized by Scanning Electron Microscope the seeds of *L. orientalis* were seen to be oblong to obovate with Smooth-rough, variously ridges or wrinkled with projections, granules, margins were wavy slightly straight or deeply dentate, anticlinal wall was raised, grooved with apex acuminate while the outer-periclinal wall convex with fine texture as shown in Figure 31.

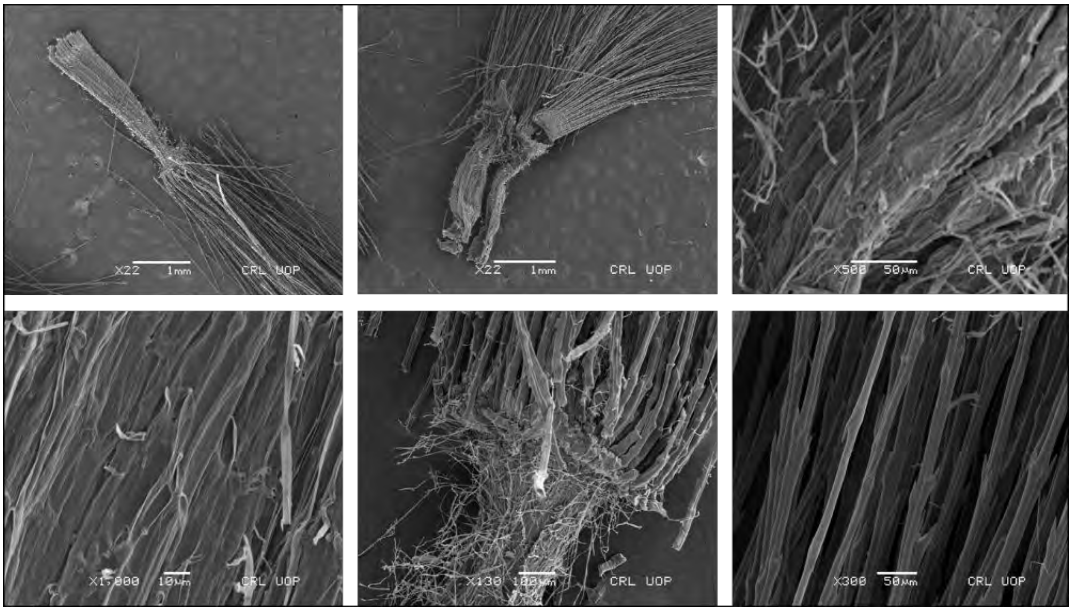

Figure 31: Scanning Electron Microscope of *L. orientalis* Seeds.

3.3.2 Preliminary Phytochemical Screening of *L. orientalis* Seeds

The result of the phytochemical screening of the crude seed extracts of *L. orientalis* revealed the presence or absence of phenolic compounds, saponins, steroids, alkaloid and tannins. Early detection demonstrated variation in the presence of these compounds (+++ strongly present; ++ moderately present; + weakly present) based on solvent used for the extraction. Table 14 revealed that methanol and ethanol extracts showed strongly presence of flavonoids, glycosides, phenols, steroids and saponins as compared to the terpenoids and alkaloids. While tannins and amino acids were observed in all extracts of *L. orientalis*. Phenolics, tannins, terpenoids, steroids and saponins present in this plant explained wide range uses of its species in traditional medicines as these phytochemicals are famous for their medicinal and pharmacological potential (Toma et al., 2019). Our findings were in good harmony with the findings of Qin et al (2018) provide detail phytochemical analysis of *L. sativa* and confirmed the existence of phenol, flavonoids, steroids and glycosides in six different extracts of *L. sativa.*

Table 14: Preliminary Phytochemical Analysis of *L. orientalis* Seeds Extracts.

Plant Extracts	Alkaloids	Flavonoids	Phenols	Terpenoids	Steroids	Glycosides	Saponins	Tannins	Amino acids
LOSM	+	+++	+++	+	+++	+++	+++	+++	+
LOSE	++	+++	++	++	-	+	+	+	+
LOSC	++	+++	+++	+++	-	-	++	++	+
LOSEA	++	++	++	-	+	+	++	+	++
LOSH	-	+	+	++	-	-	+	+	+
LOSAq	-	++	++	+	++	++	-	+	+

+++ Strongly present; ++ Moderately present; + Weakly present; - :Absent. LOSM: *L. orientalis* seed methanol, LOSE: *L. orientalis* seed ethanol, LOSC: *L. orientalis* seed chloroform, LOSEA: *L. orientalis* seed ethyl acetate, LOSH: *L. orientalis* seed n-hexane, LOSAq: *L. orientalis* seed aqueous extracts.

3.3.3 Quantitative Phytochemical Analysis of *L. orientalis* Seeds

Pharmacological potential of any plant depends on composition of secondary metabolites and phenolic compounds which were actively involved in maintaining enzymes responsible for detoxification (Baidez et al., 2007). The total phenolic contents of the extract was determined from Gallic acid calibration curve. Methanol seeds extract of *L. orientalis* showed highest TPC value of 95.76±3.71 GAE/g. TPC values showed decreasing trend as polarity of the solvent is decreasing and maximum results are shown in methanol, ethanol and water respectively (Figure 32). Similar results were show by (Stojakowska et al., 2018)) the *L. orientalis* extracts shows the good amount of phenolic contents. These result indicated notable quantity of phenolic compounds in polar plant extract. Different research studies concluded that TPC values are influenced by the use of different solvents accordingly (Uddin et al., 2018).

Flavonoids are polyphenolic molecules known for their antioxidant and anti-inflammatory health benefits. The flavonoids provide protection to the living systems by stabilizing the free radicals (Karmakar et al., 2019). The content of flavonoid was estimated from the QT standard curve and results were stated as QE/g (Figure 33). The methanol seeds extract of *L. orientalis* showed maximum amount of flavonoid contents followed by ethanol and aqueous extract. Similar to our findings other members of genus lactuca also possess good amount of flavonoids (Bohm and Stuessy, 2001). On the basis of solvent polarity, the range of total flavonoid contents in various plant extracts varies (Ahmad et al. 2016; Zohra et al., 2019).

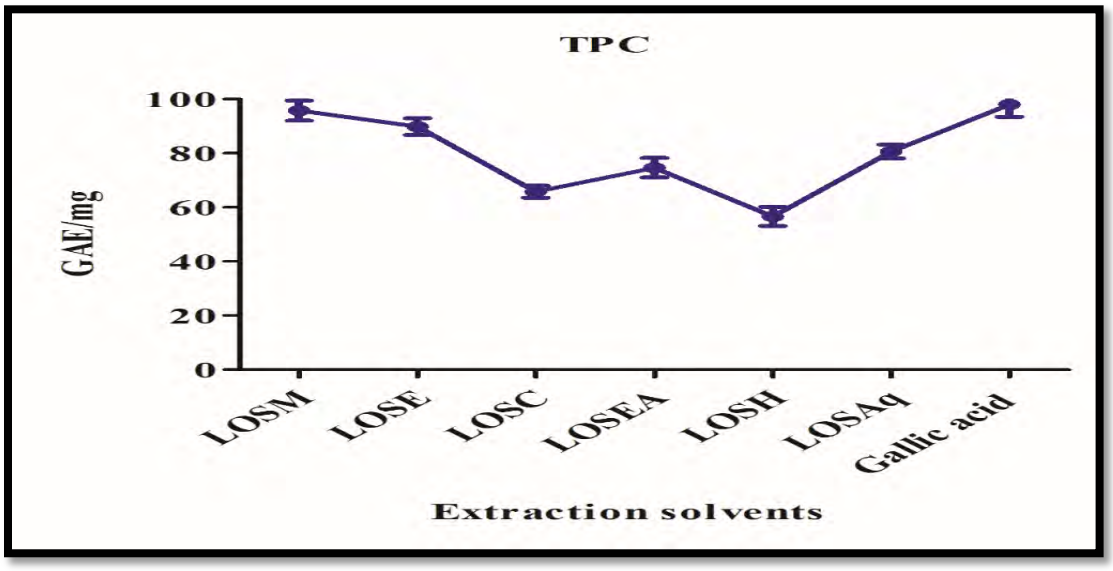

Figure 32: Total phenolic contents of *L. orientalis* seeds different extracts.Values (mean ± SD) was obtained through triplicate analysis. LOSM: *L. orientalis* seed methanol; LOSE: *L. orientalis* seed ethanol; LOSC: *L. orientalis* seed chloroform; LOSEA: *L. orientalis* seed ethyl acetate, LOSH: *L. orientalis* seed n-hexane, LOSAq: *L. orientalis* seed aqueous extracts.

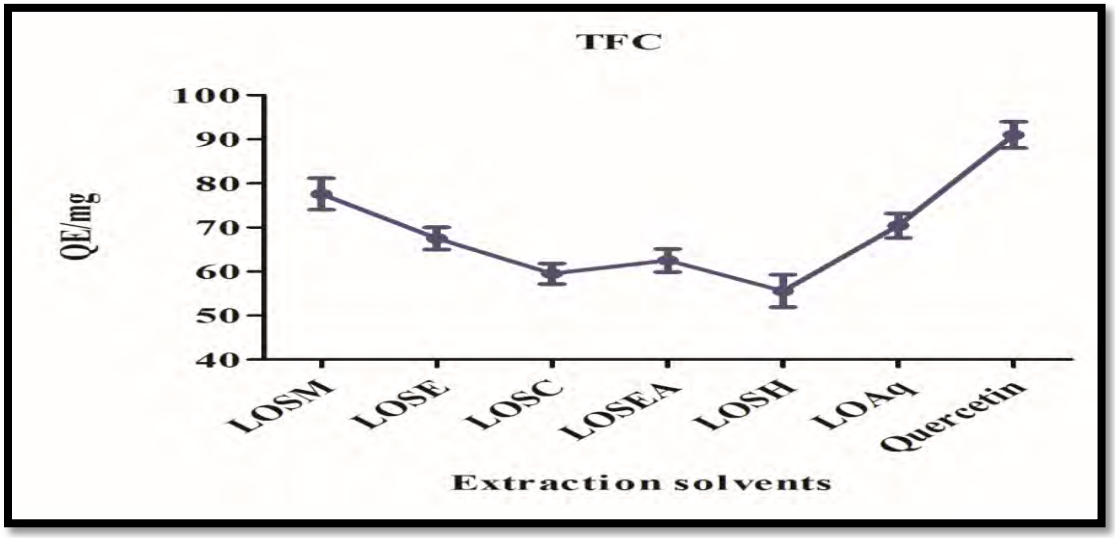

Figure 33: Total flavonoid contents of *L. orientalis* seeds different extracts.Values (mean ± SD) was obtained through triplicate analysis. LOSM: *L. orientalis* seed methanol; LOSE: *L. orientalis* seed ethanol; LOSC: *L. orientalis* seed chloroform; LOSEA: *L. orientalis* seed ethyl acetate, LOSH: *L. orientalis* seed n-hexane, LOSAq: *L. orientalis* seed aqueous extracts.

3.3.4 FTIR Spectral Analysis (cm^{-1}) of *L. orientalis* Seeds

FTIR analysis exposed the occurrence of numerous functional groups with strong and medium peak intensities. At first sight, C-H stretch was observed in the sample indicating the presence of alkanes. One broad peak is explicitly visible showing the presence of alcohols and phenols in *L. orientalis* Seeds. Some strong and medium peaks were also observed which indicated the existence of C=O stretch, C-H bend, C-H wag, C-N, C-Br and C-Cl stretch and hence confirmed the presence of alkanes, carboxylic acids, aliphatic amines and alkyl halides. FTIR spectra of *L. orientalis* are shown in Figure 34. The availability of these different functional groups showed that these biomolecules play significant role in the biological potentials of LO-mediated extracts.

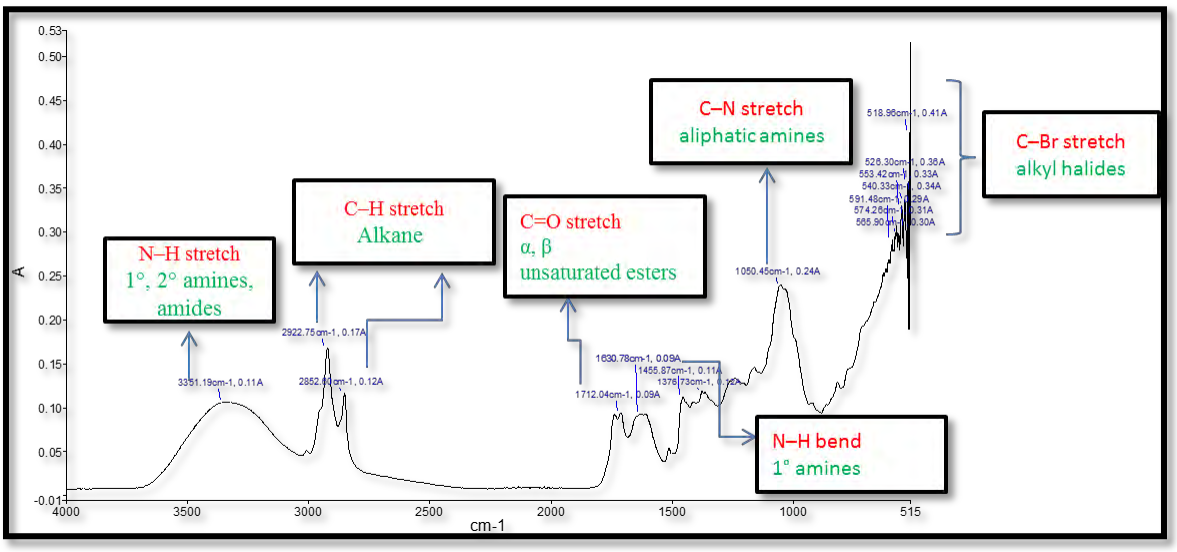

Figure 34: FTIR Spectral Analysis (cm-1) of *L. orientalis* Seeds.

3.3.5 Antioxidant Potential of *L. orientalis* Seeds

DPPH revealed the free radical scavenging potential of the *L. orientalis* from its crude extracts i.e. 82 % at 250 mg/mL. Scavenging potential has direct relation to the crude extracts concentration as it decreased up to 60 % at 6 mg/mL. GraphPad Prism software was used to determine %age DPPH scavenging activity graph using linear regression data. The resulting graphs and Equations are in Figure 35 for %age DPPH scavenging activity. Reactive oxygen species cause oxidative damage which can be alleviated by phenolic compounds which ultimately reduces the risk of serious health

problems. These findings are in agreement with the previous studies that plant extracts having highest value of phenolic compounds were good antioxidants (Ali et al., 2016; Mehwish et al 2019).

Medicinal Plants being rich source of antioxidants can decrease the oxidative stress, thus beneficial in the cure of numerous human ailments such as inflammation, cancer, and heart ailments (Krishnaiah et al., 2011). Figure 36 shows the measurement of TAC of *L. orientalis* extracts and stated it as ascorbic acid equivalent (E µg/mg dry weight of extract). LOSM and LOSE extracts have shown antioxidant potential of 90.60±1.55 and 84.41±1.43 µg E/mg (Figure 5) respectively whereas LOSH and LOSC show low TAC value as compare to polar and aqueous extract. Different research studies concluded that TAC values are influenced by the use of different solvents (Kumar and Jain, 2015; Mehwish et al., 2019).

The plant extracts LOSM and LOSE showed the highest TRP values 94.44±1.38 µg E/mg and 83.43±2.13 µg E/mg respectively. Parallel correlation was observed between TAC and TRP (Figure 36).These findings are in agreement with previous reports which unveiled that positive correlation exists between reducing power of plant extracts and total antioxidant activity. This might occur as antioxidants are electron or proton donors thus reducing ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) through electron donation (Jafri et al., 2014). In addition, our results unravelled that variation in reducing power of plant extracts might be due to difference in solvent used for the extraction as reported earlier (Mehwish et al., 2019).

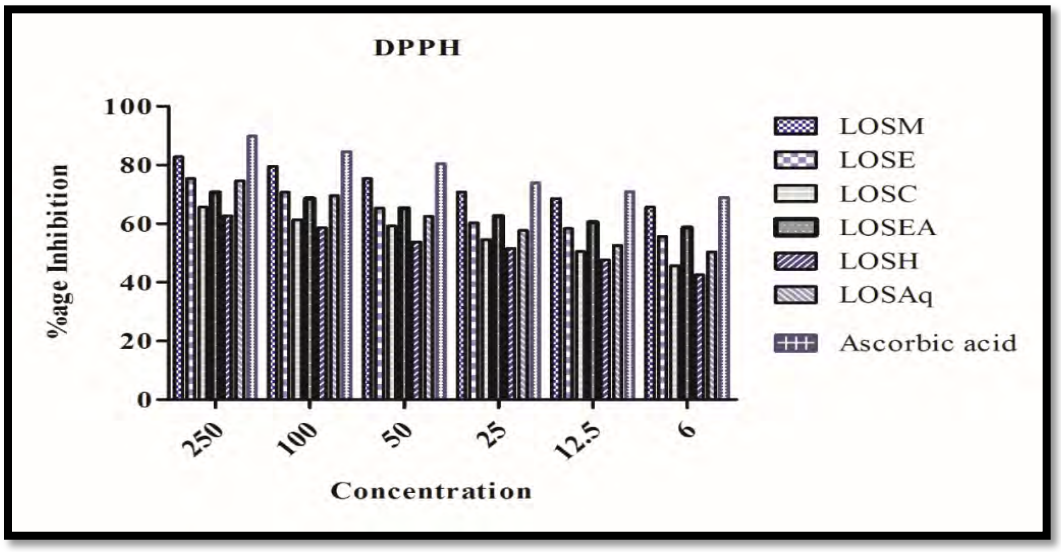

Figure 35: DPPH assay of different extracts of *L. orientalis* seeds different extracts.Values (mean ± SD) was obtained through triplicate analysis. LOSM: *L. orientalis* seed methanol; LOSE: *L. orientalis* seed ethanol; LOSC: *L. orientalis* seed chloroform; LOSEA: *L. orientalis* seed ethyl acetate; LOSH: *L. orientalis* seed n-hexane; LOSAq: *L. orientalis* seed aqueous extracts.

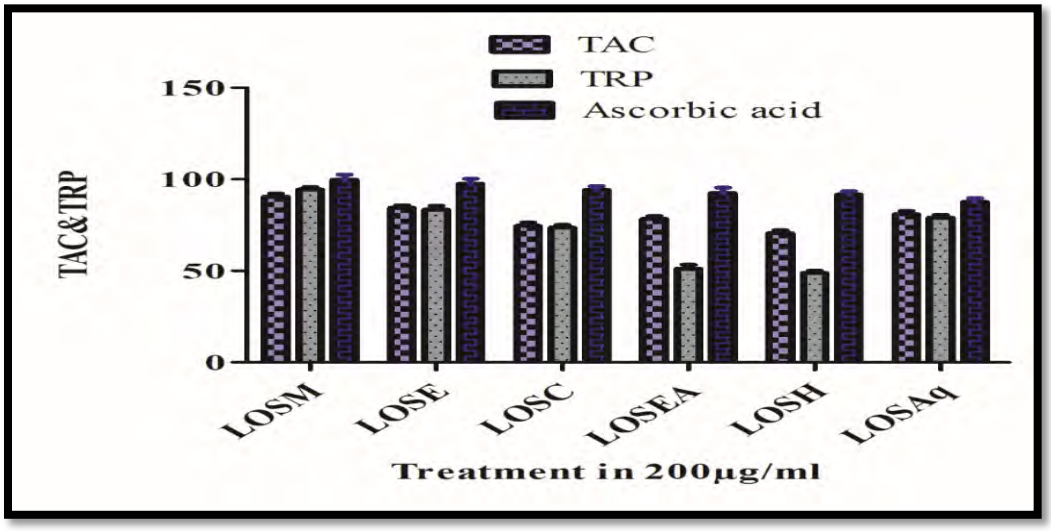

Figure 36: Total antioxidant capacity and total reducing power of different extracts of *L. orientalis* seeds different extracts.Values (mean ± SD) was obtained through triplicate analysis. LOSM: *L. orientalis* seed methanol; LOSE: *L. orientalis* seed ethanol; LOSC: *L. orientalis* seed chloroform; LOSEA: *L. orientalis* seed ethyl acetate; LOSH: *L. orientalis* seed n-hexane; LOSAq: *L. orientalis* seed aqueous extracts.

3.3.6 Antimicrobial Activity of *L. orientalis* Seeds

The antibacterial potential of *L. orientalis* seed extracts were evaluated against gram positive strains (*B. subtilis, S. aureus*) and gram negative strains (*E. coli* , *K. pneumonia* and *P. aeruginosa*. There was significant variation in the antibacterial potential measured by zone of inhibition at 250 µg/mL concentration of different plant extracts. Plant extracts which inhibit bacterial strains and have more than 11 mm zone of inhibition have been further analysed. MIC values have been checked across different concentration (100–11.11 µg/mL). Various MIC values were calculated for bacterial strains *B. subtilis* (33.33 µg/mL), *S. aureus* (11.11 µg/mL) and gram negative strains *E. coli* (33.33 µg/mL), *K. pneumonia* (11.11 µg/mL) and *P. aeruginosa* (100 µg/mL) as shown in Table 15. *K. pneumonia* and *S. aureus* were the most susceptible bacterial strains (MIC=11.11 µg/mL) while *Pseudomonas aeruginosa* having MIC= 100 µg/mL was found to be the least susceptible strain. The present study showed dose dependent antibacterial response.

In current study, different seed extracts of *L. orientalis* were analyzed for antifungal activity using different fungal strains. *L. orientalis* crude extracts showed moderate effects against fungal strains except Hexane extract (LOSH). The LOSH extract showed less susceptibility for the tested fungal strains. On the other hand, none of tested samples of *L. orientalis* have shown % inhibition greater than Clotrimazole (positive control). Table 16 showed MIC values for different fungal strains. Direct relationship was observed between tested plant extract and their concentration. The fungicidal potential was increasing with increase in plant extract concentration. In current study, significant relationship between antifungal activities and solvent used for the extraction has been reported. This fungicidal potential may be varied due to solvent use as reported by Tariq et al. (2019).

Table 15: Inhibition Zones and MIC of *L. orientalis* Extracts against Bacterial Strains.

Micro-organisms	*E. coli* ATCC 33456		*P. aeruginosa* ATCC 90271		*K. pneumonia* ATCC 1705		*B. subtilus ATCC* 19659		*S. aureus* ATCC 6538	
Plant Extracts	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/ mL)	ZOI (mm)	MIC (µg/mL)
LOSM	10± 1.13	100	NI		NI	100	10± 1.35		NI	
LOSE	11± 1.43	100	NI		19± 1.97	11.11	12± 1.46	100	NI	
LOSC	NI		10± 1.97	100	NI		NI		10± 2.09	100
LOSEA	11±2.12	100	NI		18± 2.15	11.11	19±2.43	11.11	NI	
LOSH	NI		NI		14± 1.12	33.33	15±2.43	33.33	NI	
LOSAq	10±2.43	100	NI		NI		NI		17± 1.24	33.33

--- : No activity . LOSM: *L. orientalis* seed methanol, LOSE: *L. orientalis* seed ethanol, LOSC: *L. orientalis* seed chloroform, LOSEA: *L. orientalis* seed ethyl acetate, LOSH: *L. orientalis* seed n-hexane, LOSAq: *L. orientalis* seed aqueous extracts.

Table 16: Inhibition zones and MIC of *L. orientalis* Seeds Extracts against Fungal Strains.

Fungus strains	***M. racemosus*** FCBP 0300		***C. albicans*** FCBP 478		***A. niger*** FCBP 0918		***F. solani*** FCBP 0291		***A. flavus*** FCBP 0064	
Plant Extracts	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)
LOSM	NI		NI		13±1.7	100	NI		19±1.8	33.33
LOSE	13±2.4	100	NI		12±2.6	100	NI		8±2.6	
LOSC	NI		NI		NI		NI		18±2.6	33.33
LOSEA	16±1.54	33.33	10± 2.63	100	NI		14±2.5	100	13±1.6	100
LOSH	NI		7±2.8		NI		11±1.6	100	NI	
LOSAq	NI		6±2.8		17±2.8	33.33	NI		NI	

---: No activity. LOSM: *L. orientalis* seed methanol, LOSE: *L. orientalis* seed ethanol, LOSC: *L. orientalis* seed chloroform, LOSEA: *L. orientalis* seed ethyl acetate, LOSH: *L. orientalis* seed n-hexane, LOSAq: *L. orientalis* seed aqueous extracts.

In present study, anti-leishmanial potentials of various *L. orientalis* extracts were studied and percent (%) mortality of the *L. tropica* strain is shown in Figure 37. The present results revealed that at 250 µg/mL concentration, all extracts showed substantial anti-leishmanial activity except LOSH extract (51.89± 2.90 %). Our results showed dose-dependent response and %age mortality reduced with decrease in concentration of different plant extract. These results verified the findings of previous studies on comparative use of polar solvents for better isolation of antimicrobial compounds (Khademvatan et al., 2019).

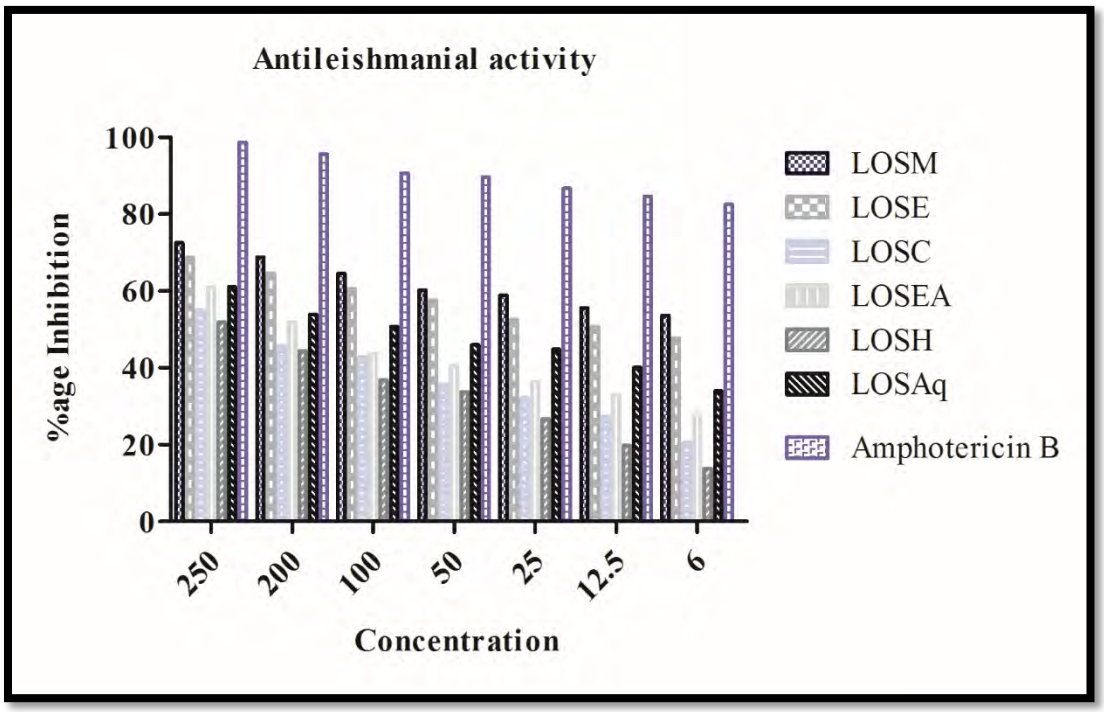

Figure 37: Anti-leishmanial assay of different extracts of of *L. orientalis* seeds different extracts.Values (mean ± SD) was obtained through triplicate analysis. LOSM: *L. orientalis* seed methanol; LOSE: *L. orientalis* seed ethanol; LOSC: *L. orientalis* seed chloroform; LOSEA: *L. orientalis* seed ethyl acetate; LOSH: *L. orientalis* seed n-hexane; LOSAq: *L. orientalis* seed aqueous extracts.

3.3.7 Cytotoxicity Assessment and Anticancer potential of *L. orientalis*

The BSCT is a suitable assay to determine the cytotoxic potential of medicinal plants extracts and associated compounds (Husin et al., 2019). The percentage (%) cytotoxicity of different extracts. Among all *L. orientalis* extracts LOSH and LOSEA showed high % mortality of *Artemia salina* 94 % and 84 % having LD_{50}=13.03 and 23.01 (μg/mL) respectively (Table 17). The results showed concentration dependent response and increase in extract concentration resulted in increase in mortality rate of brine shrimps while mortality decreases with decrease in concentration of plant extracts (Wakawa et al., 2017).

Different solvent based extracts of *L. orientalis* were investigated against (*Streptomyces* 85E) through protein kinase inhibition assay. The tested samples showed effective PK inhibition potential as shown in Table 17. LOSM and LOSC extract with 20±2.4 mm and 18±2.1 mm showed significant activity while LOSAq give no results. Protein kinase is significantly involved in the phosphorylation of important amino acids (serine, tyrosine and threonine residues). This phosphorylation phenomenon is also involved in the regulation of different cellular processes (proliferation, apoptosis and metabolism differentiation). Therefore, any substance with ability to PKI activity have significant importance in cancer research. The present results revealed the presence of potent phytochemicals in different extracts such as aqueous, methanol and ethanol which is reported to be involved in protein kinases inhibition (Naz et al., 2019; Tabassum et al., 2019).

The cytotoxic potency of the *L. orientalis* seed extract against prostrate cell line (PC3) was demonstrated using MTT assay. The result of LOSEA extract has confirmed reduction in metabolic activity of PC3 cells. The % inhibition was achieved at three different concentrations (100, 33.33 and 11.11) µg/mL as shown in Figure 38. The reduced metabolic activity has shown that LOSEA extract might have strong anticancer potential. The cytotoxicity induced by LOSEA extract at lower doses could be due to the plant cytotoxic active functional groups and these results confirmed the *L. sativa* anticancer studies (Qin et al., 2018)

Table 17: Brine Shrimp lethality and Protein kinase Inhibition Assay.

Samples	Brine shrimp lethality (µg/ml)		Protein kinase inhibition (µg/ml)		
	% Mortality 250	LD_{50} (µg/ml)	Diameter (mm) at 100 µg/disc Clear zone	Bald zone	MIC
LOSM	67.3±1.43	79.4±2.37	----	20±2.4mm	11.11
LOSE	54.2±2.34	88.3±1.28	----	13±1.9mm	100
LOSC	40.6±2.75	123.4±2.28	----	18±2.1mm	100
LOSEA	84±1.26	23.01 ±1.36	----	15±1.7mm	33.33
LOSH	94±2.47	13.03 ±1.14	----	11±2.5mm	100
LOSAq	80.8±2.87	27.15±2.23	---	---	----

Values (mean ± standard deviation) was obtained through from triplicate analysis. ---: No activity. LOSM: *L. orientalis* seed methanol, LOSE: *L. orientalis* seed ethanol, LOSC: *L. orientalis* seed chloroform, LOSEA: *L. orientalis* seed ethyl acetate, LOSH: *L. orientalis* seed n-hexane, LOSAq: *L. orientalis* seed aqueous extracts

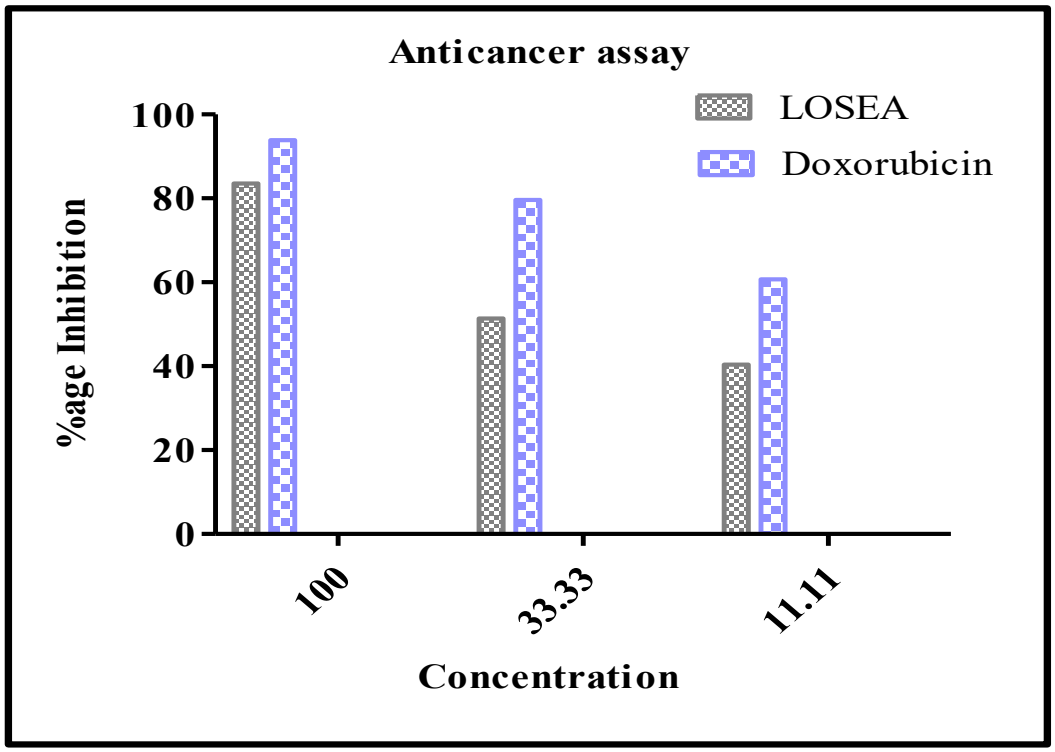

Figure 38: Anticancer Activity of *L. orientalis* Seed Extract against PC-3.

3.3.8 Antidiabetic Potential of *L. orientalis* Seeds Extracts.

The Alpha-amylase enzyme (AAE) play an important role in the conversion of carbohydrates into glucose, henceforth the inhibition of AA blocked the conversion of carbohydrates into glucose consequently it signifies a key area in diabetes research (Dineshkumar et al., 2010). In current study, we reported the AAE inhibition potential of the *L. orientalis* crude extracts. Significant alpha-amylase Inhibition activity was determined. The antidiabetic assay showed highest alpha-amylase inhibition potential (78.20±1.58 % and 77.30±1.97 %) at 250 mg/mL in extracts LOSM and LOSE respectively (Table 18). Though, the AAE inhibition potential considerably reduced with a decrease in concentration. Our results are in agreement with the findings of Taslimi et al (2020) where polar extracts (methanolic extract) showed potential antidiabetic activities. These extracts could be used as an alternative treatment option for the development of novel drugs in pharmaceutical industries.

Table 18: Antidiabetic Potential of *L. orientalis* Seeds Extracts.

Plant Extracts	%age inhibition of alpha-amylase	IC_{50}
LOSM	78.20 ±1.58	25.54
LOSE	77.30 ±1.97	27.45
LOSEA	74.30 ±1.65	28.36
LOSC	61.37 ±2.54	38.87
LOSH	59.57 ±2.59	40.65
LOSAq	74.76 ±2.93	28.87

Values (mean ± standard deviation) was obtained through from triplicate analysis. LOSM: *L. orientalis* seed methanol, LOSE: *L. orientalis* seed ethanol, LOSC: *L. orientalis* seed chloroform, LOSEA: *L. orientalis* seed ethyl acetate, LOSH: *L. orientalis* seed n-hexane, LOSAq: *L. orientalis* seed aqueous extracts.

SECTION 4: *Polygonum affine* D. Don.

3.4 *Polygonum affine* D. Don.

Polygonum affine D.Don. belongs to family Polygonaceae familiarly known as knotweed or smartweed family (Figure 39). *P. affine* is found in Pakistan, India, Canada, United States of America, France, Estonia and Spain (https :// www .gbif .org /occurrence /search?offset=20&q=Polygonum%20affine). *Polygonum donianum* Spreng is well-known synonym of this plant (http://www.thplantlist.org/tpl1.1/record/tro-50133713). The word *Polygonum* comes from the Greek poly means many and gonu, knee or joint, due to the appearance of swollen jointed stem. Total number of *Polygonum* species across the world are estimated to be 300 and mostly found in North temperate climate (Manasa et al., 2016).

Figure 39: Field Photograph of *Polygonum affine* D. Don.

The *polygonum* species are diverse in means of habit, ranging from prostrate annual herbaceous plants to erect perennial herbaceous plants, rarely shrubby that mostly branched stems. The arrangements of leaves are alternative, less than 2 cm in length, ovate or elliptic, linear-lanceolate and sessile-petiolate. They have usually six stamens and three (occasionally two) styles. In Pakistan round about 20 species of *Polygonum* were recoded (Yasmin et al., 2009). Plants belonging to this genera contain a wide range

of bioactive secondary metabolites that have therapeutic potential including triterpenoids, tannins, coumarins, lignans, flavonoids, phenylpropanoides and anthraquinones (Granica et al., 2013). *P. affine* is the prostrate annual herb which has small elliptic leaves and most widely distributed in all over the world in the temperate regions (Salama and Marraiki, 2010; Granica, 2015). In the present research, *P. affine* was collected from Nagar, Gilgit-Baltistan, and Northern Areas of Pakistan.

In traditional medicinal system, medicinal plants plays an important role in primary health care, can be used to treat many ailments. Infusions prepared from *P. affine* have been utilized in the treatment of respiratory disorders, skin infections and inflammation. Whole herb has been used for medication of hypertension, stomachache, dysentery, jaundice, enuresis, colic pain, headache, arthritis and in the treatment of nephrolithiasis (Granica et al., 2013; Kwon et al., 2015; Park et al., 2018; Saremi et al., 2018; Shevchenko et al., 2019). Roots have been consumed as fooder and act as best remedy for diarrhea, diuretic, malarial fever, pulmonary infections as well as used for urinary bladder stones. Leaves and stems are used against diarrhea, hemorrhoids, chancroids, obesity and hypertension (Akbar, 2020). The present research is based on the pharmacological evaluation of *P. affine*. Therapeutic potential of this medicinally important specie was found by using different bio-assays.

3.4.1 Preliminary Phytochemical Analysis of *P. affine* Extracts

Phytochemicals obtained from plants and their identification as well as its standardization is needed for drug synthesis (Chaudhary et al., 2020; Mulat et al., 2020; Sethi, 2020). The initial phytochemical screening of herbal plants is necessary in order to find novel therapeutic agents (Patel and Mishra, 2020). *P. affine* extracts showed the presence of alkaloids, flavonoids, phenols, saponins, tannins and amino acids in all PAf extracts. Alkaloids, flavonoids and phenols were present in all PAf extracts however these were more concentrated in PAfM, PAfE and PAfC extracts. Glycosides were present in PAfE, PAfA and PAfQ and missing in PAfM, PAfC and PAfH. Carbohydrates were more concentrated in PAM while absent in PAfA (Table 19). Our study showed that PAf extracts revealed the existence of phytochemicals alkaloids, flavonoids, amino acids, phenols, glycosides tannins, steroids and saponins which is in harmony with the findings of Salama and Marraiki (2010) in which authors studied phytochemical potential of aerial parts of *P. affine.*

Table 19: Preliminary Phytochemical Analysis of *P. affine* Extracts.

Plant Extracts	Alkaloids	Flavonoids	Phenols	Carbohydrates	Steroids	Glycosides	Saponins	Tannins	Amino acids
PAfM	+++	+++	+++	+++	+	-	-	+	+
PAfE	++	++	++	++	-	+	+	+	-
PAfC	++	++	++	+	+	-	++	++	+
PAfA	+	+	+	-	-	+	-	+	++
PAfH	+	+	+	++	-	-	+	-	+
PAfQ	+	+	+	+	++	++	+	+	+

+++ Strongly present; ++ Moderately present; + Weakly present; -: Absent. PAfM: *P. affine* methanol, PAfE: *P. affine* ethanol, PAfC: *P. affine* chloroform, PAfA: *P. affine* ethyl acetate, PAfH: *P. affine* n-hexane, PAfQ: *P. affine* aqueous extract

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3.4.2 Quantitative Phytochemical Analysis of *P. affine* Extracts

In human health benefits the phenolic compounds have an essential role because of their antioxidant potential (Kubalt, 2016; Van Hung, 2016). The data of phenolic contents of PAf extracts were showed the trend PAfM>PAfE>PAfC>PAfA>PAfQ>PAfH as summarized in Figure 40. PAfM had highest phenolic contents (93.81 mg GAE/g), followed by PAfE (90.88 mg GAE/g), PAfC (83.51 mg GAE/g), PAfA (71.65 mg GAE/g), PAfQ (63.87 mg GAE/g) and PAfH (60.66 mg GAE/g). Similar findings were reported by Jiao et al. (2018) for phenolic contents of *P. affine* extracts.

Flavonoids are the plant secondary metabolites with diverse subgroup of compounds including flavonols, anthocyanidins, chalcones, flavanones, pterocarpanes and isoflavonois (Lin et al., 2016; Tungmunnithum et al., 2018). Total flavonoid contents of *P. affine* extracts were summarized in Figure 41. PAfM has highest flavonoid contents (81.71±1.98 mg QE/g), followed by PAfE (54.64 mg QE/g), PAfC (47.06 mg QE/g), PAfA (46.36 mg QE/g), PAfQ (43.76 mg QE/g) and PAfH extract (16.25 mg QE/g). Present findings are in agreement with the previous studies of Ridzuan et al. (2019) in which researchers indicated that *P.minus* extracts have high flavonoid contents.

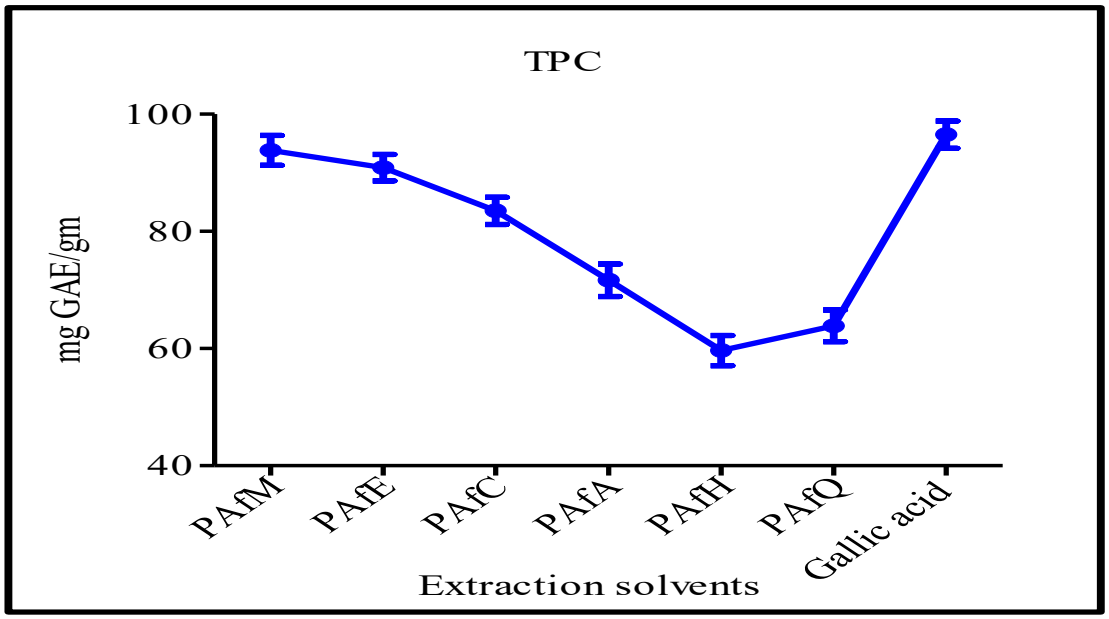

Figure 40: Total phenolic contents of *P. affine*. PAfM: *P. affine* methanol, PAfE: *P. affine* ethanol, PAfC: *P. affine* chloroform, PAfA: *P. affine* ethyl acetate, PAfH: *P. affine* n-hexane, PAfQ: *P. affine* aqueous extract.

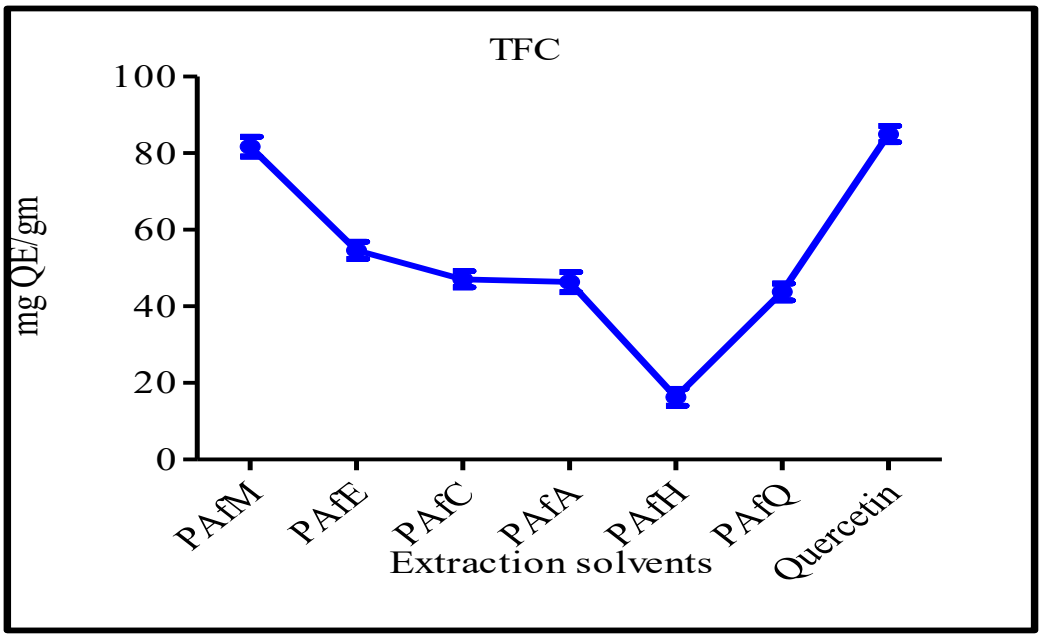

Figure 41: Total flavonoid contents of *P. affine*. PAfM: *P. affine* methanol, PAfE: *P. affine* ethanol, PAfC: *P. affine* chloroform, PAfA: *P. affine* ethyl acetate, PAfH: *P. affine* n-hexane, PAfQ: *P. affine* aqueous extract.

3.4.3 FTIR Spectral Analysis (cm^{-1}) of *P. affine*

The data of FTIR spectra has shown the presence of several functional groups with strong and medium peak intensities. C-H stretch was observed in the sample indicating the presence of alkanes. Some strong and medium peaks were observed which indicated the existence of N-H stretch, C=O stretch, C-N, C-Br, N-H bend and hence confirmed the presence of aromatics, carboxylic acids, aliphatic amines and alkyl halides. FTIR spectra of *P. affine* is shown in Figure 42. The availability of these different functional groups showed that these biomolecules play significant role in the biological potentials of PAf-mediated extracts.

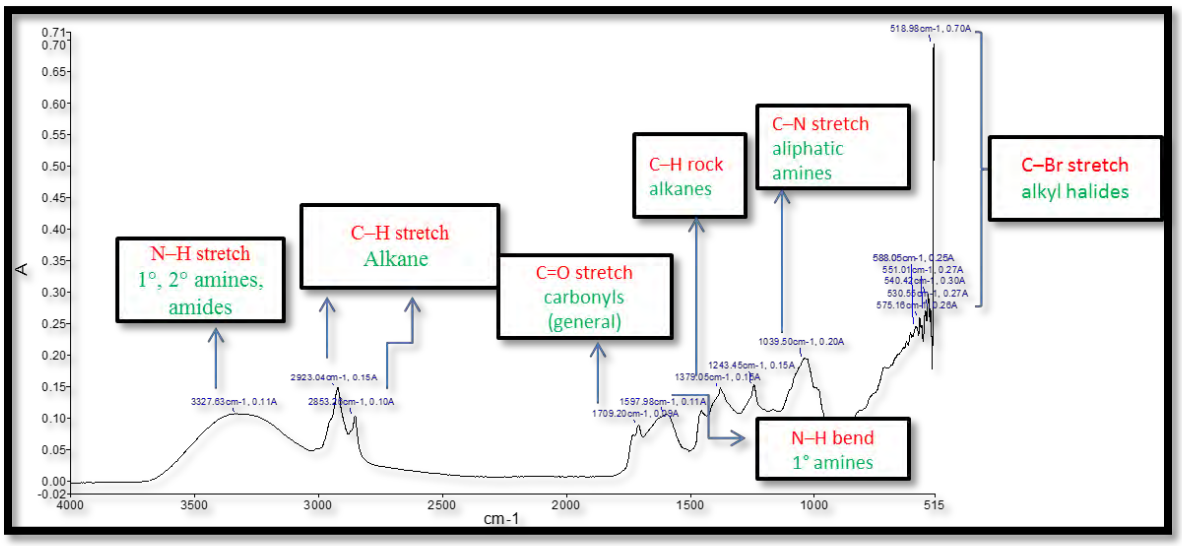

Figure 42: FTIR Spectral Analysis (cm-1) of *P. affine*.

3.4.4 Antioxidant Potential of *P. affine* Extracts

The antioxidant therapies protect the biological system by removing the free radicals (Hassan et al., 2017; Al-Laith et al., 2019). Different *in-vitro* antioxidant assays are now used to determine the antioxidant activity in plants (Kooti and Daraei, 2017; Saleem et al., 2020). The IC_{50} values for DPPH radical scavenging ability of *P. affine* extracts has given noticeable results. The noticed order of *P. affine* IC_{50} values was P f M < P f E < P f C < P f A < P f Q< P f H. PAfM showed highest value (40.32±2.1µg/mL) followed by PAE (55.23±1.3 µg/mL), PAfC (58.21±2.3 µg/mL), PAfA (64.18±2.7 µg/mL), PAfQ (71.54±2.7 µg/mL) and PAfH (88.76±1.2 µg/mL)

(Figure 43). Similar results were demonstrated by Eslami et al. (2017) for *P. convolvulus* methanol extracts showing good antioxidant activity by DPPH assay.

The data of total antioxidant capacity of *P. affine* extracts is given in Figure 44. Highest antioxidant capacity was given by PAfM (89.38±1.53 mg AAE/g sample) followed by PAfE (69.73±1.65 mg AAE/g sample), PAfC (65.99±1.94 mg AAE/g sample), PAfA (58.56±2.44 mg AAE/g sample), PAfQ (56.19±1.43 mg AAE/g sample) and PAfH (50.87±1.87 mg AAE/g sample) and was found to decrease in the order of PAfM > PAfE > PAfC > PAfA > PAfQ > PAfH. Our findings have been in agreement of Mahmoudi et al. (2019) in which authors described that *P. equisetiforme* extracts exhibited good antioxidant capacity.

The antioxidant potential of plants were accessed by their electron donation ability that's result into the reduction of ferric ions to ferrous ions (Bui et al., 2019). PAfM showed the maximum reducing power with 83.65±1.43 mg AAE/g sample measured at 200 µg/mL of extract followed by P f E (73.54±1.74 mg AAE/g sample), PAfC (71.82±1.38 mg AAE/g sample), PAfA (68.05±2.37 mg AAE/g sample) PAfQ (67.43±2.98 mg AAE /g sample) and PAfH (60.78±2.49 mg AAE/g sample) as shown in Figure 44. This assay findings were followed in the pattern of P f M > P f E > P f C > P f A > P f Q > P f H at 200 µg/mL.

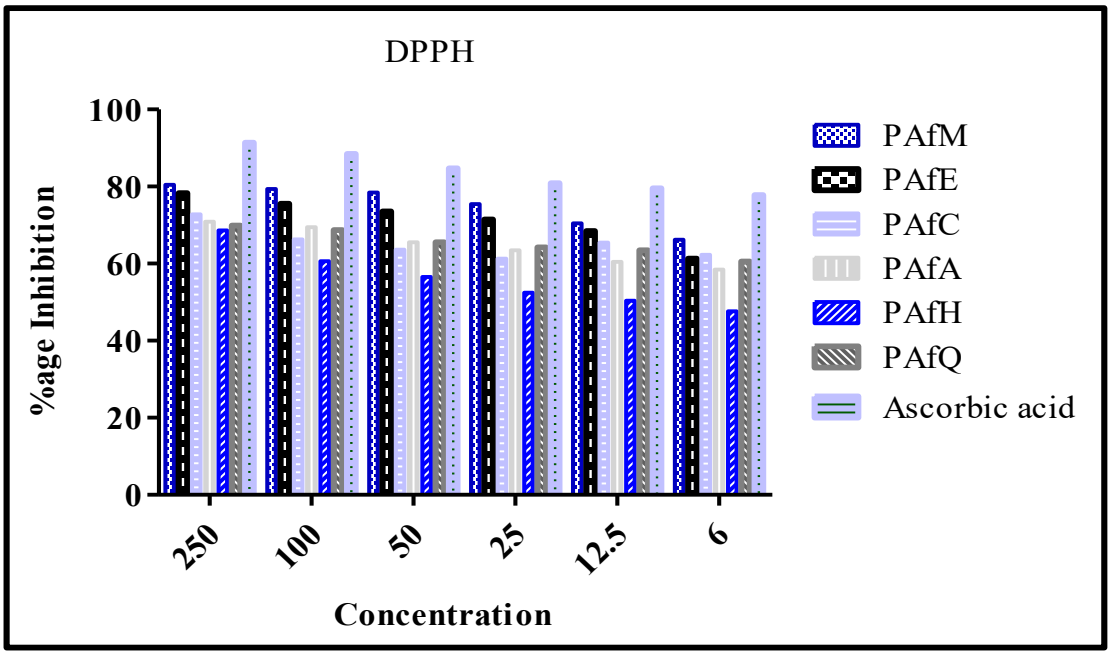

Figure 43: DPPH activity on different extracts of *P. affine*. PAfM: *P. affine* methanol, PAfE: *P. affine* ethanol, PAfC: *P. affine* chloroform, PAfA: *P. affine* ethyl acetate, PAfH: *P. affine* n-hexane, PAfQ: *P. affine* aqueous extract.

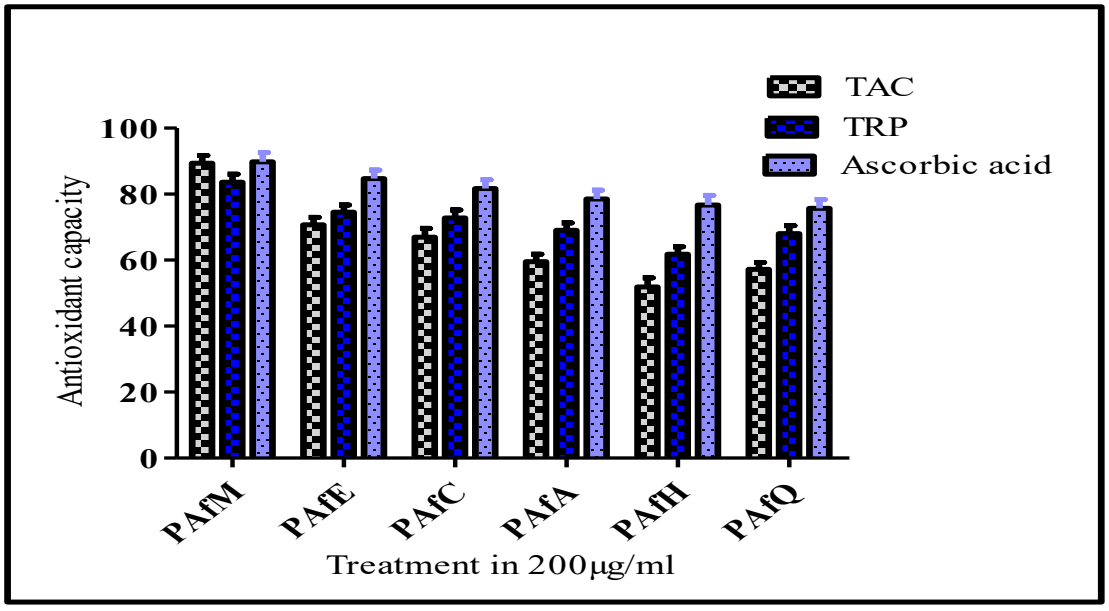

Figure 44: Total antioxidant capacity and total reducing power activity of different extracts of *P. affine*. PAfM: *P. affine* methanol, PAfE: *P. affine* ethanol, PAfC: *P. affine* chloroform, PAfA: *P. affine* ethyl acetate, PAfH: *P. affine* n-hexane, PAfQ: *P. affine* aqueous extract.

3.4.5 Antimicrobial Activity of *P. affine* Extracts

Traditional medicines extracted from plants can be used to develop novel antimicrobial drugs that have new mechanism of action and may provide a protective barrier against multidrug resistance microbial strains (Joshi et al., 2020; Mulat et al., 2020).The anti-bacterial screening and MIC values is given in Table 20. PAfA have good anti-bacterial activity against selected gram positive as well as gram negative bacterial strains *B. subtilus* (20±1.43 mm) *S. aureus* (19±2.46 mm) and *E. coli* (17±2.26 mm) *P. aeruginosa* (16±2.43 mm) and *K. pneumonia* (14±2.15 mm). PAfC extract showed anti-bacterial acitivity against *S. aureus* (18±2.09 mm) and *B. subtilus* (14±2.87 mm) and *K. pneumonia* (11±1.54 mm). While PAfQ showed minimum antibacterial activity having ZOI 8±1.24 mm and 3±2.45 mm against *S. aureus* and *P. aeruginosa*. Present findings were in line with the finding of Desouky et al. (2018) in which authors stated the antibacterial property of *P. plebeium* ethyl-acetate extract.

P. affine extracts showed significant antifungal property (Table 21). PAfA, PAfC, PAfE and PAfM showed significant ZOI whereas PAfQ and PAfH have given moderate results. PAfA inhabited growth of all tested fungal strains and most susceptible fungal strain towards PAfA was *F. solani* (16±2.3 mm) while the less susceptible strain was *A. flavus* (5±1.43 mm). Moreover, PAfC inhibited growth of four studied fungal strains and the more susceptible fungal strain was *A. flavus* (15±2.4 mm). PAfH and PAfQ plant extract were showed least potential against two fungal strains *C. albicans* (2±2.7 mm) and *A. niger* (10±2.43 mm). Present findings are in agreement with the findings of Salama and Marraiki (2010) and Jovanovic et al. 2018 indicating the antifungal activities of *P. affine* and *P. maritimum.*

Table 20: Inhibition Zones and MIC of *P. affine* Extracts against Bacterial Strains.

Micro-organisms	*E. coli* ATCC 33456		*P. aeruginosa* ATCC 90271		*K. pneumonia* ATCC 1705		*B. subtilus ATCC* 19659		*S. aureus* ATCC 6538	
Plant Extracts	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/ mL)	ZOI (mm)	MIC (µg/mL)
PAfM	NI		4±2.32		6±2.43		10± 1.35	100	12±2.54	100
PAfE	8± 1.43		6±1.43		NI		12± 1.46	100	10±2.43	100
PAfC	NI		NI		11±1.5	100	14±2.87	100	18± 2.09	33.33
PAfA	17±2.26	33.33	16±2.43	33.33	14±2.1	100	20±1.43	11.11	19±2.46	11.11
PAfH	2±1.65		NI		4± 1.12		9±2.12	100	NI	
PAfQ	NI		3±2.45		NI		NI		8± 1.24	

Values (mean ± standard deviation) was obtained through from triplicate analysis. NI: no activity, PAfM: *P. affine methanol,* PAfE: *P. affine ethanol,* PAfC: *P. affine* chloroform, PAfA: *P. affine ethyl acetate,* PAfH: *P. affine n-hexane,* PAfQ: *P. affine aqueous* extract.

Table 21: Inhibition Zones and MIC of *P. affine* Extracts against Fungal Strains.

Fungus strains	*M. racemosus* FCBP 0300		*C. albicans* FCBP 478		*A. niger* FCBP 0918		*F. solani* FCBP 0291		*A. flavus* FCBP 0064	
Plant Extracts	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)
PAfM	10±2.43	100	NI		13±1.5	100	NI		2±1.6	
PAfE	2±2.16		NI		NI		15±2.4	33.33	14±2.4	100
PAfC	9±2.43	100	13±1.43	100	NI		5±2.43		15±2.4	33.33
PAfA	12±1.65	100	11± 2.43	100	14±1.4	100	16±2.3	33.33	5±1.43	
PAfH	NI		2±2.7		9±2.4	100	NI		NI	
PAfQ	NI		6±2.5		11±2.6	100	NI		NI	

Values (mean ± standard deviation) were obtained through from triplicate analysis. NI: no activity, PAfM: *P. affine* methanol, PAfE: *P. affine* ethanol, PAfC: *P. affine* chloroform, PAfA: *P. affine* ethyl acetate, PAfH*: P. affine* n-hexane, PAfQ*: P. affine* aqueous extract.

Several studies proposed that the natural extracts display a large variety of therapeutic applications (Badirzadeh et al., 2020; Dias et al., 2020). Noticeable inhibition in growth of *L. tropica* was displayed by all six extracts. All PAf extracts had percentage inhibition greater than fifty and could be used as a source of anti-leishmanial drug. PAfH showed highest percentage inhibition with IC_{50} of 55±1.14 µg/mL, follow by P f with IC_{50} 69 ±2.32 µg/mL, P f C with IC_{50} 88±2.24 µg/mL, P f E having IC_{50} 105±2.23 µg/mL, P f M having with IC_{50} 113±2.77 µg/mL and PAfQ with IC_{50} 187±2.32 µg/mL. Present findings were in line with the previous report on *P. glabrum* stem extracts showing effective anti-leishmanial activity (Rahman et al., 2015).

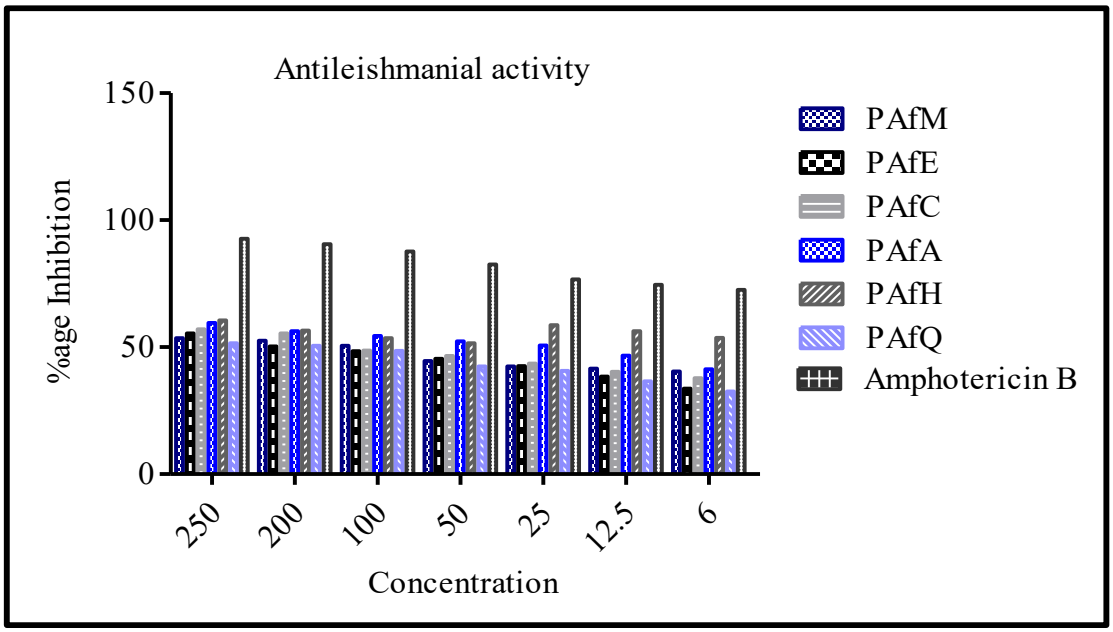

Figure 45: Anti-leishmanial assay on different extracts of *P. affine*. PAfM: *P. affine* methanol, PAfE: *P. affine* ethanol, PAfC: *P. affine* chloroform, PAfA: *P. affine* ethyl acetate, PAfH: *P. affine* n-hexane, PAfQ: *P. affine* aqueous extract.

3.4.6 Cytotoxicity Assessment and Anticancer Potential of *P. affine*

Plant based products are the major target of researcher in order to develop new anticancer drugs (Sharma et al., 2019; Beeby et al., 2020). From the data of cytotoxic potential of PAf six organic extracts screen for plant, 55 % extracts displayed LD_{50} value under 60 µg/mL and were identified to be extremely cytotoxic where as 45 % considered as low cytotoxic (Table 22). Among all tested extracts, PAfC was found to be the best cytotoxic with LD_{50} 47.65±2.42 µg/mL demonstrating that in *P. affine* case the partial polar solvents moving towards the non-polar solvents are highly effective in the extraction of cytotoxic compounds as compared to polar solvents. Present findings matches with the previous studies of Ayaz et al. (2016) which demonstrated that the partial polar extract of *P. hydropiper* shows best brine shrimp cytotoxicity.

Many natural compounds from medicinal plants have been identified and used against PKC activity against tumor cells (Matias et al., 2016; Isakov, 2018). Direct relationship was found among different concentrations of tested plant extracts and PK inhibition activity. The significant bald area zone (17±2.1 mm) and MIC (33.33 µg/mL) was measured around PAfC loaded disc which is followed by PAfA (15±1.8 mm), PAfH (13±2.5 mm), PAfQ (10±1.5 mm) and PAfE (4±2.5 mm). PAfM gives no PKI activity (Table 32). Similar with the findings of anticancerous activity of *P. affine*, other plant species belonging to genus *Polygonum* such as *P. minus* and *P. tinctorium* also possess anticancerous activity (Ahmad et al., 2018; Chung et al., 2018).

The result of PAfC extract has confirmed the reduction in metabolic activity of PC3 cells as shown in Figure 46. The reduced metabolic activity has shown that PAfC extract might have strong anticancer potential. The cytotoxicity induced by PAfC extract at lower doses might be linked to the plant cytotoxic functional groups. These results verified the findings of previous studies on anticancer potential of *P.barbatum* (Farooq et al., 2017)

Table 22: Brine Shrimp Lethality and Protein Kinase Inhibition Assay.

Samples	Brine shrimp lethality (µg/mL)		Protein kinase inhibition (µg/mL)		
	% Mortality	LD_{50} (µg/mL)	Diameter (mm) at 100 µg/disc		MIC (µg/mL)
	250		Clear zone	Bald zone	
PAfM	49.5±2.47	105±1.34	----	----	
PAfE	51.4±2.12	97±2.54	----	4±2.5mm	
PAfC	85.5±2.32	34±2.38	----	17±2.1mm	33.33
PAfA	79.3±2.35	47.65±2.65	----	15±1.8mm	33.33
PAfH	74.2±2.54	55±2.42	----	13±2.5mm	100
PAfQ	67.4±2.28	84±1.65	----	10±1.5 mm	100

Values (mean ± standard deviation) were obtained through from triplicate analysis. PAfM; *P. affine* methanol, PAfE: *P. affine* ethanol, PAfC: *P. affine* chloroform, PAfA: *P. affine* ethyl acetate, PAfH: *P. affine* n-hexane, PAQ: *P. affine* aqueous extract.

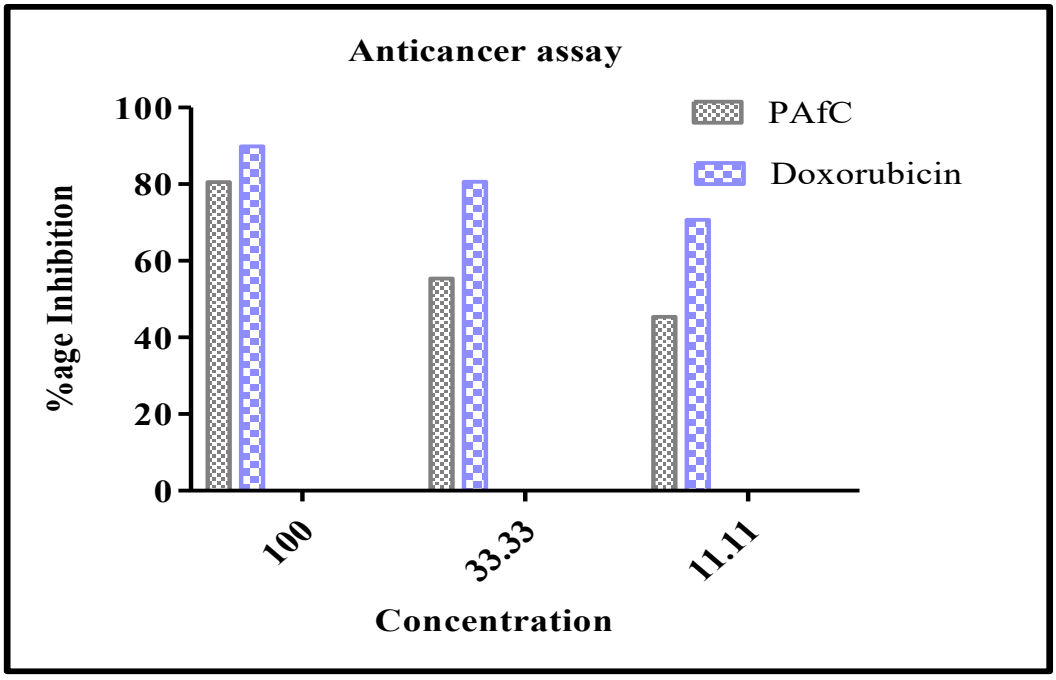

Figure 46: Anticancer Activity of *P. affine* Extract Against PC-3 Cells.

3.4.7 Antidiabetic Potential of *P. affine* Extracts

Plant based medicine is thought to be safe and effective approach used in the treatment of diabetes (Agarwal and Gupta, 2016; Ali, 2016). Among tested extracts PAfC showed significant activity (73.54±2.54 %). The rest of the extracts have shown the following order PAfA, PAfH, PAfE, PAfQ and PAfM 54.20±2.65 %, 49.7±1.54 %, 47.60±1.23 %, 41.36±2.42 % and 40.47±2.54 (Table 23). Present findings were in line with previous reports based on antidiabetic potential of different species of *Polygonum* such as *P. maritimum* and *P. cuspidatum* (Rodrigues et al., 2017; Zhao et al., 2017).

Table 23: Antidiabetic Potential of *P. affine* Extracts and Respective IC_{50} values.

Plant Extracts	%age inhibition of alpha-amylase	**IC_{50} µg/mL**
PAfM	40.47 ±2.54	------
PAfE	47.60 ±1.23	------
PAfC	73.54 ±2.54	87.54
PAfA	54.20 ±2.65	105.43
PAfH	49.77 ±1.54	119.54
PAfQ	41.36 ±2.43	**------**

Values (mean ± standard deviation) were obtained through from triplicate analysis. ----- : no activity. PAfM: *P. affine* methanol, PAfE: *P. affine* ethanol, PAfC *P. affine* chloroform, PAfA: *P. affine* ethyl acetate, PAfH: *P. affine* n-hexane, PAfQ*: P. affine* aqueous extract.

SECTION 5 : *Rhodiola imbricata* Edge

3.5 *Rhodiola imbricata* Edgew.

Rhodiola imbricata Edgew. is the perennial plant of family Crassulaceae (Figure 47). Naturally found in Nepal, India, Bhutan, China and Tajikistan (https: //www. gbif.org/occurrence/search? Occurrence_status=present&q=Rhodiola%20 imbricata % 20 Edgew.) *Rhodiola* plants, most commonly known as rose root and found in mountainous areas of Europe and Asia (Nootropic, 2017). Mostly grows on higher mountain areas with altitude of 1500 and height of 3000–4000 m. Genus *Rhodiola* with 90 species that are mostly grow in the Northern Hemisphere, closer to the North pole and polar region, and few species grows outside the equator. Himalaya (3rd cold pole of earth) is a major geographic center and suitable area for the growth of genus *Rhodiola* (Cunningham et al., 2020).

Figure 47: Field Photograph of *Rhodiola imbricata* Edgew.

For thousands of years, about 48 *Rhodiola* species were used in traditional Chinese medicinal system. Local community of China, use *Rhodiola* roots as a tonic for the treatment of tonic, daptogen, antistress and antidepressant, while leaves and flowers were reported to be used to cure diuresis, liver disease, poisoning and fever (Wu et al.,

2002). In the present research *R. imbricata* **(**which is one of the biologically active member of genus *Rhodiola*) was collected from the region of Deosai, Northern Areas of Pakistan.

In traditional medicinal system, different *Rhodiola* species were used to treat different ailments. The genus *Rhodiola* is known for phytochemicals such as flavonoids, coumarins and phenyl glycosides. The root of *R.imbricata* was found to possess radio-protective, cytoprotective, wound healing and immunomodulatory activities. In a dose dependent study an aqueous extract of *R.imbricata* roots was found to possess potent adaptogenic activity in animals and post-stress recovery. It was indicated that the adaptogenic activity of root aqueous extract was due to improved metabolism and better anabolic state. The antioxidant and free radical scavenging properties of the studied aqueous extract of *R.imbricata* root was evaluated using *in-vitro* model and it was observed that the plant is a rich source of natural antioxidant (Gupta et al., 2010). Recently, various phytochemicals have been extracted from different members of genus *Rhodiola*, including flavonoids, alkaloids, glycosides (Li et al., 2017). However, no literature regarding the detailed biological potential of *R. imbricata* was seen. Therefore, it is important to explore this ethnomedicinally important herb. This is the first comprehensive research that was based on phytochemical analysis, antioxidant potential, antimicrobial, cytotoxic, anti-leishmanial and antidiabetic potential of this highly ethno-medical important herb.

3.5.1 Preliminary Phytochemical Analysis of *R. imbricata*

Phytochemicals revealed significant influence on many therapeutic products thus defining their pharmacological potential (Batool et al., 2019; Kamalarajan et al., 2019). Phytochemical screening of *R. imbricata* extracts showed the presence of alkaloids, flavonoids, phenols and carbohydrates in all the six RIM, RIE, RIC, RIA, RIH and RIQ extracts. Glycosides and sterol were present in RIM, RIE, RIC, RIA, RIQ but were missing in RIH extract. Tannins were absent in RIC, RIA and RIH while amino acids was missing in RIC and RIE and saponins were absent in RIC, RIA and RIH (Table 24). Our study showed that *R. imbricata* extracts revealed the existence of phytochemicals alkaloids, flavonoids, amino acids, phenols, glycosides tannins, steroids and saponins which is in accordance with the findings of Zhong et al. (2020) showing the presence of different phytochemicals in various extracts of *R. crenulate.*

Table 24: Preliminary phytochemical analysis of *R. imbricata* different extracts.

Plant Extracts	Alkaloids	Flavonoids	Phenols	Carbohydrates	Steroids	Glycosides	Saponins	Tannins	Amino acids
RIM	++	+++	+++	++	++	+++	+++	+++	+++
RIE	++	++	++	++	++	++	+++	+++	++
RIC	+	++	+++	+++	+	++	-	-	-
RIA	++	+	+	++	-	++	-	-	-
RIH	++	+	+	++	-	-	-	-	+
RIQ	+	++	+	+		++	+++	+++	++

+++: Strongly present; ++: Moderately present; +: Weakly present; -: Absent. RIM: *R. imbricata* methanol, RIE: *R. imbricata* ethanol, RIC: *R. imbricata* chloroform, RIA: *R. imbricata* ethyl acetate, RIH: *R. imbricata* n-hexane and RIQ: *R. imbricata* aqueous extracts.

3.5.2 Quantitative Phytochemical Analysis of *R. imbricata* Extracts

Phytochemicals are produced in all plant parts and have positive applications on human health (Ruwali and Negi, 2019). Findings of phenolic contents of *R.imbricata* extracts were summarized in Figure 48. The extracts of RI have followed the trend RIM>RIE>RIQ>RIC>RIA>RIH. It was found that RIM has highest phenolic contents (94.76 mg GAE/g). Our TPC data is in accordance with the report of Pace and Watnick (2020) indicating that the *R.imbricata* methanol extract contain highest amount of phenolic compounds.

Total flavonoid contents of *R.imbricata* extracts are summarized in Figure 49. It was seen that RIM has highest flavonoid contents (65.98 mg QE/g), followed by RIE (56.53 mg QE/g), RIQ (47.95 mg QE/g), RIC (18.26 mg QE/g), RIA (14.61 mg QE/g) and RIH extract (14.76 mg QE/g). Yatoo et al. (2017) has described the total amount of total phenolic and flavonoid contents of various extracts of different species of *Rhodiola* High flavonoid contents in methanolic extract may be due to more solubility of these bio-compounds in respective solvent. Our findings are in agreement with a previous report of Ahmad et al. (2020) demonstrating methanol as a best solvent for phenolic and flavonoid contents.

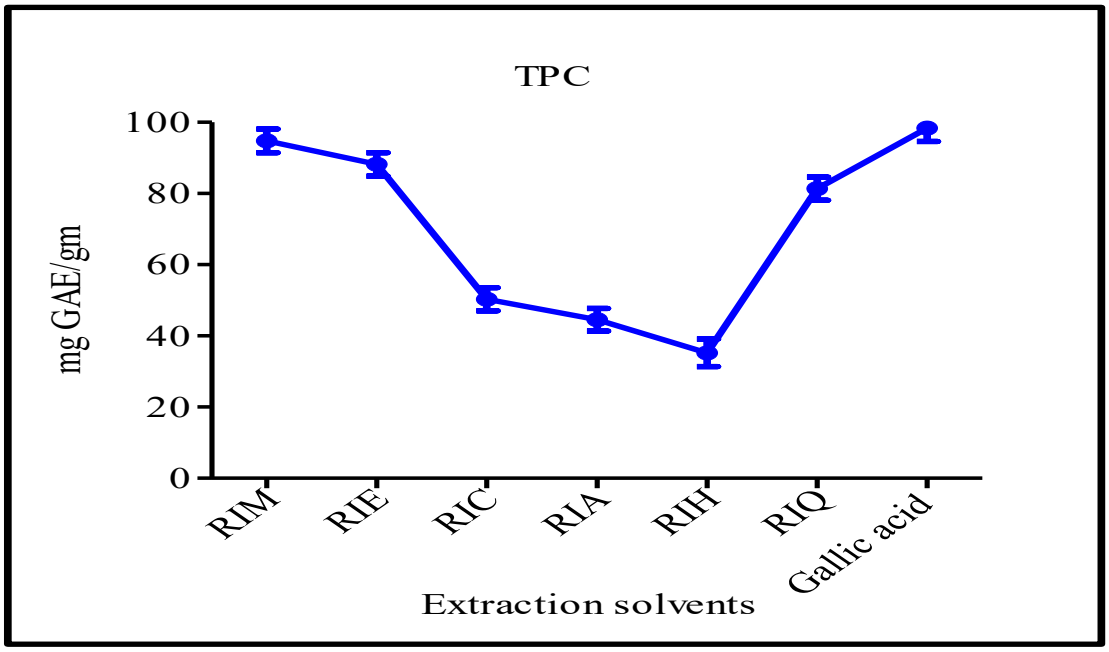

Figure 48: Total phenolic contents of *R. imbricata*. RIM: *R. imbricata* methanol, RIE: *R. imbricata* ethanol, RIC: *R. imbricata* chloroform, RIA: *R. imbricata* ethyl acetate, RIH: *R. imbricata* n-hexane, RIQ: *R. imbricata* aqueous extract.

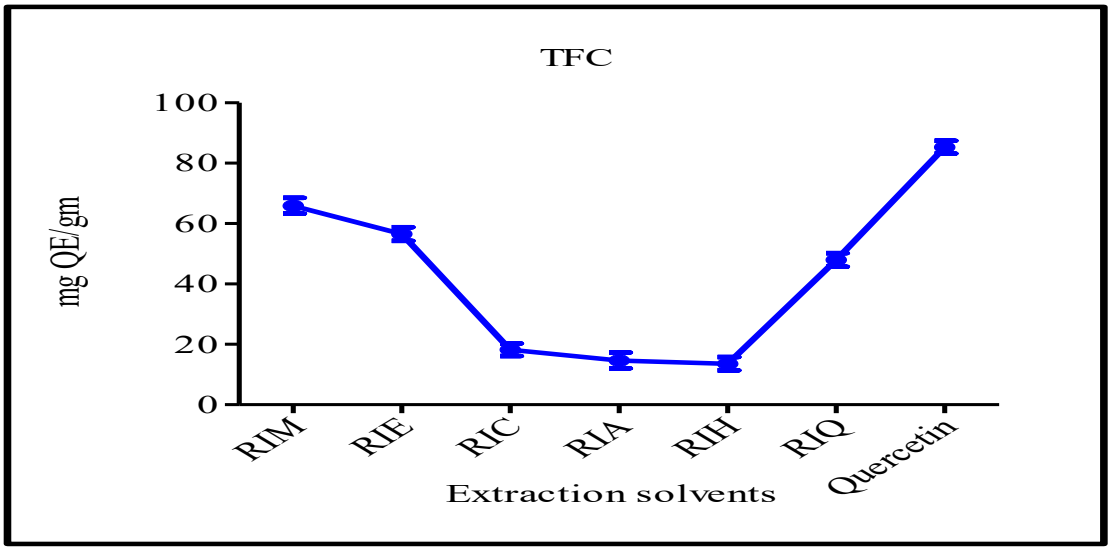

Figure 49: Total flavonoid contents of *R. imbricata*. RIM: *R. imbricata* methanol, RIE: *R. imbricata* ethanol, RIC: *R. imbricata* chloroform, RIA: *R. imbricata* ethyl acetate, RIH *R. imbricata* n-hexane, RIQ: *R. imbricata* aqueous extract.

3.5.3 FTIR spectral analysis (cm^{-1}) of *R.imbricata*

FTIR spectra shows the presence of numerous functional groups with strong and medium peak intensities. N-H strectch was observed indicating presecnce of amines and C-H stretch was also observed in the sample indicating the presence of alkanes. One broad peak is explicitly visible showing the presence of alcohols and phenols in *R.imbricata*. Some medium peaks were observed which indicated the existence of N-O stretch, C-H bend, C-H wag, C-N and C-Br stretch and hence confirmed the presence of alkanes, carboxylic acids, aliphatic amines and alkyl halides (Figure 50). The availability of these different functional groups showed that these biomolecules play significant role in the biological potentials of RI-mediated extracts.

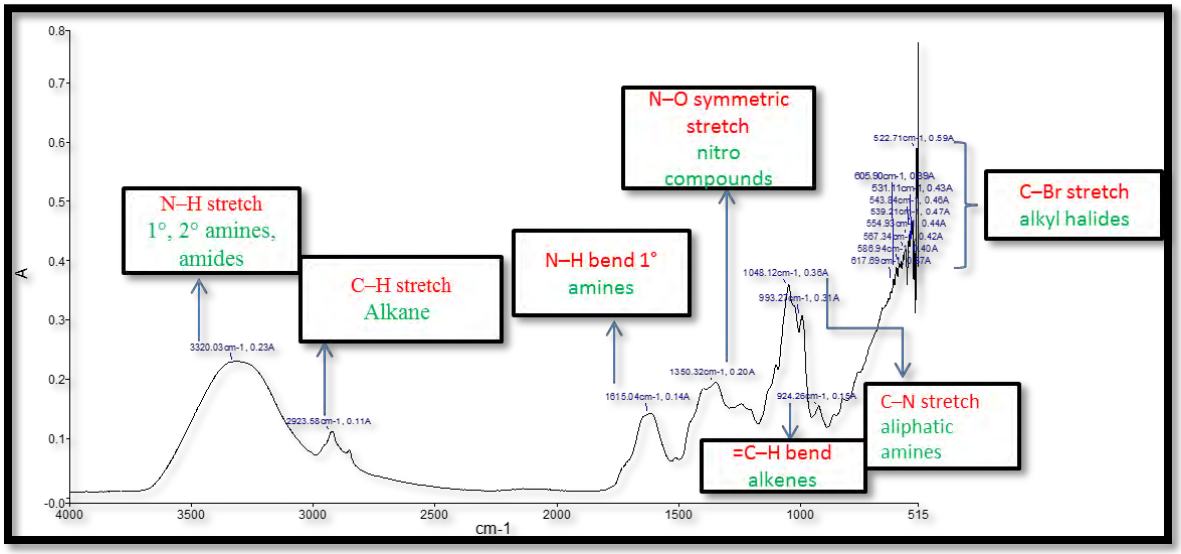

Figure 50: FTIR Spectral Analysis (cm-1) of *R. imbricata*.

3.5.4 Antioxidant Potential of *R. imbricata* Extracts

The scavenging ability of antioxidants present in extracts reduces the free radicals by electron donation (Alam et al., 2013). The IC_{50} values for DPPH radical scavenging ability of *R. imbricata* extracts displayed reasonable results. The noticed order of IC_{50} values for different extracts of *R. imbricata* was RIM < RIE < RIQ < RIC < RIA < RIH. IC_{50} values for RIM showed best results (87.55±2.7 µg/mL) followed by RIE (96.54±2.5 µg/mL), RIQ (107.4±3.2 µg/mL), RIC (142.6 ± 2.2 µg/mL), RI (264.5±3.5 µg/mL) and RIH (295.6±3.3 µg/mL) (Figure 51). Our findings are in coherence with Nahak and Sahu (2010) that there is direct connection among the phenolic contents and antioxidant capacity.

Data of TAC followed an order of RIM > RIE > RIQ > RIC > RIA> RIH. Highest antioxidant capacity for RI extract was given by RIM (60.33±2.34 mg AAE /g sample) followed by RIE (44.06±2.24 mg AAE /g sample), RIQ (38.14±2.1 mg AAE /g sample), RIC (34.98±2.26 mg AAE /g sample), RIA (24.39± 2.76 mg AAE /g sample) and RIH (12.87±2.76 mg AAE /g sample). RIM has the best antioxidant capacity due to the presence of high phenolic and flavonoid contents. Our findings are in good agreement by the previous study of Sikder et al. (2010) describing that plant extract in methanol exhibited good antioxidant capacity.

Findings of TRP of RI extracts showed that RIM displayed maximum reducing power with 82.96±2.37 mg followed by RIE (72.98±2.11 mg AAE/g sample), RIQ (64.44±2.42 mg AAE/g sample), RIC (50.11±2.11 mg AAE/g sample) RIA (46.47±2.23 mg AAE/g sample) and RIH (39.81±2.23 mg AAE/g sample) (Figure 52). The result of this assay was considerably correlated with TFC and TPC. Findings of this assay have followed the pattern of RIM > RIE > RIQ> RIC > RIA > RIH at 200 µg/mL. Our results are in accordance with a previous report demonstrating that the amount of TPC in extract is linked with reducing power capacity (Jafri et al., 2017).

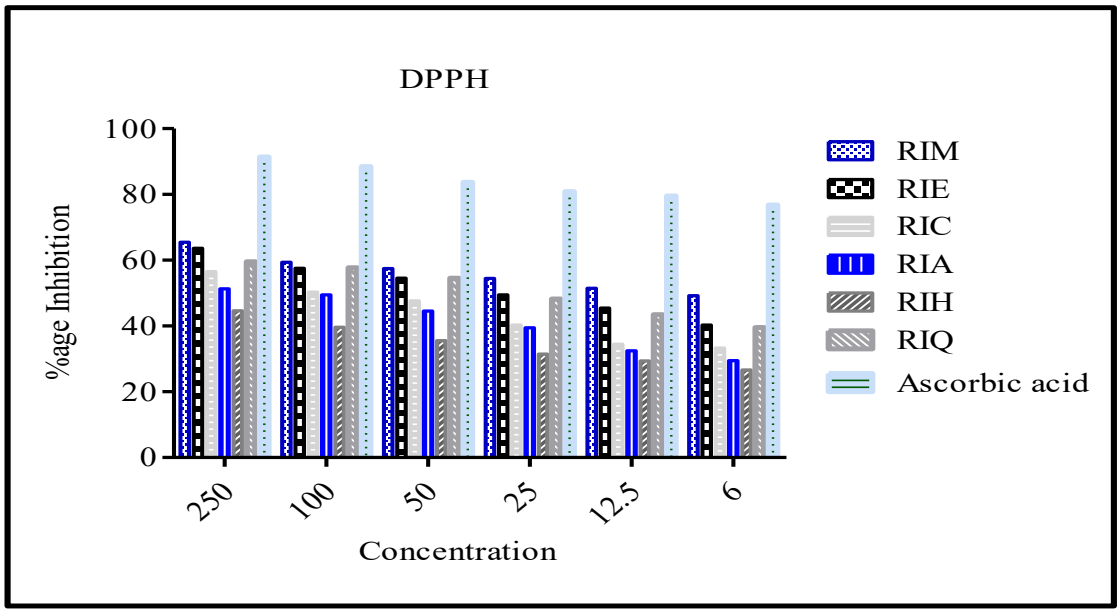

Figure 51: DPPH assay of different extracts of *R. imbricata*. RIM: *R. imbricata* methanol, RIE: *R. imbricata* ethanol, RIC: *R. imbricata* chloroform, RIA: *R. imbricata* ethyl acetate, RIH: *R. imbricata* n-hexane, RIQ: *R. imbricata* aqueous extract.

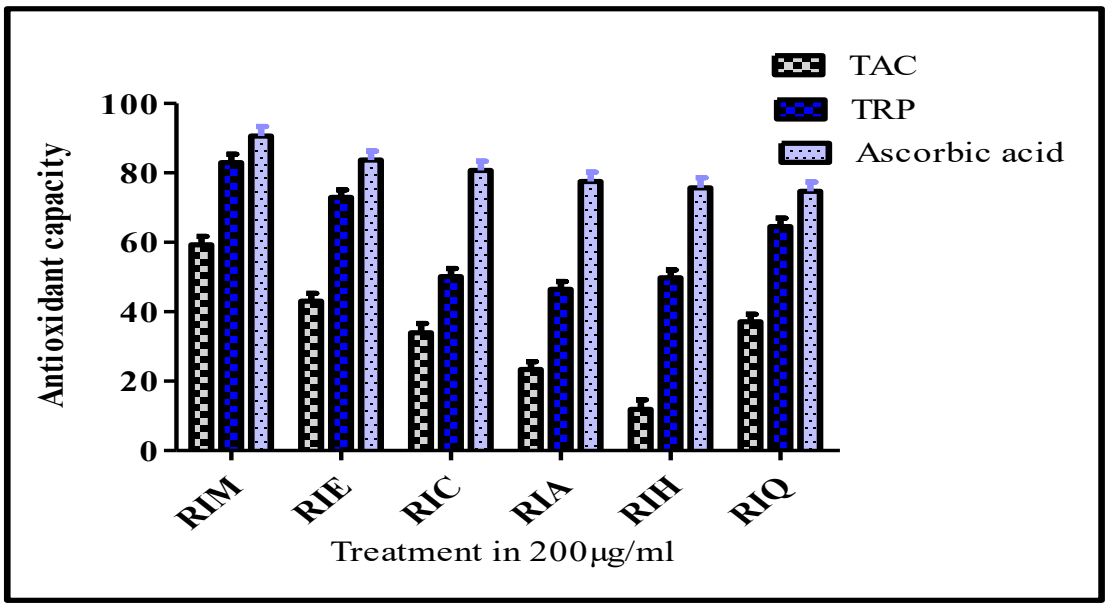

Figure 52: Total antioxidant capacity and total reducing power of different extracts of *R. imbricata*. RIM *R. imbricata* methanol, RIE *R. imbricata* ethanol, RIC *R. imbricata* chloroform, RIA *R. imbricata* ethyl acetate, RIH *R. imbricata* n-hexane, RIQ *R. imbricata* aqueous extract.

3.5.5 Antimicrobial Activity of *R. imbricata* Extracts

The data for antibacterial screening of *R. imbricata* extracts is given in Table 25. The largest and smallest ZOI was shown by RIC 25± 2.23 mm against *S. aureus* and 15±1.53 mm against *K. pneumonia* respectively. RIQ showed least antibacterial activity against studied bacterial strains while RIC revealed good antibacterial activity against studied bacterial strains except *E. coli.* Present findings were in line with the data reported by Kosakowska et al. (2018) in which authors stated the antibacterial property of *R. rosea.*

Data of antifungal activities of all extracts of *R.imbrica* displayed significant ZOI for RIM, RIE and RIQ. Whereas RIC, RIA and RIH give activity only against *C. albicans*, *M. racemosus* and *A. niger* (Table 26). Most susceptible fungal strain towards RIM was *M. racemosus* having the ZOI 24±1.21 mm while the less susceptible strain was *C. albicans* with ZOI 18±1.33 mm. RIE inhibited growth of *C. albicans* with ZOI 20±1.11 mm. It was noticed that RIM, RIE and RIQ contain saponin and tannins and showing good antifungal property as compared to RIC, RIA and RIH which has no saponin and tannins compounds and showing no antifungal properties. Our findings are in settlement with the Ohadoma et al. (2014) demonstrating that the presence of tannins and saponins in plant extracts may become responsible for antimicrobial property of extracts. Present findings are in compliance with the report on methanolic extract of *J. oxycedrus*, in which strong antimicrobial activity was linked with polar solvants (Karaman et al., 2003)

Table 25: Inhibition Zones and MIC of *R. imbricata* Extracts against Bacterial Strains.

Micro-organisms	***E. coli*** ATCC 33456		***P. aeruginosa*** ATCC 90271		***K. pneumonia*** ATCC 1705		***B. subtilus*** *ATCC* 19659		***S. aureus*** ATCC 6538	
Plant Extracts	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)
RIM	NI		21± 1.42	11.11	17±2.31	33.33	NI		10±2.11	100
RIE	14±1.3	100	NI		NI		NI		13±1.23	100
RIC	NI		18±2.31	33.33	15±1.53	33.33	17±1.13	33.33	25±2.23	11.11
RIA	10± 1.1	100	19± 2.14	33.33	NI		NI		NI	
RIH	10±2.3	100	NI		11±1.81	100	NI		19±2.24	33.33
RIQ	NI		11±1.17	100	NI		NI		NI	

Values (mean ± standard deviation) was obtained through from triplicate analysis. NI: no activity. RIM: *R.imbricata* methanol, RIE: *R. imbricata* ethanol, RIC: *R. imbricata* chloroform, RIA: *R. imbricata* ethyl acetate, RIH: *R. imbricata* n-hexane, RIQ: *R. imbricata* aqueous extract.

Table 26: Inhibition Zones and MIC of *R.imbricata* Extracts against Fungal Strains.

Fungus strains	***M. racemosus*** FCBP 0300		***C. albicans*** FCBP 478		***A. niger*** FCBP 0918		***F. solani*** FCBP 0291		***A. flavus*** FCBP 0064	
Plant Extracts	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)
RIM	24±1.21	11.11	18±1.3		20±1.6	11.11	NI		19±1.3	33.33
RIE	20.7±1.4	11.11	20±1.1		NI		11±1.2		13±2.21	100
RIC	NI		10±2.1	100	NI		NI		NI	
RIA	17±1.2	33.33	NI		NI		NI		NI	
RIH	NI		NI		17±1.32	33.33	NI		NI	
RIQ	11±1.81	100	12±1.21	100	14±1.42	100	NI		20±1.21	11.11

Values (mean ± standard deviation) was obtained through from triplicate analysis. NI no activity, . RIM *R.imbricata* methanol, RIE *R. imbricata* ethanol, RIC *R. imbricata* chloroform, RIA *R. imbricata* ethyl acetate, RIH *R. imbricata* n-hexane, RIQ *R. imbricata* aqueous extract.

A well marked inhibition in growth of *L. tropica* was indicated by all the extracts of *R. imbricata*. RIH, RIA, RIC with IC_{50} 111±1.32 µg/mL, 153±1.12 µg/mL and 182±1.21 µg/mL repectively showed significant activity. Reasonable activity was also revealed by RIE extract with IC_{50} around 199±1.21 µg/mL. Whereas the least activity was found in RIQ and RIM. Figure 53 showed that those compounds that were involved in most potent activity were concentrated in non-polar extracts.Our findings are in correlation with Zahra et al. (2017) in which authors reported anti-leishmanial activity of non-polar solvents.

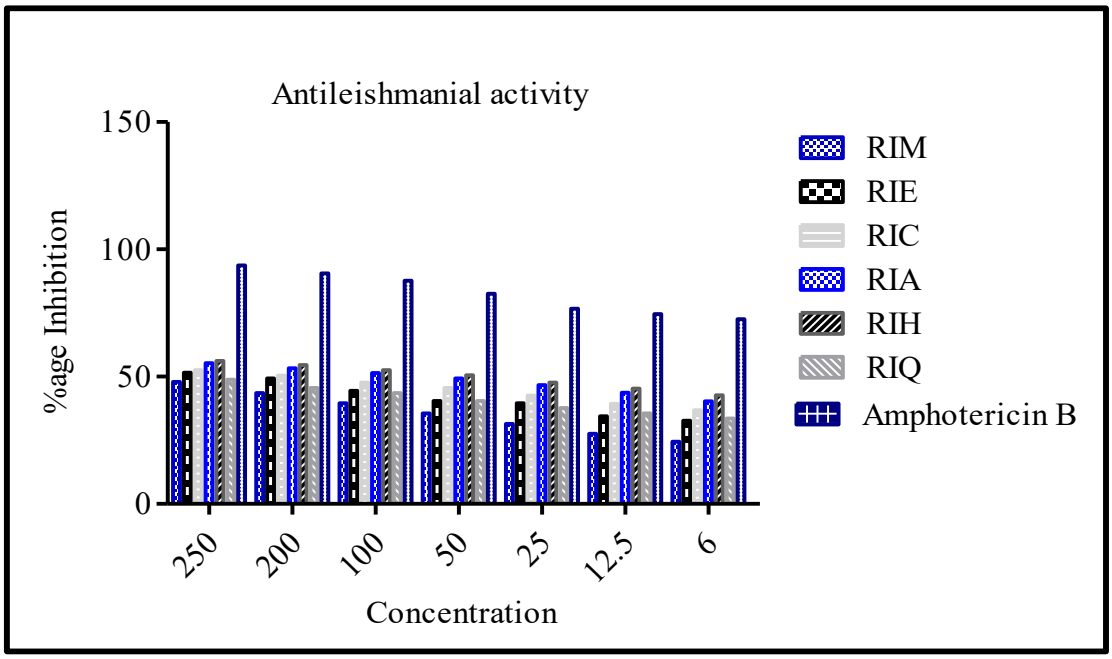

Figure 53: Anti-leishmanial activity of different extracts of *R. imbricata*. RIM: *R. imbricata* methanol, RIE: *R. imbricata* ethanol, RIC: *R. imbricata* chloroform, RIA: *R. imbricata* ethyl acetate, RIH: *R. imbricata* n-hexane, RIQ: *R. imbricata* aqueous extract.

3.5.6 Cytotoxicity Assessment and Anticancer Potential of *R.imbricata*

R.imbricata extracts were screened for cytotoxic potential, 16 % extracts displayed LD_{50} value under 60 µg/mL and were identified to be extremely cytotoxic where as 82 % considered as low cytotoxic with LD_{50} values >200 µg/mL (Table 27). Brine shrimp lethality activity of *M. azedarach* methanol extract (Zahoor et al., 2015) is in agreement with our findings.

Direct relationship was noticed among concentrations of tested plant extracts and PKI activity. Among all the tested samples, a significant bald area zone with ZOI 25±1.6 mm and MIC = 11.11 µg/mL was measured around RIE loaded disc that was followed by RIQ with bald zone 14±1.2 mm and RIC with bald zone 10±2.2 mm (Table 27). In case of RIA, clear zone was noticed with ZOI 19±1.1 mm. Our findings were in settlement with Ahmed et al. (2017) in which *Q. dilatata* extract showed PKI activity.

Prostate cancer frequency and death rates fluctuate worldwide (Rawla, 2019). The cytotoxic potency of the RIE extract against prostrate cell line (PC3) was demonstrated using MTT assay. The result of RIE extract has confirmed reduction in metabolic activity of PC3 cells. The percentage inhibition was achieved at three different concentrations (100, 33.33 and 11.11) µg/mL as shown in Figure 54. The reduced metabolic activity has shown that RIE extract might have strong anticancer potential. The cytotoxicity induced by RIE extract at lower doses might be likned with cytotoxic active functional groups as mentioned in previous report on *R. Linnaeus* (Eghianruwa et al., 2019).

Table 27: Brine Shrimp Lethality and Protein Kinase Inhibition Assay.

Samples	Brine shrimp lethality (µg/mL)		Protein kinase inhibition (µg/mL)		
	% Mortality	LD_{50} (µg/mL)	Diameter (mm) at 100 µg/disc		MIC(µg/mL)
	250		Clear zone	Bald zone	
RIM	39.43±1.80	>200	----	---	
RIE	19.56±1.94	>200	----	27±1.6mm	11.11
RIC	19.76±1.80	>200	----	10±2.2mm	100
RIA	69.54±1.44	66.65± 1.67	19±1.1mm	----	33.33
RIH	29.54±1.5	>200	----	----	----
RIQ	34.65±2.13	>200		14±1.2 mm	100

RIM: *R. imbricata* methanol, RIE: *R. imbricata* ethanol, RIC: *R. imbricata* chloroform, RIA: *R. imbricata* ethyl acetate, RIH: *R. imbricata* n-hexane, RIQ: *R. imbricata* aqueous extract.

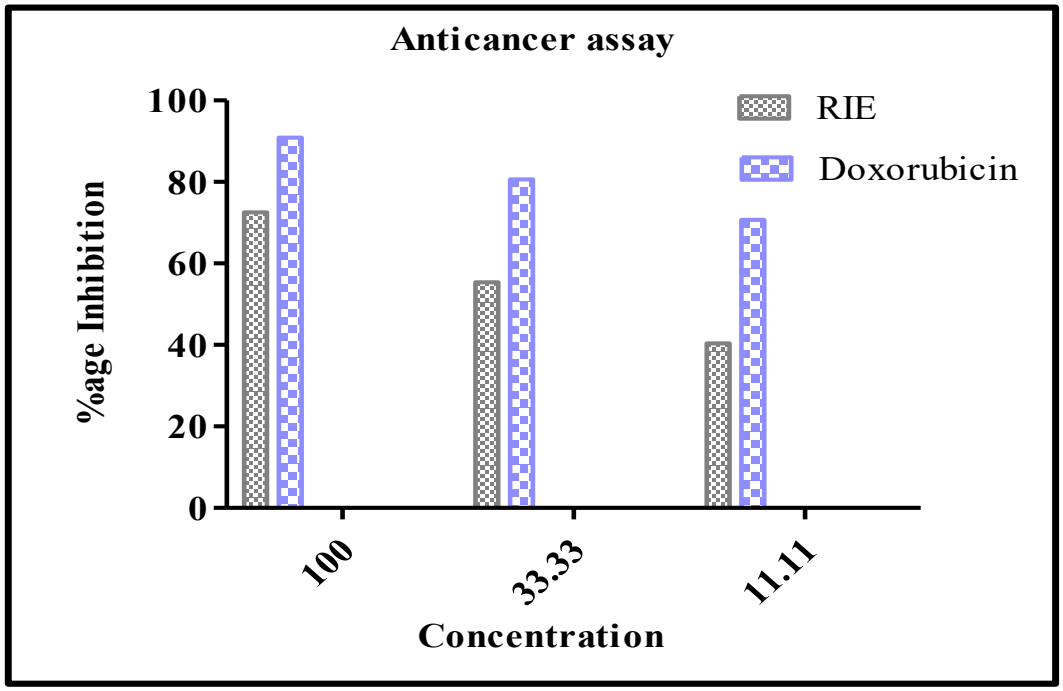

Figure 54: Anticancer activity of *R. imbricate* extract against PC-3 Cells.

3.5.7 Antidiabetic Potential of *R.imbricata*

The antidiabetic potential of *R.imbricata* showed moderate results (Table 28). Among all extracts, RIH showed highest activity with the value of 73.10±2.65 %. However, RIA, RIC and RIE have shown alpha-amylase inhibition of 47.10±2.43%, 37.57±1.36 % and 37.27±3.32 % respectively. Present findings are in accordance to a previous report of Zahra et al. (2017) in which authors stated that non-polar extracts have rich phytochemicals responsible for the antidiabetic activity.

Table 28: Antidiabetic Potential of *R. imbricata* Extracts and Respective IC50 values.

Plant Extracts	%age inhibition of alpha-amylase	IC_{50} µg/mL
RIM	37.97 ±2.45	-----
RIE	38.27 ±3.32	-----
RIC	38.57 ±1.36	-----
RIA	48.10 ±2.43	-----
RIH	74.10 ±2.65	68.76±1.43
RIQ	36.76 ±1.32	-----

Values (mean ± standard deviation) were obtained through from triplicate analysis. -: no activity, RIM: *R. imbricata* methanol, RIE: *R. imbricata* ethanol, RIC: *R. imbricata* chloroform, RIA: *R. imbricata* ethyl acetate, RIH: *R. imbricata* n-hexane, RIQ: *R. imbricata* aqueous extract.

SECTION 6 : *Salix planifolia* Pursh

3.6 *Salix planifolia* Pursh

Salix planifolia Pursh belongs to family Salicaceae (Figure 55) and naturally found in Canada and United States of America (https://www.gbif. org/occurrence /search? offset= 80&q =Salix%20planifolia%20Pursh. No synonyms were recorded for this species (http://www. theplantlist. org /tpl1.1/record/kew-182822). *S. planifolia* plant is most commonly known as diamond leaf willow, tea-leafed and planeleaf willow. The *Salix* comes from the Latin word that means gender neutral. Total number of *Salix* species across the world is estimated to be 526. *Salix* species are extensively found in the Northern Hemisphere while only a few of them found in the Southern Hemisphere. The genus includes deciduous trees and shrubs.

Figure 55: Field Photograph of *Salix planifolia* Pursh.

In the present study, *Salix planifolia* that is first time reported in Pakistan and was collected from the Deosai, Gilgit-Baltistan, Northern Areas of Pakistan. Deosai plateau is one of the major and second highest alpine plateau of the world and heritage region with diverse climate conditions for natural reserve of unique vegetation. Deosai National Park

in summer, provides a valuable source of medicinal plants to the local communities of Gilgit-Baltistan (Shaheen et al., 2019). In traditional medicinal system medicinal plants plays an important role in primary health care, can be used to treat many ailments. Plants are the richest source of bio-active compounds that have therapeutic values (Gupta and Prakash, 2019). Traditional knowledge about the use of medicinal plants has gained much importance in local communities (Vinagre et al., 2019). Traditionally different species of *Salix* were used to treat different aliments such as flu, cold, wound healing, stomach ulcer, liver and rheumatism problems. *Salix* extracts or its constituents were used as antimicrobial, antitumor, antioxidant, cardiovascular and cytotoxic agents. Several *Salix* species are used in herbal teas and as flavoring agent, in perfume, cosmetics and in pharmaceutical industries (Prashith et al., 2017).

S. purpurea, the plant with diverse medicinal properties have been used for the treatment of rheumatic diseases (Sulima and Przyborowski, 2019). *S. tetrasperma* used to cure diabetes, rheumatism, piles, fever, epilepsy, swellings, ear pain, wound, dysentery, stones in bladder, cough and cold (Prashith et al., 2017). *S. alba* is used in the treatment of acute inflammation, pain, chronic infection and fever (Bussmann et al., 2020). Inhabitors of Skardu (Gilgit-Baltistan) Northern Pakistan, locally use *S. planifolia* for the treatment of stomach problems, vomiting, fever, diabetes and flu.

3.6.1 Preliminary Phytochemical Analysis of *S. planifolia*

Initial phytochemical screening of *S. planifolia* extracts showed the presence of alkaloids, flavonoids, phenols, saponins, tannins, amino acids in all the six SPM, SPE, SPC, SPA, SPH and SPQ extracts. Alkaloids, flavonoids and phenols were present in all SP extracts however these were more concentrated in SPM and SPE extracts. Glycosides were present in SPE, SPA, SPQ while amino acids were missing in SPE and glycosides were absent in SPM, SPC and SPH (Table 29). Our study showed that SP extracts revealed the existence of phytochemicals alkaloids, flavonoids, amino acids, phenols, glycosides tannins, steroids and saponins which is in harmony with the findings of Javed et al. (2020) reporting detailed phytochemical analysis of aerial parts of *S. alba.*

Table 29: Preliminary Phytochemical Analysis of *S. planifolia* Extracts.

Plant Extracts	Alkaloids	Flavonoids	Phenols	Carbohydrates	Steroids	Glycosides	Saponins	Tannins	Amino acids
SPM	++	+++	+++	+++	+	-	-	+	+
SPE	++	++	++	++	-	+	+	+	-
SPC	+	+	+	+	+	-	++	++	+
SPA	+	+	+	-	-	+	-	+	++
SPH	+	+	+	++	-	-	+	-	+
SPQ	+	+	+	+	++	++	+	+	+

+++: Strongly present; ++: Moderately present; + :Weakly present; -: Absent. SPM: *S. planifolia* methanol, SPE: *S. planifolia* ethanol, SPC: *S. planifolia* chloroform, SPA: *S. planifolia* ethyl acetate, SPH: *S. planifolia* n-hexane, SPQ: *S. planifolia* aqueous extract.

3.6.2 Quantitative Phytochemical Analysis of *S. planifolia* Extracts

Quantitative phytochemical examination of *S. planifolia* has proposed the presence of phenolic and flavonoid contents in good amount that can be of used in the treatment of several diseases. Total phenolic contents in various extracts of SP were evaluated and summarized in Figure 56. The data obtained from present finding of total phenolic of SP extracts showed following order SPM>SPE>SPC>SPA>SPQ>SPH. SPM has highest phenolic contents (65.96 mg GAE/g) and least in SHH (45.80 mg GAE/g). Similar results were shown in a report on phenolic contents of *S. alba* leaves (Piatczak et al., 2020)

The result of total flavonoid of *S. planifolia* extracts is mentioned in Figure 57. SPM has highest flavonoid contents (76.12mg QE/g), followed by SPE (70.15mg QE/g), SPC (63.76mg QE/g), SPA (61.03mg QE/g), SPQ (55.76mg QE/g) and least in SPH extract (46.05mg QE/g). Other members of *Salix* such as *S. babylonica* also possess good amount of flavonoids (Chen, H. et al., 2018). Present findings are in agreement with the previous studies of Keyue (2008) who reported that *S. babylonica* methanol extract contain highest flavonoid contents and had strong antioxidant capacity.

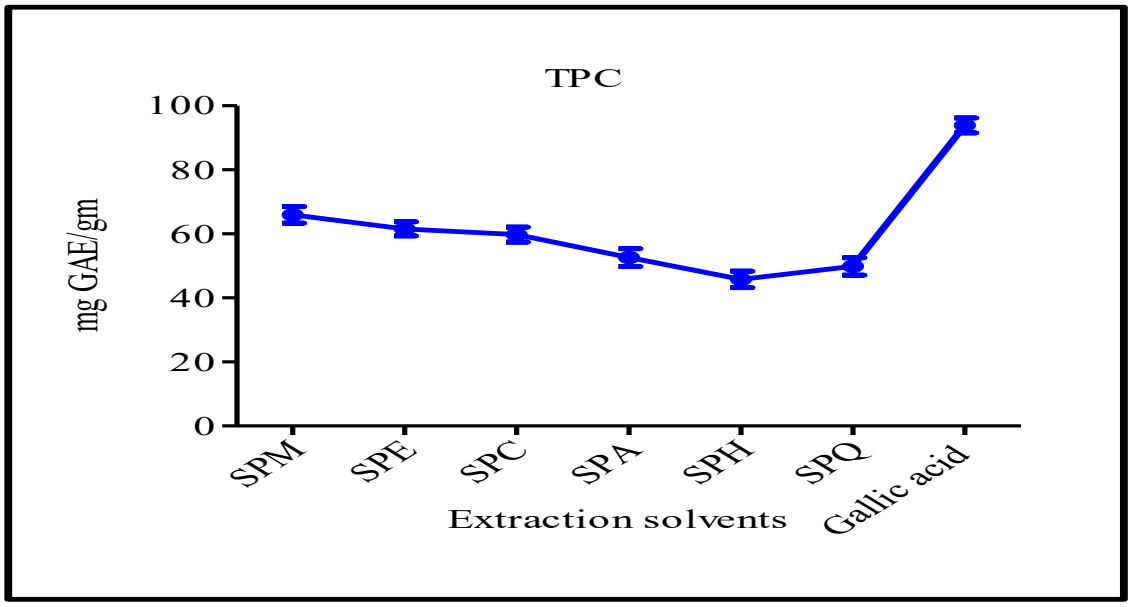

Figure 56: Total phenolic contents of *S. planifolia*. SPM: *S. planifolia* methanol, SPE: *S. planifolia* ethanol, SPC: *S. planifolia* chloroform, SPA: *S. planifolia* ethyl acetate, SPH: *S. planifolia* n-hexane, SPQ: *S. planifolia* aqueous extract.

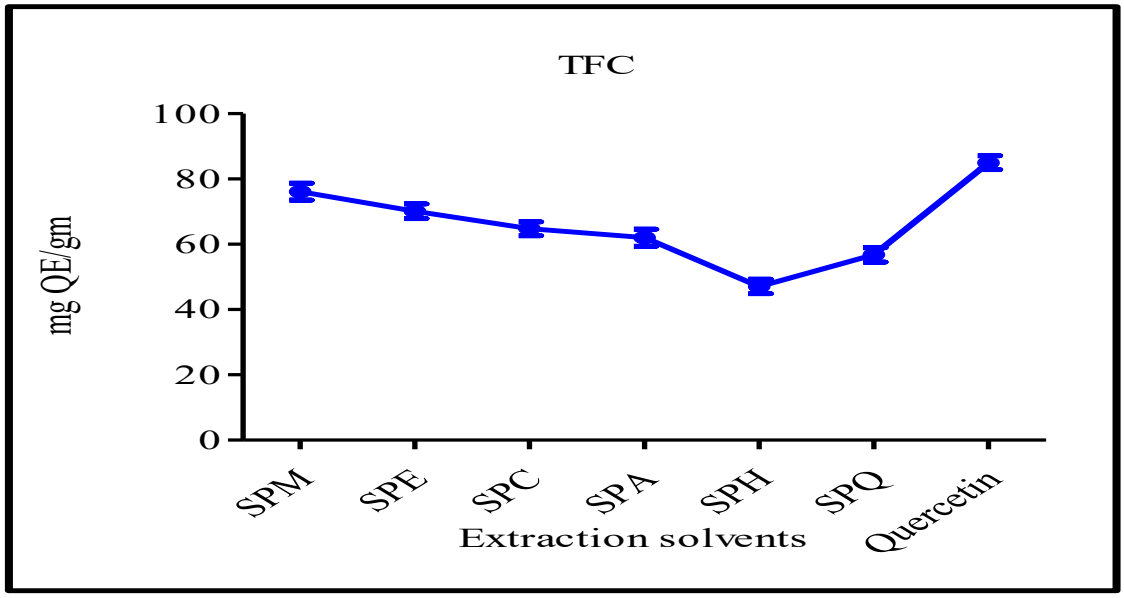

Figure 57: Total flavonoid contents of *S. planifolia*. SPM: *S. planifolia* methanol, SPE: *S. planifolia* ethanol, SPC: *S. planifolia* chloroform, SPA: *S. planifolia* ethyl acetate, SPH: *S. planifolia* n-hexane, SPQ: *S. planifolia* aqueous extract.

3.6.3 FTIR Spectral Analysis (cm^{-1}) of *S. planifolia*

FTIR analysis shows the presence of numerous functional groups with strong and medium peak intensities. At first sight, C-H stretch was observed in the sample indicating the presence of alkanes. Some strong and medium peaks were observed which indicated the existence of N-H stretch, N-H bend, C-C, C-N and C-Br stretch and hence confirmed the presence of aromatics, carboxylic acids, aliphatic amines and alkyl halides. The availability of these different functional groups showed that these biomolecules play significant role in the biological potentials of SP-mediated extracts.

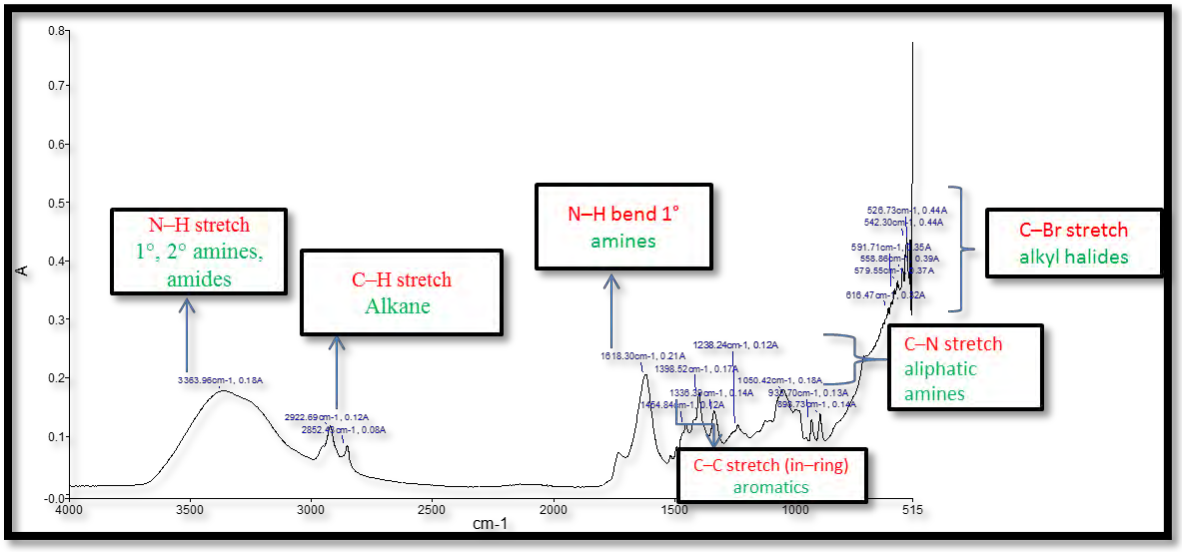

Figure 58: FTIR Spectral Analysis (cm-1) of *S. planifolia*.

3.6.4 Antioxidant Potential of *S. planifolia* Extracts

IC_{50} values for DPPH radical scavenging aassay of *S. planifolia* extracts have shown reasonable results. The noticed order of IC_{50} values for different extracts of *S. planifolia* was SPM < SPE < SPC < SPA < SPQ < SPH (Figure 59). IC_{50} values for SPM showed best results (65.5±1.5 µg/mL) that were followed by SPE (78.5±1.6 µg/mL), SPC (109.5±2.1 µg/mL), SPA (89.5±2.4 µg/mL), SPQ (108.6±2.1 µg/mL) and SPH (119.6±2.5 µg/mL). DPPH radical scavenging ability of given extracts exhibited correlation with TPC and TFC. Similar results were demonstrated by Kohler et al. (2020) in which authors reported the antioxidant potential of *S. purpurea* bark extract.

Total antioxidant capacity (TAC) of *S. planifolia* extracts is shown in Figure 60. Highest antioxidant capacity was given by SPM (72.33±1.56 mg AAE/g sample) followed by SPE (69.45±1.63 mg AAE/g sample), SPC (65.26±2.64 mg AAE/g sample), SPA (57.26±2.24 mg AAE/g sample), SPQ (51.87±2.44 mg AAE/g sample) and SPH (41.19±1.77 mg AAE/g sample) and was found to decrease in the order of SPM > SPE > SPC > SPA > SPQ > SPH. Our present finding were in line with the previous findings of El-Sayed et al. (2015) showing good antioxidant capacity of *S. mucronata*.

SPM showed the maximum reducing power with 60.61±1.43 mg AAE/g sample measured at 200 µg/mL of extract followed by SPE (59.50±1.87 mg AAE/g sample), SPC (53.77±1.67 mg AAE/g sample), SPA (46.45±1.59 mg AAE/g sample) SPQ (43.87±1.76 mg AAE/g sample) and SPH (35.10±1.94 mg AAE/g sample) as shown in Figure 60. The result of this assay was considerably correlated with TFC and TPC. This assay findings were following the pattern of SPM > SPE > SPC > SPA > SPQ > SPH at 200 µg/mL. Present study is in agreement with Nauman et al. (2020) describing the reducing power activity on different extracts of *S. aegyptiaca.*

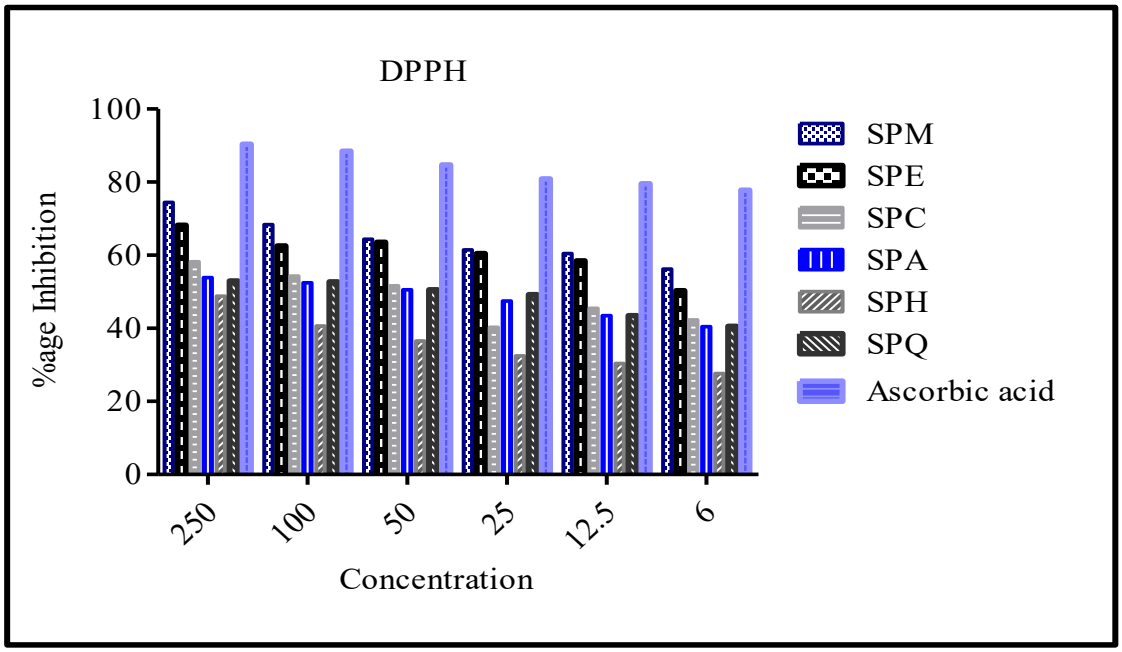

Figure 59: DPPH assay of different extracts of *S. planifolia*. SPM: *S. planifolia* methanol, SPE: *S. planifolia* ethanol, SPC: *S. planifolia* chloroform, SPA: *S. planifolia* ethyl acetate, SPH: *S. planifolia* n-hexane, SPQ: *S. planifolia* aqueous extract.

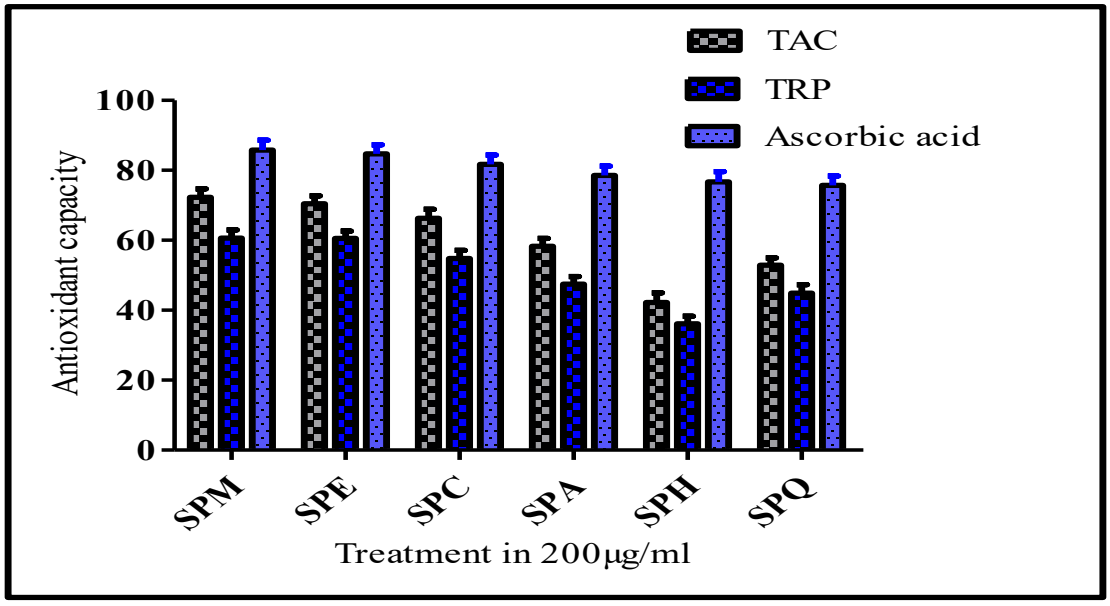

Figure 60: Total antioxidant capacity and total reducing power of different extracts of *S. planifolia*. SPM: *S. planifolia* methanol, SPE: *S. planifolia* ethanol, SPC: *S. planifolia* chloroform, SPA: *S. planifolia* ethyl acetate, SPH: *S. planifolia* n-hexane, SPQ: *S. planifolia* aqueous extract.

3.6.5 Antimicrobial activity *S. planifolia* Extracts

Antibacterial activity of *S. planifolia* extracts was evaluated and data is given in Table 30. SPM has good anti-bacterial capacity beside all tested bacterial strains. The extreme sensitive bacterial strain proved to be gram positive strain *S. aureus* and *B. subtilus* displaying 22±1.43 mm and 19±2.4mm. *K. pneumonia* has shown least activity that exhibits the ZOI 5±1.43 mm. SPE inhibited the growth of all tested bacterial strains, presenting zone 14±1.65mm against *B. subtilus* and least against *E. coli* 3±1.32 mm. SPC revealed antibacterial activity against four tested bacterial strains excluding *P. aeruginosa.* Our finding is in accordance with the report of antibacterial activities of different *Salix* species such as *S. babylonica* (Gonzalez-Alamilla et al., 2019).

Antifungal activity of *S. planifolia* extracts shows positive results (Table 31). SPM inhabited growth of all fungal strains and most susceptible fungal strain towards SPM was *A. niger* with ZOI 17±1.6 mm while the less susceptible strain was *F. solani* with ZOI 4±2.4 mm. SPE inhibited growth of four studied fungal strains, the more susceptible fungal strain was *A. flavus* 18±1.4 mm. In case of SPC the maximum ZOI was 17±2.4 mm against *A. flavus* and minimum ZOI was 2±1.5mm against *A. niger*. SPA extract was found to be active against three fungal strains, the utmost sensitive fungal strains was *C. albicans* (17±2.43mm). For SPH plant extract, it was sensitive against two fungal strains and insensitive against *C. albicans*, *F. solani*, *A. flavus*. Whereas SPQ gave positive results against three fungal stains and showed insensitivity against *M. racemosus* and *F. solani*. Earlier it was reported that other *Salix* species such *S. subserrata* also show strong antifungal activity (Hussain et al., 2011).

Table 30: Inhibition Zones and MIC of *S. planifolia* Extracts against Bacterial Strains.

Micro-organisms	***E. coli*** ATCC 33456		***P. aeruginosa*** ATCC 90271		***K. pneumonia*** ATCC 1705		***B. subtilus*** *ATCC* 19659		***S. aureus*** ATCC 6538	
Plant Extracts	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)
SPM	11±1.34	100	12± 2.4	100	5±1.43		19±2.4	33.33	22±1.4	11.11
SPE	3± 1.32		13± 2.44	100	10±2.2	100	14± 1.65	100	6± 1.14	
SPC	6±1.25		NI		3± 2.03		21±2.54	11.11	17±2.1	33.33
SPA	13± 1.36	100	4±1.67		NI		11± 2.29	100	16± 1.22	100
SPH	NI		3±2.44		NI		2±1.46		12±2.44	100
SPQ	NI		NI		10± 1.3	100	11±1.76	100	15±2.54	100

Values (mean ± standard deviation) was obtained through from triplicate analysis.NI no activity. SPM: *S. planifolia* methanol, SPE: *S. planifolia* ethanol, SPC: *S. planifolia* chloroform, SPA: *S. planifolia* ethyl acetate, SPH: *S. planifolia* n-hexane, SPQ: *S. planifolia* aqueous extract.

Table 31: Inhibition Zones and MIC of *S. planifolia* Extracts against Fungal Strains.

Fungus strains	***M. racemosus***		***C. albican***		***A. niger***		***F. solani***		***A. flavus***	
	FCBP 0300		FCBP 478		FCBP 0918		FCBP 0291		FCBP 0064	
Plant Extracts	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)
SPM	15±2.5	100	14±2.6	100	17±1.6	33.33	4±2.4		13±1.6	100
SPE	13±2.6	100	6±2.6		NI		14±2.5	100	18±1.4	33.33
SPC	3±2.6		10±1.6	100	2±1.5		NI		17±2.4	100
SPA	NI		17±2.4	33.33	10±2.7	100	NI		11±1.5	100
SPH	4±2.8		NI		9±1.34	100	NI		NI	
SPQ	NI		14±2.5	100	15±2.8	100	NI		10±2.3	100

Values (mean ± standard deviation) was obtained through from triplicate analysis. NI: no activity. SPM: *S. planifolia* methanol, SPE: *S. planifolia* ethanol, SPC: *S. planifolia* chloroform, SPA: *S. planifolia* ethyl acetate, SPH: *S. planifolia* n-hexane, SPQ *S. planifolia* aqueous extract.

The leishmania parasite has caused a wide range of diseases such as mucocutaneous, visceral and cutaneous leishmaniasis (Coelho et al., 2016; Shaddel et al., 2018). Due to the adverse side effects, cost, toxicity and resistance against available leishmaniasis drugs, achieving natural biologically active compounds with less toxicity, high efficacy and lesser side effects has gained much attention and this leads to more attention in medicinal plants (Hamid et al., 2019). Result showed that the noticeable inhibition in growth of *L. tropica* was found in case of all six extracts used in this study (Figure 61). All SP extracts had percentage inhibition greater than 50 and could be used as a source of anti-leishmanial drug. SPA shows highest %age inhibition with IC_{50} of 87±1.78 µg/mL, followed by SPC with IC_{50} 96±1.65 µg/mL, SPE with IC_{50} 107±1.71 µg/mL, SPM having IC_{50} 126±2.11 µg/mL and SPH having with IC_{50} 146±2.34 µg/mL, SPH with IC_{50} 156±1.65. The data showed that the compounds for potent anti-leishmanial activity were more concentrated in polar extracts. Present findings were similar with the scientific report mentioning that phytochemical extracted from *S. niga* revealed effective anti-leishmanial activity (Ahmed et al. 2018).

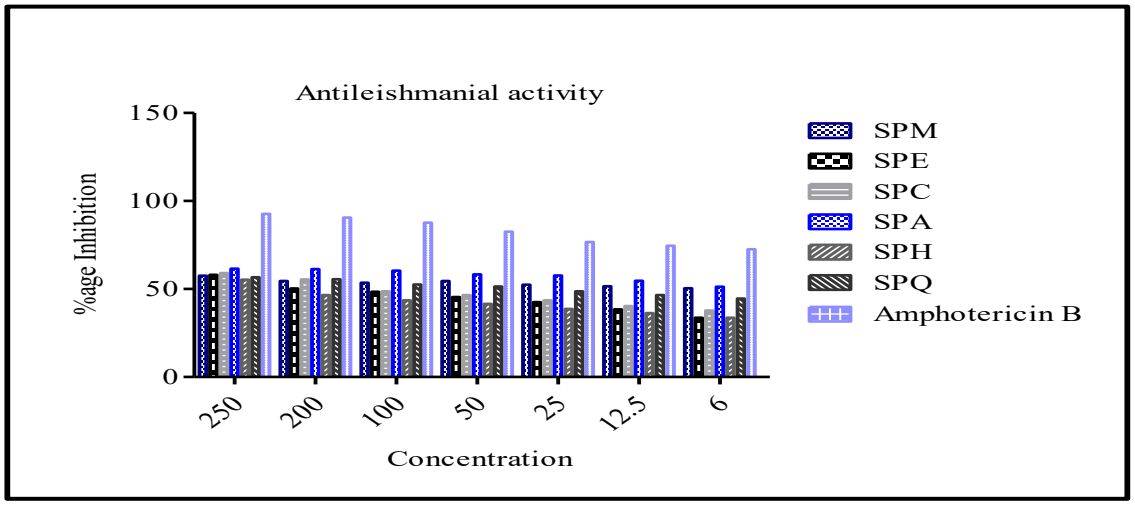

Figure 61: Anti-leishmanial assay of different extracts of *S. planifolia.* SPM: *S. planifolia methanol,* SPE: *S. planifolia ethanol, SPC: S. planifolia chloroform,* SPA: *S. planifolia ethyl acetate,* SPH: *S. planifolia n-hexane,* SPQ*: S. planifolia aqueous extract.*

3.6.6 Cytotoxicity Assessment and Anticancer Potential of *S. planifolia*

S. planifolia extracts was screened for plant cytotoxic potential, 65.7 % extracts displayed LD_{50} value under 60 µg/mL and were identified to be extremely cytotoxic where as 32.3% considered low cytotoxic. Cytotoxic results of SP extracts against larvae of *A. salina* were shown in Table 32. Among all tested extracts, SHH was found to be the best cytotoxic with LD_{50} 37.54±1.54 µg/mL demonstrating that in case of *S. planifolia* the non-polar solvents are highly effective in the extraction of cytotoxic compounds. Similar to *S. planifolia* other *Salix* members were also evaluated for cytotoxicity and present findings are in line with the previous study of Deniau et al. (2019), which reported the cytotoxic potential of *S. cortex.*

S. planifolia extracts were evaluated against inhibiting protein kinases (Table 32). Through plate analysis, three different kinds of measurements were noticed, clear zones, cloudy zones or no zones. The significant bald area zone with ZOI 21±2.7 mm and MIC 11.11 µg/mL was measured around SPA loaded disc. Different extracts like SPH (17±1.9 mm), SPC (15±2.3 mm) and SPE (11±2.4 mm). SPM gives no PKI activity and SPQ results in clear zone with ZOI 13±1.3mm. Our findings are in settlement with the finding of Khan et al. (2018) in which researchers checked PKI activity for *F. ananassa* leaves extracts.

Prostate cancer frequency and death rates fluctuate worldwide (Gillessen et al., 2020). The cytotoxic potency of the SPA extract against prostrate cell line (PC3) was demonstrated using MTT assay. The result of SPA extract has confirmed reduction in metabolic activity of PC3 cells (Figure 62). The reduced metabolic activity of SPA extract might have anticancer potential as reported for *S. safsaf* (Aboul-Soud et al., 2020)

Table 32: Brine Shrimp Lethality and Protein Kinase Inhibition Assay.

Samples	Brine shrimp lethality (µg/mL)		Protein kinase inhibition (µg/mL)		
	% Mortality	LD_{50} (µg/mL)	Diameter (mm) at 100 µg/disc		MIC
	250		Clear zone	Bald zone	
SPM	56.43±1.23	59.3±2.54	----	----	----
SPE	69.56±2.35	53.2±1.58	----	11±2.4mm	100
SPC	76.32±1.85	39.54±2.43	----	15±2.3mm	100
SPH	79.54±2.36	37.65±1.77		17±1.9mm	33.33
SPA	86.54±1.56	31.54±1.54	----	21±2.7mm	11.11
SPQ	52.65±1.13	60±2.54	13±1.3mm	----	----

Values (mean ± standard deviation) was obtained through from triplicate analysis. ----: no activity, SPM: *S. planifolia* methanol, SPE: *S. planifolia* ethanol, SPC: *S. planifolia* chloroform, SPA: *S. planifolia* ethyl acetate, SPH: *S. planifolia* n-hexane, SPQ: *S. planifolia* aqueous extract.

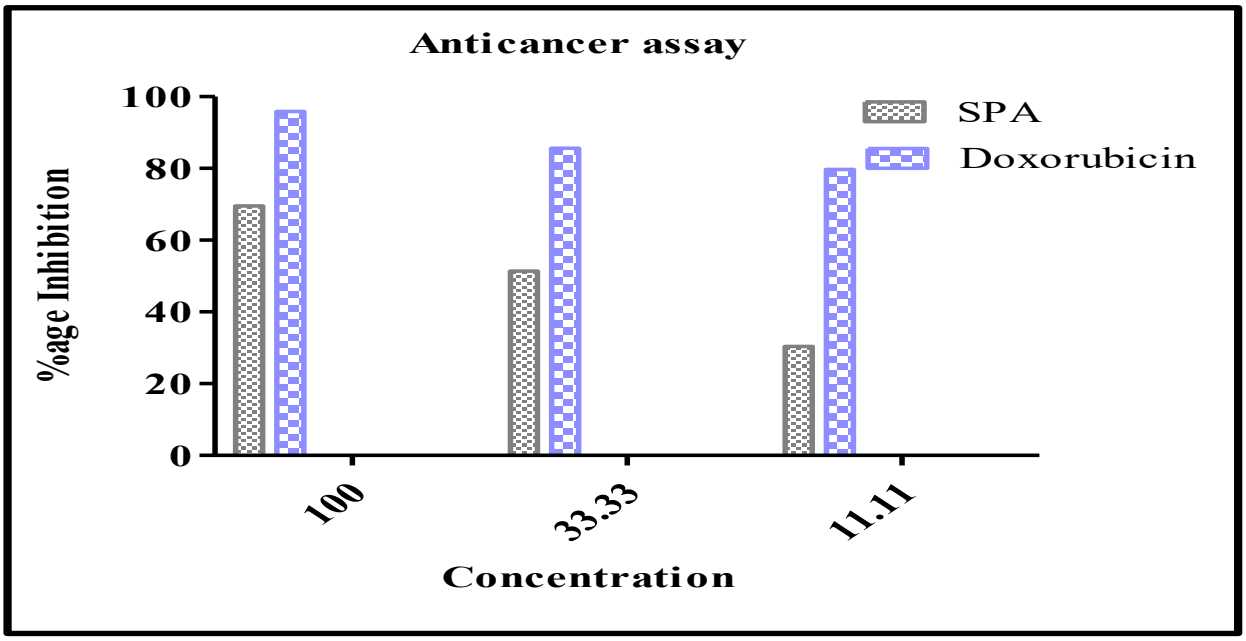

Figure 62: Anticancer activity of *S. planifolia* extract against PC-3 Cells.

3.6.7 Antidiabetic Potential of *S. planifolia* Extract

In order to analyze antidiabetic effect of *S. planifolia* extracts alpha-amylase Inhibition (AAI) assay was used. The results are given in the Table 33. SPH shows the good activity as compared to other SP extracts, with the value of 74.07 ±2.54 % alpha-amylase inhibition. Different extracts like SPE, SPC, SPQ, SPM and SPA have shown alpha-amylase inhibition of 73.10±1.65 %, 42.24±1.98 %, 39.86±2.86 %, 37.30±2.43 % and 35.10±1.54 % respectively. It means that bioactive components responsible for antidiabetic potential are concentrated in the SPH extract. Present findings are in line with the previous outcome of Bais and Choudhary (2017) in which antidiabetic potential of *S. alba* was reported.

Table 33: Antidiabetic Potential of *S. planifolia* Extracts and Respective IC_{50} values.

Plant Extracts	%age inhibition of alpha-amylase	IC_{50} µg/mL
SPM	37.30 ±2.43	-----
SPE	73.10 ±1.65	34.43
SPC	42.24 ±1.98	-----
SPA	35.10 ±1.54	-----
SPH	74.07 ±2.54	32.25
SPQ	39.86 ±2.86	-----

Values (mean ± standard deviation) was obtained through from triplicate analysis. ---- :no activity, SPM: *S. planifolia* methanol, SPE: *S. planifolia* ethanol, SPC: *S. planifolia* chloroform, SPA: *S. planifolia* ethyl acetate, SPH: *S. planifolia* n-hexane, SPQ: *S. planifolia* aqueous extract.

SECTION 7: *Saxifraga flagellaris* Willd.

3.7 *Saxifraga flagellaris* Willd.

Saxifraga flagellaris Willd. (SF) belongs to family Saxifragaceae and local name is ~~–t~~umburuk" (Figure 63). Mostly grows in moist places on gravel or in moss carpets. The whole plant is more or less red, stems are single, erect and leafy, usually has one terminal flower, with golden yellow petals. SF widely distributed in Pakistan (Deosai), US, Canada, Russian Federation, Norway, India, China and Greenland (https://www.gbif.org/occurrence/h?q=Saxifraga%20flagellaris%20Willd). According to the previous literature, variety of *Saxifraga* species has been investigated for their antimicrobial and anti-inflammatory potential and promising results were obtained. According to ethnobotanical survey in the local community, this plant is used for the treatment of renal calculi and as a general body tonic. *S. flagellaris* decoction is used to relieve backache. Previously, promising anti-bacterial and anti-fungal activities of *S. flagellaris* have been reported (Rehman et al., 2019).

Figure 63: Field Photograph of *Saxifraga flagellaris* Willd.

3.7.1 Preliminary Phytochemical Analysis of *S. flagellaris* Extracts

Phytochemical screening gives the necessary information about the secondary metabolites diversity present in different plant extracts (Gibbons, 2004; Patil, 2009). Qualitative analysis confirmed the presence of flavonoids, alkaloids, tannins, steroids, phenol, saponins, in all *S. flagellaris* extracts except in case of SFH saponins, amino acids and tannins were missing. Whereas quinones were absent in chloroform extract and tannins were absents in n-hexane extract (Table 34). SF extracts revealed the existence of phytochemicals such as alkaloids, flavonoids, amino acids, phenols, glycosides tannins, steroids and saponins which is in harmony with the recorded data of Zhang et al. (2018) in which authors described the preliminary phytochemical analysis of *S.luoxiaensis* extracts.

Table 34: Preliminary Phytochemical Analysis of *S. flagellaris* Extracts.

Plant Extracts	Alkaloids	Flavonoids	Phenols	Carbohydrates	Steroids	Glycosides	Saponins	Tannins	Amino acids
SFM	+	+++	+++	+	+	+	++	+	+++
SFE	++	++	++	+	+	++	+	+	++
SFC	+	++	+++	+	+	+	+	+	+++
SFA	+	++	+	++	+	++	++	+	
SFH	++	+	+	++	++	+	-	-	-
SFQ	++	++	+	+	++	++	+++	++	++

+++ Strongly present; ++ Moderately present; + Weakly present; -: Absent. *S. flagellaris* different extracts. SFM: *S. flagellaris* methanol, SFE: *S. flagellaris* ethanol, SFC: *S. flagellaris* chloroform, SFA: *S.* n *flagellaris* ethyl acetate, SFH: *S. flagellaris* n-hexane and SFQ : *S. flagellaris* aqueous extract.

3.7.2 Quantitative Phytochemical Analysis of *S. flagellaris*

Total phenolic contents of *S. flagellaris* showed following order SFM>SFE> SFA>SFC>SFH>SFQ order. The SFM possessed highest quantity of total phenolic compounds 94.76 mg GAE/g followed by SFE (88.89 mg GAE/g), SFA (81.33 mg GAE/g), SFC (70.14 mg GAE/g), SFH (50.14 mg GAE/g) while the lowest amount of total phenolic contents (35.51 mg GAE/g) was observed in SFQ extract as shown in Figure 64. Present findings are in harmony with Howlader et al. (2016) that methanol act as a best solvent for extraction of phenolic contents.

Total flavinoid contents of studied plant extracts was determined by aluminium chloride chlorimetric method. Aluminium chloride react with hydroxyl group of flavonoids of tested extract and gives results in formation of yellow coloured in solution (Upadhyaya et al., 2019). The *S. flagellaris* different extracts contained reasonable amount of flavonoids content. The SFM had the highest concentration of flavonoids content (85.69 mg QE/g) followed by SFE (83.69 mg QE/g) and SFA (76.37 mg QE/g), SFC (71.37 mg QE/g) and SFH (66.37 mg QE/g) whereas lowest concentration of total flavonoids was observed in the SFQ (59.21 mg QE/g) (Figure 65). Present findings are in agreement with the previous studies of Youn et al. (2018), indicating that *S. stolonifera* extracts have remarkable flavonoid contents.

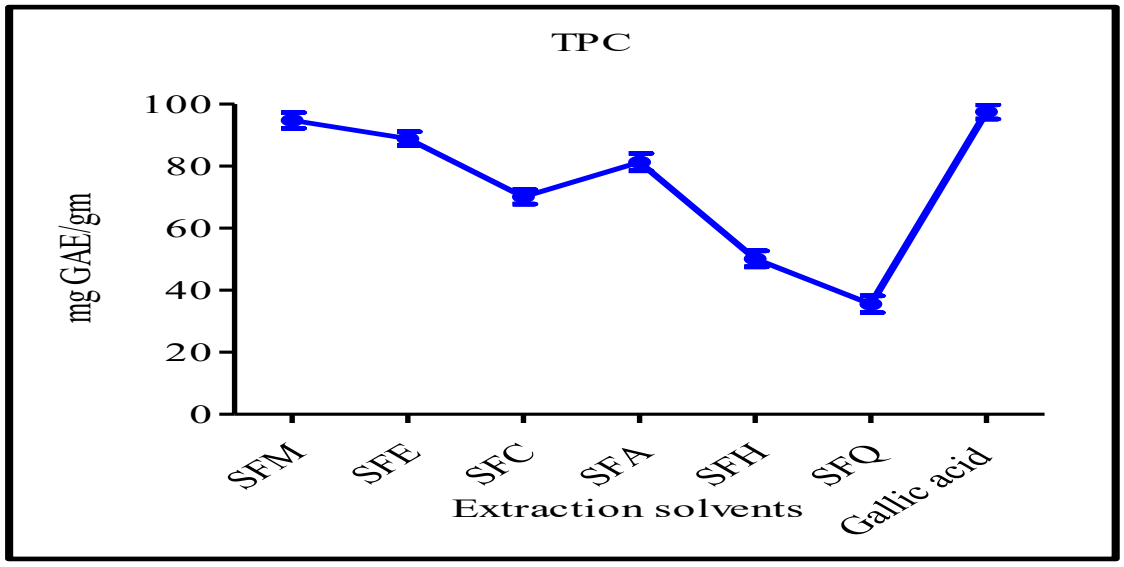

Figure 64: Total phenolic contents of *S. flagellaris* different extracts. SFM: *S. flagellaris* methanol; SFE: *S. flagellaris* ethanol; SFC: *S. flagellaris* chloroform; SFA: *S. flagellaris* ethyl acetate; SFH: *S. flagellaris* n-hexane and SFQ: *S. flagellaris* aqueous extract.

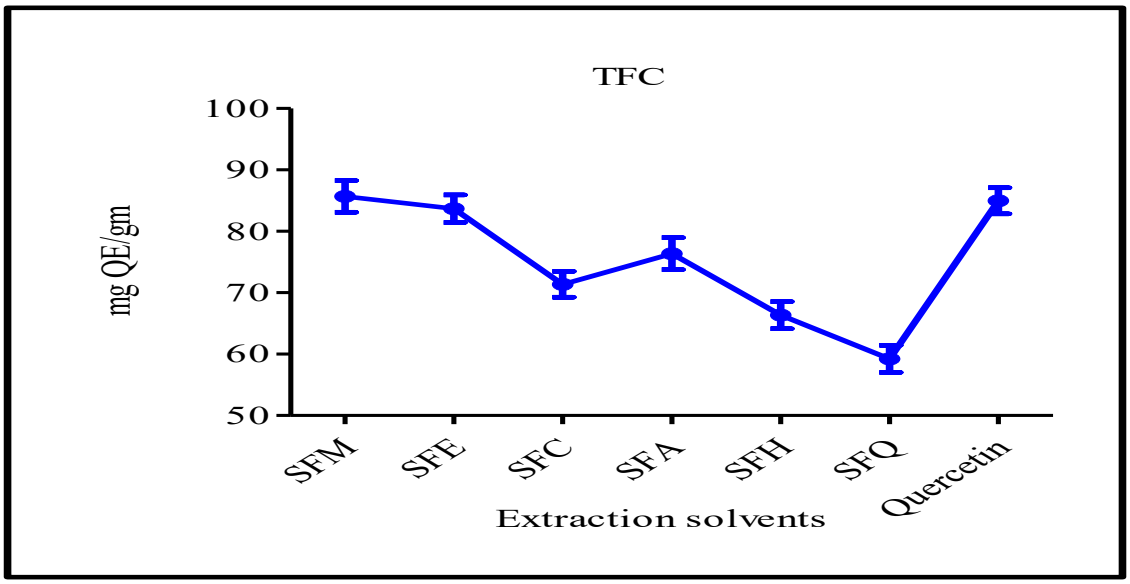

Figure 65: Total flavonoid contents of *S. flagellaris* different extracts. SFM: *S. flagellaris* methanol; SFE *S. flagellaris* ethanol; SFC: *S. flagellaris* chloroform; SFA: *S. flagellaris* ethyl acetate; SFH: *S. flagellaris* n-hexane and SFQ: *S. flagellaris* aqueous extract.

3.7.3 FTIR Spectral Analysis (cm^{-1}) of *S. flagellaris*

FTIR spectroscopy probes the vibrational properties of numerous functional groups Specific strong and medium peaks were observed which indicated the existence of C-H stretch, N-H stretch, N-H bend, C-C, C-N, C-Br and C-Cl stretch and hence confirmed the presence of aromatics, carboxylic acids, aliphatic amines and alkyl halides. FTIR spectra of *S. flagellaris* is shown in Figure 66. The availability of these different functional groups showed that these biomolecules play significant role in the biological potentials of SF-mediated extracts.

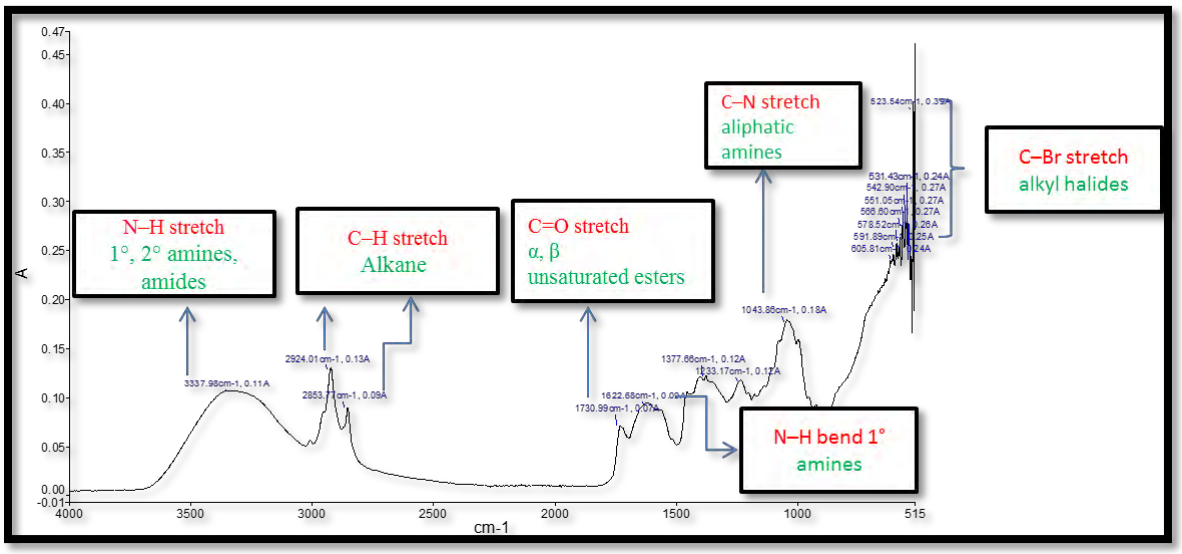

Figure 66: FTIR Spectral Analysis (cm-1) of *S. flagellaris*.

3.7.4 Antioxidant Potential of *S. flagellaris* Extracts

Antioxidants have reducing ability to scavenge free radicals. Due to their therapeutic values, these compounds are gaining a great entrust of researchers and also pharmacologists (Szymanska et al., 2018; Nasseri et al., 2019). The result of DPPH percentage inhibition showed that SFM exhibited highest antioxidant activity (IC_{50} 47.6± 2.2 µg/mL) followed by SFE (IC_{50} 52.7±2.7 µg/mL), SKA (IC_{50} 58.1± 2.3µg/mL) SFC (IC_{50} 61.4±2.6 µg/mL) and SFH (IC_{50} 77.6±2.4 µg/mL). SFQ extract had the lowest scavenging effect (IC_{50} 84.4±2.8 µg/mL). The noticed order of SF IC_{50} values for different extracts was SFM < SFE < SFA < SFC < SFH< SFQ (Figure 67). All the extracts revealed higher values of IC_{50} as compared to ascorbic acid (30.57±1.6 µg/mL).

The IC_{50} values and free radical quenching activity have inverse relationship (Khan et al., 2012). Parallel findings were verified by Dang et al. (2015) in which researchers studied *S. tangutica* methanol extracts showing antioxidant activity

Total antioxidant capacity of different extracts of *S. flagellaris* illustrated that SFM has highest antioxidant activity (89.6±1.55 AAE/mg) followed by SFE (83.40±1.43 AAE/mg), SFA (77.57±1.81 AAE/mg), SFC (73.39±1.54 AAE/mg), SFH (75.61± 1.56 AAE/mg) and SFQ showed lowest antioxidant capacity (69.12± 1.65 AAE/mg) (Figure 68). Our findings are in agreement to a previous study of Liu et al. (2012) describing that *S. stolonifera* methanol extract exhibited antioxidant capacity.

In reducing power assay, antioxidants in plant extracts can reduce the ferric (Fe^{3+}) to ferrous (Fe^{2+}) ion by electron (Mondal et al., 2019). SFM showed the maximum reducing power with 83.65±1.43 mg AAE/g sample followed by SFE (73.54±1.74 mg AAE/g sample), SFA (71.82±1.38 mg AAE/g sample), SFC (68.05±2.37 mg AAE/g sample) SFH (67.43±2.98mg AAE/g sample) and SFQ (60.78±2.49 mg AAE/g sample) as shown in Figure 68. This assay findings have followed the pattern of SFM > SFE > SF > SFC > SFH > SFQ. Dang et al. (2015) reported the similar results regarding the reducing power antioxidant activity of *S. tangutica* methanol extract. These findings are closely matching with our data.

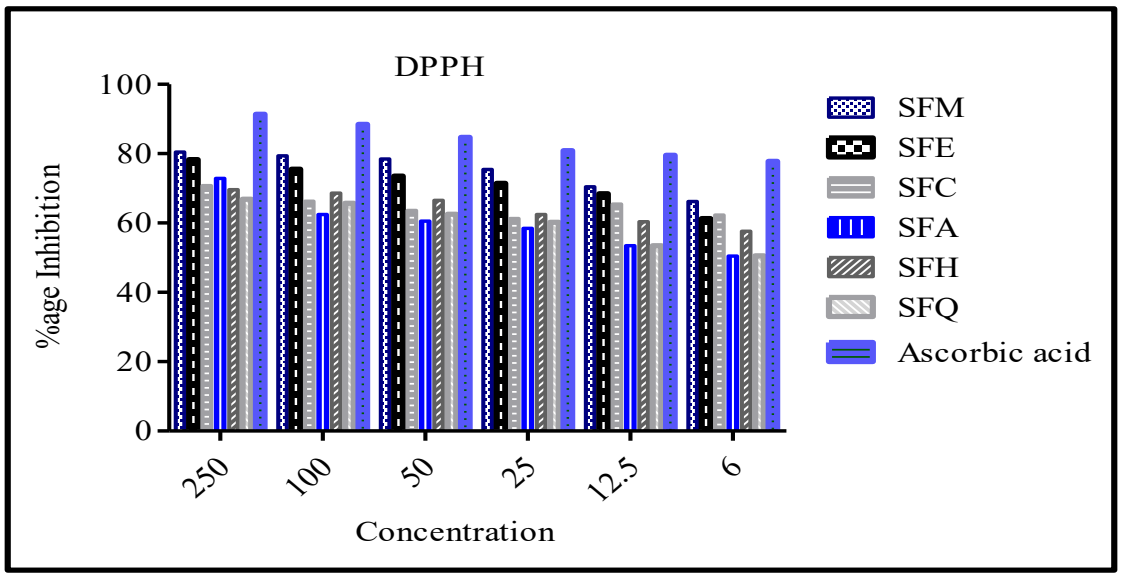

Figure 67: DPPH assay of different extracts of *S. flagellaris* different extracts. SFM: *S. flagellaris* methanol; SFE: *S. flagellaris* ethanol; SFC: *S. flagellaris* chloroform; SFA: *S. flagellaris* ethyl acetate; SFH: *S. flagellaris* n-hexane and SFQ: *S. flagellaris* aqueous extract.

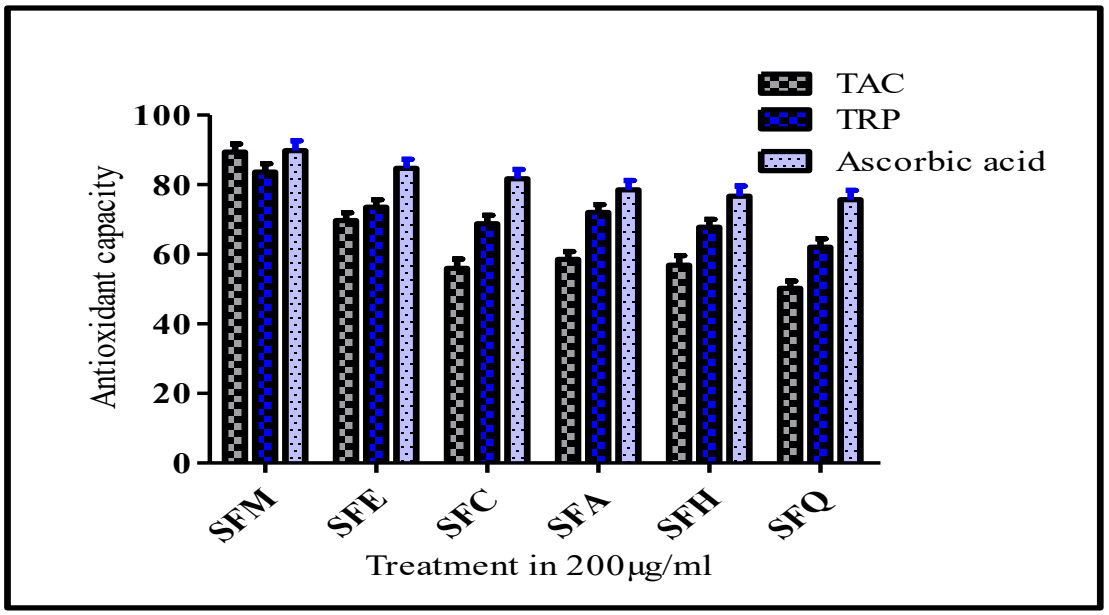

Figure 68: Total antioxidant capacity and total reducing power of different extracts of *S. flagellaris* different extracts. SFM: *S. flagellaris* methanol; SFE: *S. flagellaris* ethanol; SFC: *S. flagellaris* chloroform; SFA: *S. flagellaris* ethyl acetate; SFH: *S. flagellaris* n-hexane and SFQ: *S. flagellaris* aqueous extract.

3.7.5 Antimicrobial Activity of *S. flagellaris* Extracts

Qualitatively and quantitatively antibacterial activity of *S. flagellaris* was evaluated against five bacterial strains (Table 35). SFA showed antibacterial capacity against three studied bacterial strains namely *P. aeruginosa*, *E. coli* and *B subtilis*. SFQ inhibited growth of only one bacterial strain *K. pneumonia* (ZOI 13±1.24 mm) . Our results verified the findings of Rehman et al. (2019) in which authors studied the antibacterial potential of *S. flagellaris.*

S. flagellaris extracts showed reasonable antifungal property (Table 36). Plant extracts SFA, SFE and SFM showed significant ZOI whereas SFC, SFQ and SFH indicated moderate results. SFA inhabited growth of four fungal strains and most susceptible fungal strain was *M. racemosus* (ZOI 19±1.54 mm) while the less susceptible strain was *C. albicans* (ZOI 10±2.63 mm) and *A. niger* has given no activity. SFC plant extract showed minimum antifungal potential and inhibited *A. flavus* (ZOI of 13±2.6 mm). Like *S. flagellaris* the antifungal potential from other *Saxifraga* species such as *S. ligulata* have been previously reported (Barman et al., 2011).

Table 35: Inhibition Zones and MIC of *S. flagellaris* Extracts against Bacterial Strains.

Micro-organisms	***E. coli***		***P. aeruginosa***		***K. pneumonia***		***B. subtilus***		***S. aureus***	
	ATCC 33456		ATCC 90271		ATCC 1705		ATCC 19659		ATCC 6538	
Plant Extracts	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)
SFM	NI		NI		NI		NI		20± 1.44	11.11
SFE	7± 1.24		NI		NI		12± 1.8	100	NI	
SFC	NI		14± 1.44	100	12± 1.47	100	NI		4± 1.98	
SFA	17± 1.36	33.33	21± 1.85	11.11	NI		13± 1.9	100	NI	
SFH	18± 2.55	33.33	NI		NI		NI		13± 1.28	100
SFQ	NI		NI		13± 1.24	100	NI		NI	

Values (mean ± standard deviation) was obtained through from triplicate analysis. NI: no activity. SFM: *S. flagellaris* methanol, SFE: *S. flagellaris* ethanol, SFC: *S. flagellaris* chloroform, SFA: *S. flagellaris* ethyl acetate, SFH: *S. flagellaris* n-hexane and SFQ: *S. flagellaris* aqueous extract.

Table 36: Inhibition Zones and MIC of *S. flagellaris* Extracts against Fungal Strains.

Fungus strains	***M. racemosus*** FCBP 0300		***C. albicans*** FCBP 478		***A. niger*** FCBP 0918		***F. solani*** FCBP 0291		***A. flavus*** FCBP 0064	
Plant Extracts	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)
SFM	NI		NI		13±1.7	100	NI		19±1.8	33.33
SFE	13±2.4	100	NI		12±2.6	100	NI		8±2.6	
SFC	NI		NI		NI		NI		13±2.6	100
SFA	19±1.54	33.33	10± 2.63		NI		14±2.5	100	13±1.6	100
SFH	NI		7±2.8		NI		11±1.6	100	NI	
SFQ	NI		6±2.8		19±2.8	33.33	NI		NI	

Values (mean ± standard deviation) were obtained through from triplicate analysis. NI: no activity, SFM: *S. flagellaris* methanol, SFE: *S. flagellaris* ethanol, SFC: *S. flagellaris* chloroform, SFA: *S. flagellaris* ethyl acetate, SFH: *S. flagellaris* n-hexane and SFQ: *S. flagellaris* aqueous extract.

Anti-leishmanial activity of *S. flagellaris* extracts was evaluated against *L. tropica* Kwh_{23} among SF extracts, SFA showed highest IC_{50} value (17.39±1.89 µg/mL), followed by SFH (18.52±1.95 µg/mL), SFE (45.76±2.04 µg/mL) and SFM (54.01±1.33 µg/mL), SFQ (67.21±1.87µg/mL) while the lowest IC_{50} value was recorded for SFC (63.29±1.95 µg/mL) (Figure 69). These findings verified a recent study on comparative use of polar solvents for the isolation of compounds having anti-leishmanial potential (Al Nasr, 2020).

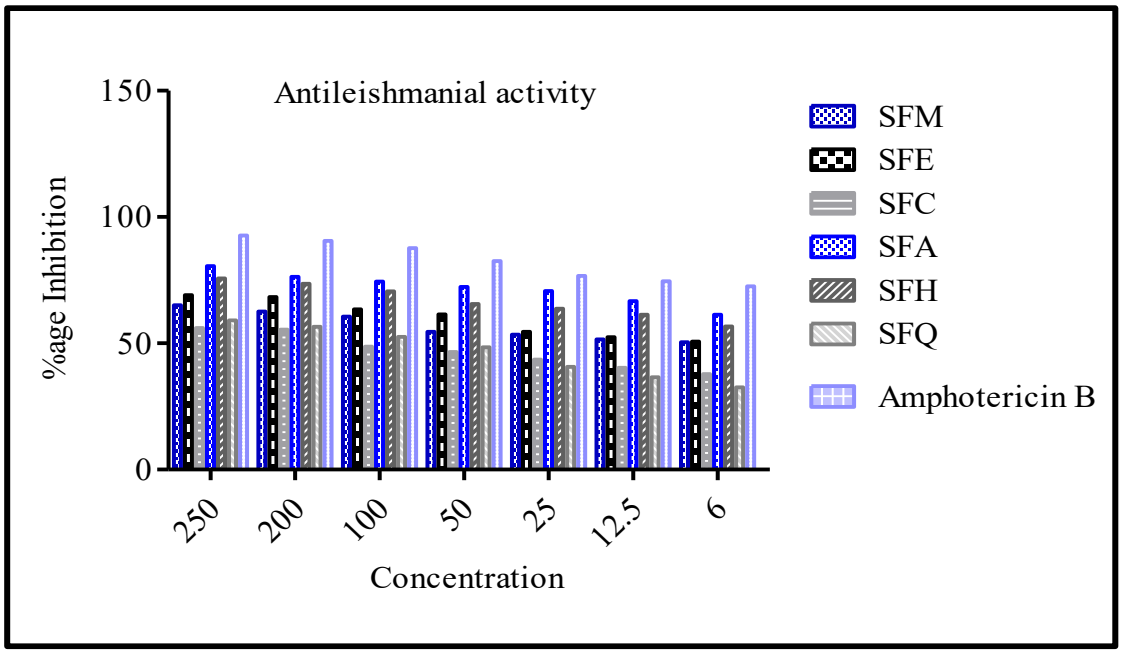

Figure 69: Anti-leishmanial assay of different extracts of *S. flagellaris* different extracts. SFM: *S. flagellaris* methanol; SFE: *S. flagellaris* ethanol; SFC: *S. flagellaris* chloroform; SFA: *S. flagellaris* ethyl acetate; SFH: *S. flagellaris* n-hexane and SFQ: *S. flagellaris* aqueous extract.

3.7.6 Cytotoxicity Assessment and Anticancer Potential of *S. flagellaris*

S. flagellaris extracts were screened for plant cytotoxic potential, 32 % extracts showed LD_{50} values under 60 µg/mL and were identified to be extremely cytotoxic where as 51 % considered as low cytotoxic while 18 % showed no cytotoxicity (Table 37). Among all tested extracts, SFH was found to be the best cytotoxic with LD_{50} 57.54±1.34 µg/mL demonstrating that in case of *S. flagellaris* the non-polar solvents are highly effective in the extraction of cytotoxic compounds as compared to polar solvents.

Many natural compounds form medicinal plants have been identified and used against PKI activity (Matias et al., 2016). The significant bald area zone with ZOI 16±2.1 mm and MIC 33.33 µg/mL was measured around SFH loaded discs, followed by SFE with bald zone 13±2.5 mm and SFQ with bald zone 11±1.5 mm, SFC with bald zone 10±2.1 mm. SFM and SFA have given no PKI activity. Present study is similar with reference to the anticancerous activity of other *Saxifraga* species reported by Khan et al. (2016) and Wang et al. (2018).

At present prostate carcinoma is the second fatal cancer for men (Culp et al., 2020). The cytotoxic potency of the SFH extract against prostrate cell line (PC3) was demonstrated using MTT assay. The result of SFH extract showed 85 % reduction in metabolic activity of PC3 cells at 100ug/mL conc. The reduced metabolic activity has shown that SFH extract might have anticancer potential (Figure 70). According to the best of our knowledge, no earlier research studies have been performed on the anticancer potential of SF mediated plant extracts

Table 37: Brine Shrimp Lethality and Protein Kinase Inhibition Assay.

Samples	Brine shrimp lethality (µg/mL)		Protein kinase inhibition (µg/mL)		
	% Mortality 250	LD_{50} (µg/mL)	Diameter (mm) at 100 µg/disc Clear zone	Bald zone	MIC
SFM	66.3±2.23	88.4±2.53	----	----	
SFE	73.7±1.32	97.3±1.18	----	13±2.5mm	100
SFC	25.6±1.84	207.43±2.28	----	10±2.1mm	100
SFA	79.1±2.12	56.65±2.43	----	----	
SFH	86.8±1.87	45.54±1.34	----	16±2.1mm	33.33
SFQ	67.8±2.23	94.15±1.13		11±1.5 mm	100

Values (mean ± standard deviation) was obtained through from triplicate analysis. ----: no activity. SFM: *S. flagellaris* methanol, SFE: *S. flagellaris* ethanol, SFC: *S. flagellaris* chloroform, SFA: *S. flagellaris* ethyl acetate, SFH: *S. flagellaris* n-hexane and SFQ: *S. flagellaris* aqueous extract.

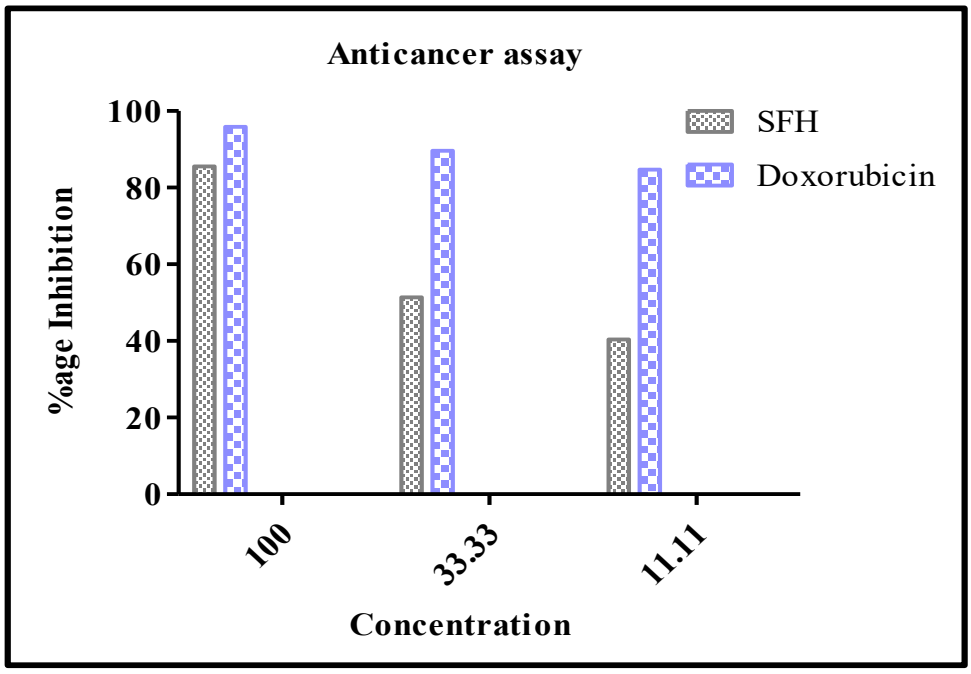

Figure 70: Anticancer activity of *S. flagellaris* extract against PC-3 Cells.

3.7.7 Antidiabetic Potential of *S. flagellaris* Extracts

Herbal drugs are receiving more importance for the treatment of diabetes, as these herbal drugs have no side effects (Justino et al., 2018). In the present study various extracts of *S. flagellaris* were evaluated for the assessment of antidiabetic potential (Table 38). SFH shows the good activity with the value of 77.34±2.65 %, followed by SFA, SFE, SFM, SFC and SFQ 76.20±2.43 %, 75.24±1.87 %, 74.20±1.43 %, 74.14±1.54 % and 73.64±2.85% alpha-amylase inhibition. Present findings were in line with previous literature based on antidiabetic potential of different species of *Saxifraga* such as *S. ligulata* (Goswami et al., 2013).

Table 38: Antidiabetic Potential of *S. flagellaris* Extracts and Respective IC_{50} Values.

Plant Extracts	%age inhibition of alpha-amylase	IC_{50} µg/mL
SFM	74.20 ±1.43	32.35
SFE	75.24 ±1.87	22.36
SFC	74.14 ±1.54	36.54
SFA	75.20 ±2.43	27.64
SFH	77.34 ±2.65	44.54
SFQ	73.64 ±2.85	26.65

SFM: *S. flagellaris* methanol, SFE: *S. flagellaris* ethanol, SFC: *S. flagellaris* chloroform, SFA: *S. flagellaris* ethyl acetate, SFH: *S. flagellaris* n-hexane and SFQ: *S. flagellaris* aqueous extract

SECTION 8 : *Sophora alopecuroides* L.

3.8 *Sophora alopecuroides* L.

Sophora alopecuroides L. Genus Sophora belongs to family Leguminosae (Figure 71), comprise of more than 70 plant species and mostly found in tropical to temperate regions (Zhou et al., 2010). The plant is rhizomatous undershrub with length of 50-100cm branched stems, dark green colored and the color of its flowers is cream and its flowering time is frequently between end of June- August. This wild edible plant has cream to light yellowish slightly round-shape seeds. (http://www.efloras.org /florataxon.aspx?flora_id=5&taxonid=242349639). *S. alopecuroides* the perennial undershrub commonly known as Kudouzi. Mostly distributed in Central and Western Asia such as Iran, China, Japan, Mongolia and Pakistan (Wang et al., 2012). Ethnomedicinaly the whole plant and its seeds are used as antibacterial, analgesic, antirheumatic agents and also used to treat gastrointestinal disorders, fever and also used to lessen the effects of heat in body and drying dampness. Recent therapeutic studies shown that *S. alopecuroides* also possesses anti-inflammatory, antitumor, cardioprotective, analgesic, antiviral, antiarrhythmic, analgesic, antioxidant and neuroprotective activities (Wang et al., 2020)

Figure 71: Field Photograph of *Sophora alopecuroides* L.

3.8.1 Scanning Electron Microscopy of *S. alopecuroides* Seeds

Seeds micro-morphological characters along with seed size and seed shape, were the diagnostic outfits for the proper identification of different plant species (Luqman et al., 2019). Seed color was cream to light yellow, with length and width of 3.76–3.85 mm and 3.36–3.54 mm When visualized by Scanning Electron Microscope the seeds of *S. alopecuroides* were seen to be ovate oblong (round shape) reticulate surface pattern with glabrous projection, random cell outline, cell arrangement was irregular, depressed and course periclinal wall surface as shown in Figure 72.

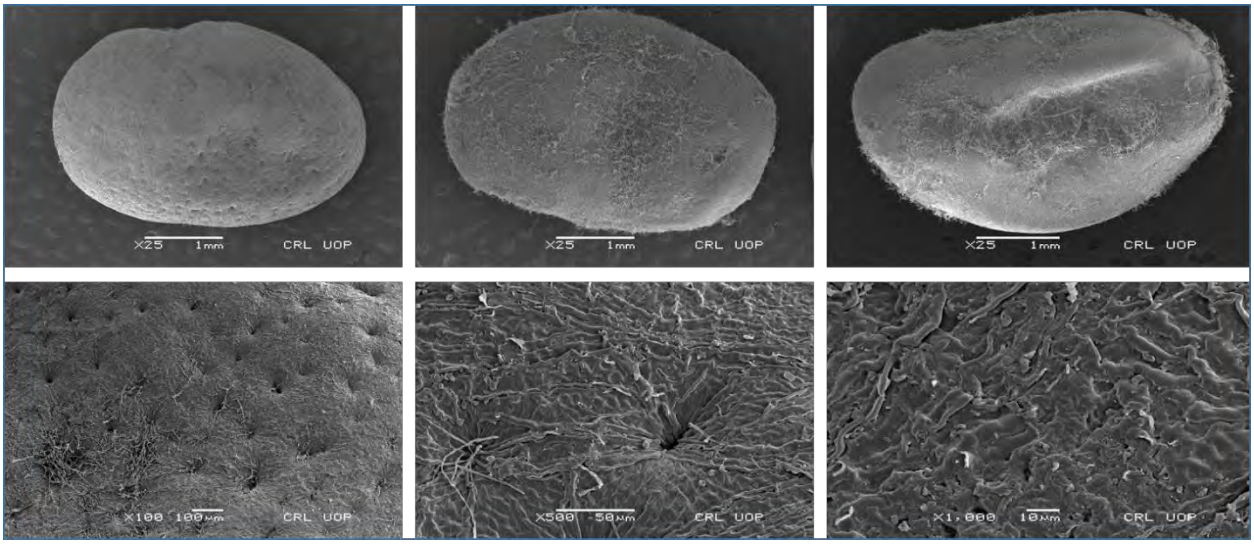

Figure 72: Scanning Electron Microscopy of the *S. alopecuroides* L Seeds.

3.8.2 Preliminary Phytochemical Screening

The result of the phytochemical screening of the crude seed extracts of *S. alopecuroides* revealed the presence or absence of phenolic compounds, saponins, steroids, alkaloid and tannins. Early detection demonstrated variation in the presence of these compounds (+++ strongly present; ++ moderately present; + weakly present) based on solvent used for the extraction. Table 39 revealed that methanol and ethanol extracts showed strongly presence of flavonoids, glycosides, phenols, steroids and saponins as compared to the terpenoids and alkaloids. While tannins and amino acids were observed in all extracts of *S. alopecuroides*. Phenolics, tannins, terpenoids, steroids and saponins present in this plant explained wide range uses of its species in traditional medicines as

these phytochemicals are famous for their medicinal and pharmacological potential (Toma et al., 2019). Our findings were in good harmony with the findings of (Moghaddam et al., 2019) provide detail phytochemical analysis of *S. alopecuroides* and confirmed the existence of phenol, flavonoids, steroids and glycosides in different extracts of *S. alopecuroides.*

Table 39: Preliminary Phytochemical Analysis of *S. alopecuroides* Seeds extracts.

Plant Extracts	Alkaloids	Flavonoids	Phenols	Terpenoids	Steroids	Glycosides	Saponins	Tannins	Amino acids
SASM	+	+++	+++	+	+++	+++	+++	+++	+
SASE	++	+++	++	++	-	+	+	+	+
SASC	++	+++	+++	+++	-	-	++	++	+
SASEA	++	++	++	-	+	+	++	+	++
SASH	-	+	+	++	-	-	+	+	+
SASAq	-	++	++	+	++	++	-	+	+

+++: Strongly present; ++: Moderately present; +: Weakly present; -: Absent. SASM: *S. alopecuroides* seed methanol, SASE: *S. alopecuroides* seed ethanol, SASC: *S. alopecuroides* seed chloroform, SASEA: *S. alopecuroides* seed ethyl acetate, SASH: *S. alopecuroides* seed n-hexane, SASAq: *S. alopecuroides* seed aqueous extracts.

3.8.3 Quantitative Phytochemical Analysis of Seed Extracts

Pharmacological potential of any plant depends on composition of secondary metabolites and phenolic compounds which were actively involved in maintaining enzymes responsible for detoxification (Baidez et al., 2007). The total phenolic contents of the extract was determined from Gallic acid calibration curve. Methanol seeds extract of *S. alopecuroides* showed highest TPC value of 93.76±2.71 GAE/g. TPC values showed decreasing trend as polarity of the solvent is decreasing and maximum results are shown in methanol, ethanol and water respectively (Figure 73). Similar results were show by (Erdenechimeg et al., 2017) the *S. alopecuroides* seed extracts shows the good amount of phenolic contents. These result indicated notable quantity of phenolic compounds in polar plant extract. Different research studies concluded that TPC values are influenced by the use of different solvents accordingly (Uddin et al., 2018). Flavonoids are polyphenolic molecules known for their antioxidant and anti-inflammatory health benefits. The flavonoids provide protection to the living systems by stabilizing the free radicals (Karmakar et al., 2019). The content of flavonoid was estimated from the QT standard curve and results were stated as QE/mg (Figure 74). The methanol seeds extract of *S. alopecuroides* showed maximum amount of flavonoid contents followed by ethanol and aqueous extract. Similar to our findings other members of genus *Sophora* also possess good amount of flavonoids (Erdenechimeg et al., 2017). On the basis of solvent polarity, the range of total flavonoid contents in various plant extracts varies (Ahmad et al. 2016; Zohra et al., 2019).

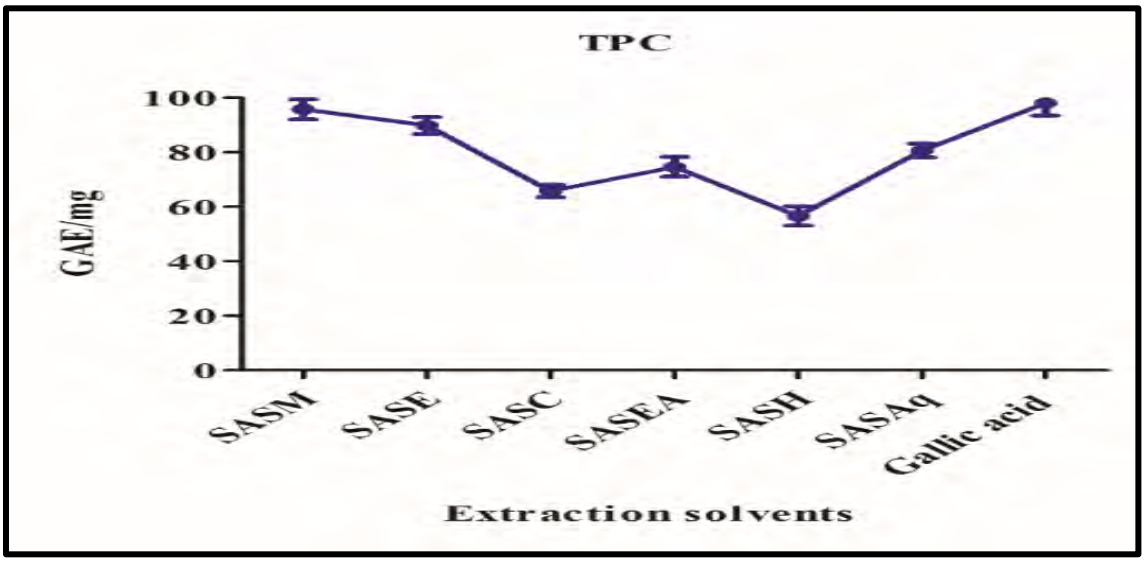

Figure 73: Total phenolic contents of *S. alopecuroides* seeds different extracts.Values (mean ± standard deviation) was obtained through triplicate analysis. SASM: *S. alopecuroides* seed methanol; SASE: *S. alopecuroides* seed ethanol; SASC: *S. alopecuroides* seed chloro chloroform, SASEA: *S. alopecuroides* seed ethyl acetate, SASH: *S. alopecuroides* seed n-hexane, SASAq: *S. alopecuroides* seed aqueous extracts.

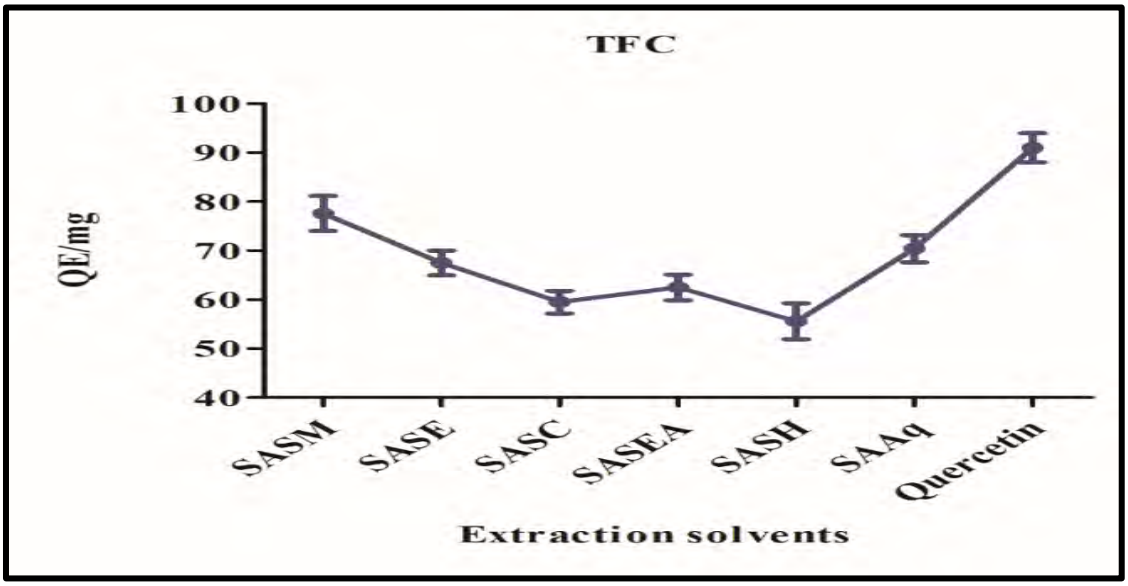

Figure 74: Total flavonoid contents of *S. alopecuroides* seeds different extracts.Values (mean ± standard deviation) was obtained through triplicate analysis. SASM: *S. alopecuroides* seed methanol; SASE: *S. alopecuroides* seed ethanol; SASC: *S. alopecuroides* seed chloro chloroform; SASEA: *S. alopecuroides* seed ethyl acetate; SASH: *S. alopecuroides* seed n-hexane and SASAq: *S. alopecuroides* seed aqueous extracts.

3.8.4 FTIR Spectral Analysis (cm^{-1}) of *S. alopecuroides*

FTIR analysis shows the presence of numerous functional groups with strong and medium peak intensities. At first sight, C-H stretch was observed in the sample indicating the presence of alkanes. Some strong and medium peaks were observed which indicated the existence of N-H stretch, N-H bend, C-C, C-N, C-Br and C-Cl stretch and hence confirmed the presence of aromatics, carboxylic acids, aliphatic amines and alkyl halides (Figure 75). The availability of these different functional groups showed that these biomolecules play significant role in the biological potentials of SA-mediated extracts.

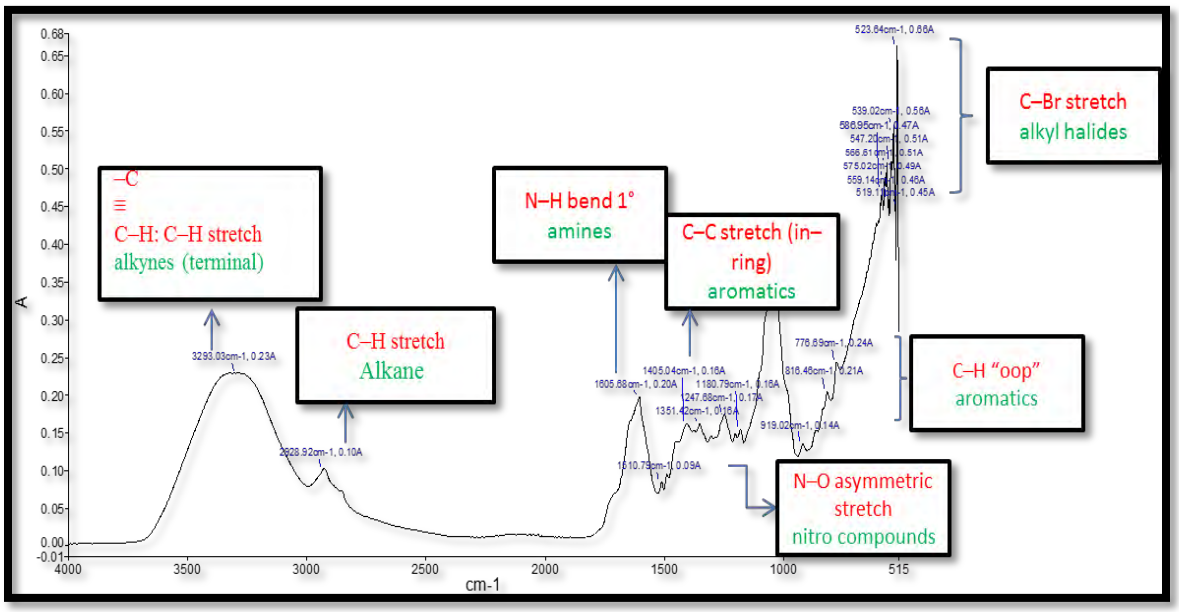

Figure 75: FTIR Spectral Analysis (cm-1) of *S. alopecuroides*

3.8.5 Antioxidant Potential of *S. alopecuroides*

DPPH revealed the free radical scavenging potential of the *S. alopecuroides* from its crude extracts i.e. 82% at 250 mg/mL. Scavenging potential has direct relation to the crude extracts concentration as it decreased up to 60% at 6 mg/mL. GraphPad Prism software was used to determine % DPPH scavenging activity graph using linear regression data. The resulting graphs and Equations are in Figure 76 for % DPPH scavenging activity. Reactive oxygen species cause oxidative damage which can be alleviated by phenolic compounds which ultimately reduces the risk of serious health problems. These findings are in agreement with the previous studies that plant extracts having highest value of phenolic compounds were good antioxidants (Ali et al., 2016; Mehwish et al 2019).

Medicinal Plants being rich source of antioxidants can decrease the oxidative stress, thus beneficial in the cure of numerous human ailments such as inflammation, cancer, and heart ailments (Krishnaiah et al., 2011). Figure 4 shows the measurement of TAC of *S. alopecuroides* extracts and stated it as ascorbic acid equivalent (E µg/mg dry weight of extract). SASM and SASE extracts have shown antioxidant potential of 90.60 ± 2.55 and 84.41 ± 2.43 µg E/mg (Figure 5) respectively whereas SASH and SASC show low TAC value as compare to polar and aqueous extract. Different research studies concluded that TAC values are influenced by the use of different solvents (Kumar and Jain, 2015; Mehwish et al., 2019).

The plant extracts SASM and SASE showed the highest TRP values 94.44 ± 1.38 µg E/mg and 83.43 ± 2.13 µg E/mg respectively. Parallel correlation was observed between TAC and TRP (Figure 4).These findings are in agreement with previous reports which unveiled that positive correlation exist between reducing power of plant extracts and total antioxidant activity. This might occur as antioxidants are electron or proton donors thus reducing ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) through electron donation (Jafri et al., 2014). In addition, our results unravelled that variation in reducing power of plant extracts might be due to difference in solvent used for the extraction as reported earlier (Mehwish et al., 2019).

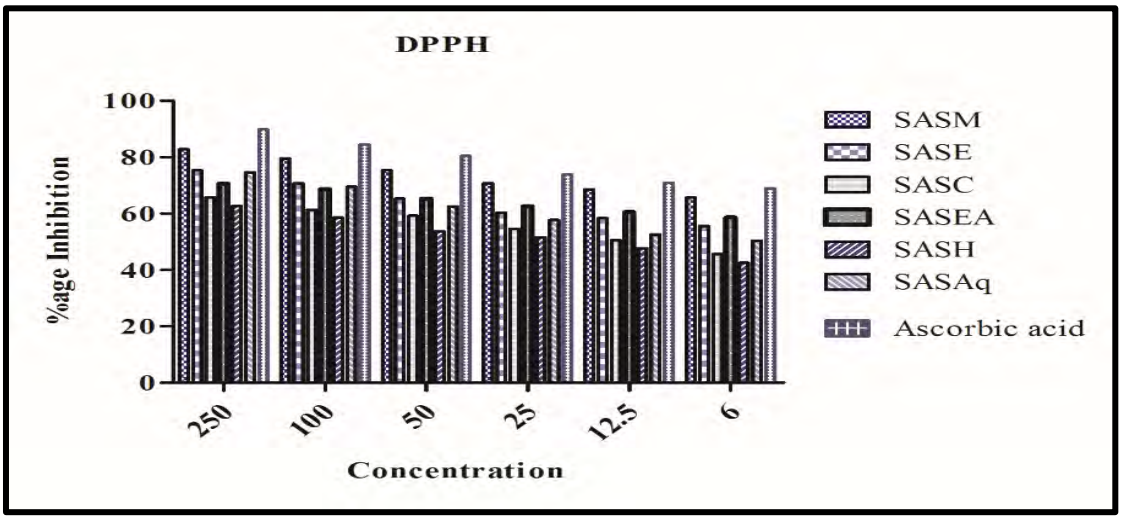

Figure 76: DPPH assay of different extracts of *S. alopecuroides* seeds different extracts.Values (mean ± standard deviation) was obtained through triplicate analysis. SASM: *S. alopecuroides* seed methanol; SASE: *S. alopecuroides* seed ethanol; SASC: *S. alopecuroides* seed chloroform; SASEA: *S. alopecuroides* seed ethyl acetate; SASH: *S. alopecuroides* seed n-hexane and SASAq: *S. alopecuroides* seed aqueous extracts.

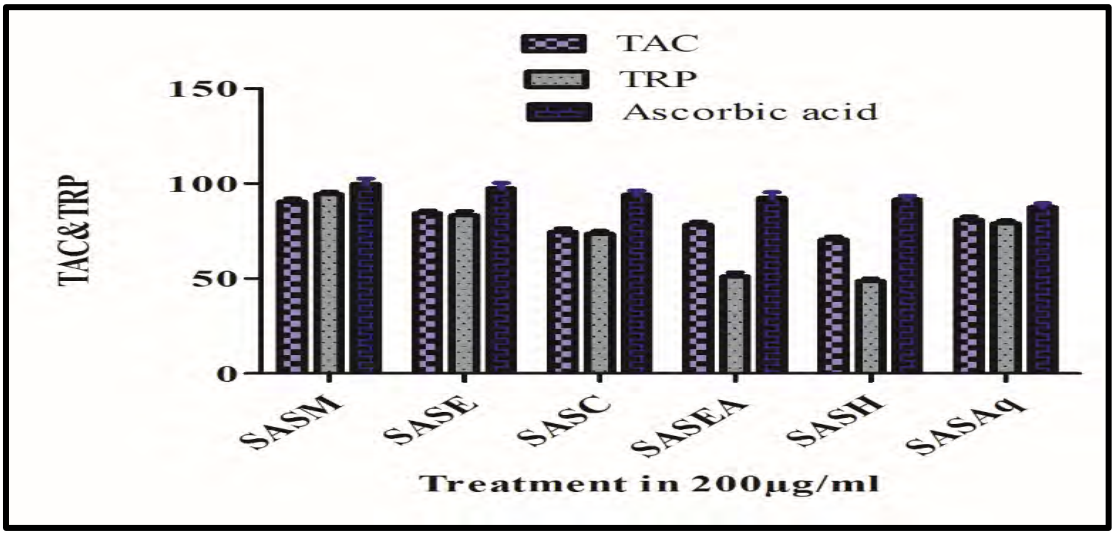

Figure 77: Total antioxidant capacity and total reducing power of *S. alopecuroides* seeds different extracts.Values (mean ± standard deviation) was obtained through triplicate analysis. SASM: *S. alopecuroides* seed methanol; SASE: *S. alopecuroides* seed ethanol; SASC: *S. alopecuroides* seed chloroform; SASEA: *S. alopecuroides* seed ethyl acetate; SASH: *S. alopecuroides* seed n-hexane and SASAq: *S. alopecuroides* seed aqueous extracts.

3.8.6 Antimicrobial Potential of *S. alopecuroides* Seed

The antibacterial potential of *S. alopecuroides* seed extracts were evaluated against gram positive strains (*B. subtilis, S. aureus*) and gram negative strains (*E. coli* , *K. pneumonia* and *P. aeruginosa*. There was significant variation in the antibacterial potential measured by zone of inhibition at 250 µg/mL concentration of different plant extracts. Plant extracts which inhibit bacterial strains and have more than 11 mm zone of inhibition have been further analysed. MIC values have been checked across different concentration (100–11.11 µg/mL). Various MIC values were calculated for bacterial strains *B. subtilis* (33.33 µg/mL), *S. aureus* (11.11 µg/mL) and gram negative strains *E. coli* (33.33 µg/mL), *K. pneumonia* (11.11 µg/mL) and *P. aeruginosa* (100 µg/mL) as shown in Table 40. *K. pneumonia* and *S. aureus* were the most susceptible bacterial strains (MIC=11.11 µg/mL) while *Pseudomonas aeruginosa* having MIC= 100 µg/mL was found to be the least susceptible strain. The present study showed dose dependent antibacterial response. Our results are in agreement with Wan et al (2015) and concluded that *S. alopecuroides* mediated extracts revealed significant antibacterial potentials against different strains.

In current study, different seed extracts of *S. alopecuroides* were analysed for antifungal activity using different fungal strains. *S. alopecuroides* crude extracts showed moderate effects against fungal strains except Hexane extract (SASH). The SASH extract showed less susceptibility for the tested fungal strains. On the other hand, none of tested samples of *S. alopecuroides* have shown % inhibition greater than Clotrimazole (positive control). Table 41 showed MIC values for different fungal strains. Direct relationship was observed between tested plant extract and their concentration. The fungicidal potential was increasing with increase in plant extract concentration. In current study, significant relationship between antifungal activities and solvent used for the extraction has been reported. This fungicidal potential may be varied due to solvent use as reported by Tariq et al. (2019). Our result confirmed the findings of Wan et al. (2015) that *S. alopecuroides* seeds possessed significant antifungal activities against different fungal strains.

Table 40: Inhibition Zones and MIC of *S. alopecuroides* Seeds Extracts against Bacterial Strains.

Micro-organisms	***E. coli*** ATCC 33456		***P. aeruginosa*** ATCC 90271		***K. pneumonia*** ATCC 1705		***B. subtilus*** *ATCC* 19659		***S. aureus*** ATCC 6538	
Plant Extracts	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)
SASM	NI	---	NI	---	NI	---	16±1.44	33.33	NI	---
SASE	7± 1.24	---	NI	---	13± 1.51	100	15± 1.8	33.33	10± 1.44	100
SASC	17± 1.48	33.33	13± 1.44	100	20± 1.47	11.11	NI	---	11± 1.98	100
SASA	12± 1.36	100	11± 1.85	100	19± 1.65	11.11	13± 1.7	100	16± 1.64	33.33
SASH	13± 2.55	100	NI	---	18± 1.74	11.11	11± 1.64	100	21± 1.28	11.11
SASAq	10± 1.44	100	NI	---	13± 1.24	100	12± 1.54	100	20± 1.74	11.11

Values (mean ± standard deviation) was obtained through from triplicate analysis.---: No activity. SASM: *S. alopecuroides* seed methanol, SASE: *S. alopecuroides* seed ethanol, SASC: *S. alopecuroides* seed chloroform, SASEA: *S. alopecuroides* seed ethyl acetate, SASH: *S. alopecuroides* seed n-hexane, SASAq: *S. alopecuroides* seed aqueous extracts

Table 41: Inhibition Zones and MIC of *S. alopecuroides* Seeds Extracts against Tested Fungal strains.

Fungus strains	***M. racemosus*** FCBP 0300		***C. albicans*** FCBP 478		***A. niger*** FCBP 0918		***F. solani*** FCBP 0291		***A. flavus*** FCBP 0064	
Plant Extracts	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)	ZOI (mm)	MIC (µg/mL)
SASM	11±1.8	100	19±2.8	11.11	13±1.7	100	20±2.8	11.11	17±1.8	33.33
SASE	16±2.7	33.33	20±2.7	11.11	12±2.6	---	19±2.6	11.11	18±2.7	33.33
SASC	13±2.1	100	15±1.8	33.33	NI	---	17±2.1	33.33	12±2.6	100
SASEA	12±1.54	100	4± 1.63	---	NI	---	12±2.7	100	NI	---
SASH	NI	---	7±1.8	---	NI	---	6±1.6	---	14±2.5	100
SASAq	11±2.4	100	19±2.8	11.11	19±2.8	11.11	16±2.2	33.33	13±2.6	100

Values (mean ± standard deviation) was obtained through from triplicate analysis. ---: No activity. SASM: *S. alopecuroides* seed methanol, SASE: *S. alopecuroides* seed ethanol, SASC: *S. alopecuroides* seed chloroform, SASEA: *S. alopecuroides* seed ethyl acetate, SASH: *S. alopecuroides* seed n-hexane, SASAq: *S. alopecuroides* seed aqueous extracts.

In present study, anti-leishmanial potentials of various *S. alopecuroides* extracts were studied and percent (%) mortality of the *L. tropica* strain is shown in Figure 7. The present results revealed that at 250 µg/mL concentration, all extracts showed substantial anti-leishmanial activity except SASH extract (51.89± 2.90 %). Our results showed dose-dependent response and % mortality reduced with decrease in concentration of different plant extract. These results verified the findings of previous studies on comparative use of polar solvents for better isolation of antimicrobial compounds (Khademvatan et al., 2019).

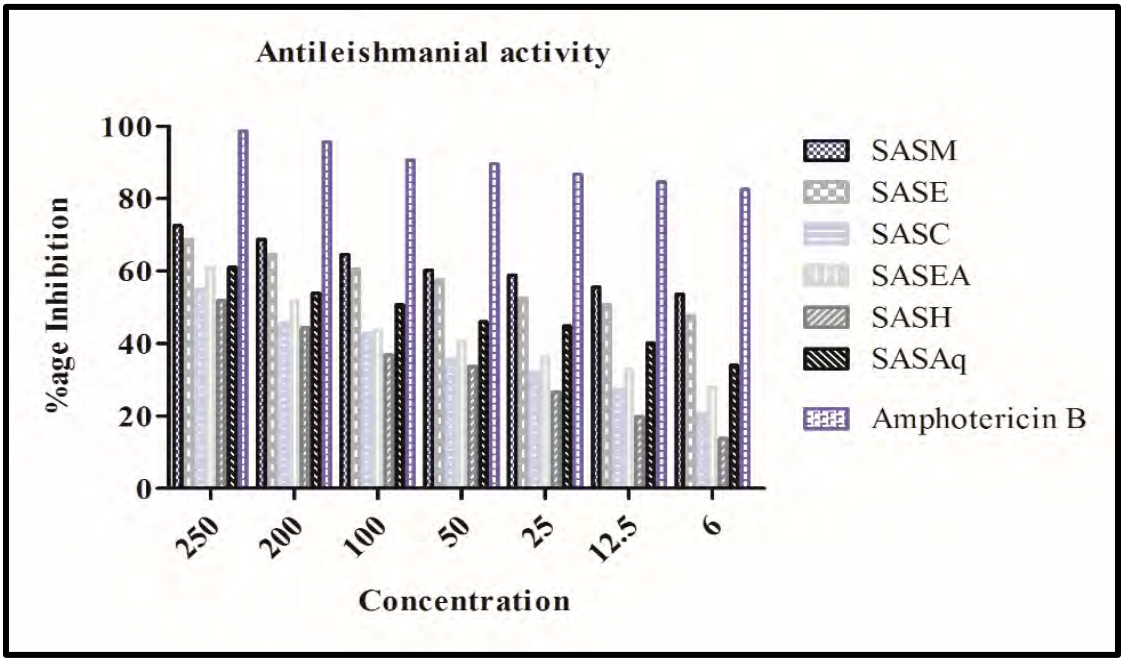

Figure 78: Anti-leishmanial assay of different extracts of *S. alopecuroides* seeds different extracts.Values (mean ± standard deviation) was obtained through triplicate analysis. SASM: *S. alopecuroides* seed methanol; SASE: *S. alopecuroides* seed ethanol; SASC: *S. alopecuroides* seed chloroform; SASEA *S. alopecuroides* seed ethyl acetate; SASH: *S. alopecuroides* seed n-hexane and SASAq: *S. alopecuroides* seed aqueous extracts.

3.8.7 Cytotoxicity Assessment and Anticancer Potential of *S. alopecuroides*

The BSCT is a suitable assay to determine the cytotoxic potential of medicinal plants extracts and associated compounds. The percentage (%) cytotoxicity of different extracts among all *S. alopecuroides* extracts SASH and SASEA showed high % mortality of *Artemia salina* 94 % and 84 % having LD_{50}=13.03 and 23.01 (μg/mL) respectively (Table 7). The results showed concentration dependent response and increase in extract concentration resulted in increase in mortality rate of brine shrimps while mortality decreases with decrease in concentration of plant extracts(Wakawa et al., 2017).

Different solvent based extracts of *S. alopecuroides* were investigated against fungal strain (*Streptomyces* 85E) through protein kinase inhibition assay. The tested samples showed effective PK inhibition potential as shown in Table 7. SASM and SASC extract with 20±2.4 mm and 18±2.1 mm showed significant activity while SASQ give no results. Protein kinase is significantly involved in the phosphorylation of important amino acids (serine, tyrosine and threonine residues). This phosphorylation phenomena is also involved in the regulation of different cellular processes (proliferation, apoptosis and metabolism differentiation). Therefore, any substance with ability to PKI activity have significant importance in cancer research. The present results revealed the presence of potent phytochemicals in different extracts such as aqueous, methanol and ethanol which is reported to be involved in protein kinases inhibition (Naz et al., 2019; Tabassum et al., 2019).

The cytotoxic potency of the *S. alopecuroides* seed extract against prostrate cell line (PC3) was demonstrated using MTT assay. The result of SASEA extract has confirmed reduction in metabolic activity of PC3 cells. The % inhibition was achieved at three different concentration (100, 33.33 and 11.11) µg/mL as shown in Figure 79. The reduced metabolic activity has shown that SASEA extract might have strong anticancer potential and confirmed the finding of *S. flavescens* plant extracts (Zhou et al., 2018)

Table 42: Brine Shrimp Lethality and Protein Kinase Inhibition Assay.

Samples	Brine shrimp lethality (µg/ml)		Protein kinase inhibition (µg/ml)		
	% Mortality	LD_{50} (µg/ml)	Diameter (mm) at 100 µg/disc		MIC
	250		Clear zone	Bald zone	
SASM	67.3±1.43	79.4±2.37	----	20±2.4mm	11.11
SASE	54.2±2.34	88.3±1.28	----	13±1.9mm	100
SASC	40.6±2.75	123.4±2.28	----	18±2.1mm	100
SASEA	84±1.26	23.01 ±1.36	----	15±1.7mm	33.33
SASH	94±2.47	13.03 ±1.14	----	11±2.5mm	100
SASAq	80.8±2.87	27.15±2.23	---	---	----

Values (mean ± standard deviation) was obtained through from triplicate analysis. ---: No activity SASM: *S. alopecuroides* seed methanol, SASE: *S. alopecuroides* seed ethanol, SASC: *S. alopecuroides* seed chloroform, SASEA: *S. alopecuroides* seed ethyl acetate, SASH: *S. alopecuroides* seed n-hexane, SASAq: *S. alopecuroides* seed aqueous extracts.

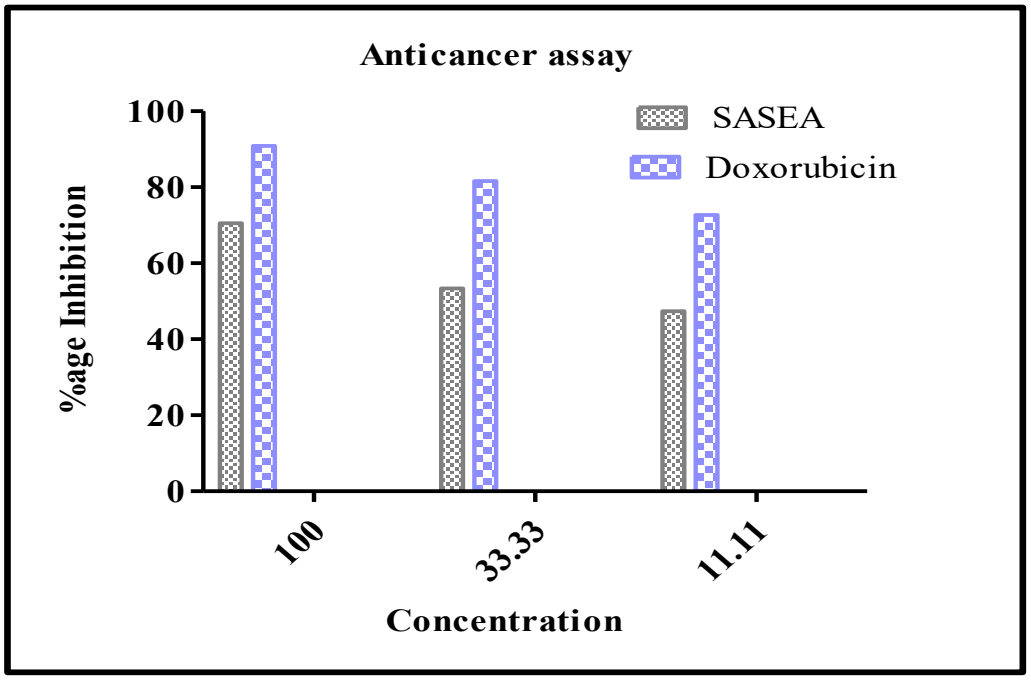

Figure 79: Anticancer activity of *S. alopecuroides* seed extract against PC-3 Cells.

3.8.8 Antidiabetic Potential of *S. alopecuroides* Seeds Extracts

The Alpha-amylase enzyme (AAE) play an important role in the conversion of carbohydrates into glucose, henceforth the inhibition of AA blocked the conversion of carbohydrates into glucose consequently it signifies a key area in diabetes research (Dineshkumar et al., 2010). In current study, we reported the AAE inhibition potential of the *S. alopecuroides* crude extracts. Significant alpha-amylase Inhibition activity was determined. The antidiabetic assay showed highest alpha-amylase inhibition potential (78.20±1.58 % and 77.30±1.97 %) at 250 mg/mL in extracts SASM and SASE respectively (Table 6). Though, the AAE inhibition potential considerably reduced with a decrease in concentration. Our results are in agreement with the findings of Taslimi et al (2020) where polar extracts (methanol extract) showed potential antidiabetic activities. These extracts could be used as an alternative treatment option for the development of novel drugs in pharmaceutical industries.

Table 43: Antidiabetic Potential of *S. alopecuroides* Seeds Extracts.

Plant Extracts	%age inhibition of alpha-amylase	IC_{50}
SASM	78.20 ±1.58	25.54
SASE	77.30 ±1.97	27.45
SASEA	74.30 ±1.65	28.36
SASC	61.37 ±2.54	38.87
SASH	59.57 ±2.59	40.65
SASAq	74.76 ±2.93	28.87

Values (mean ± standard deviation) was obtained through from triplicate analysis. SASM: *S. alopecuroides* seed methanol, SASE: *S. alopecuroides* seed ethanol, SASC: *S. alopecuroides* seed chloroform, SASEA: *S. alopecuroides* seed ethyl acetate, SASH: *S. alopecuroides* seed n-hexane, SASAq: *S. alopecuroides* seed aqueous extracts.

3.9 Conclusion

In the present study, eight novel medicinal plants have been tested for their pharmacological potential. The selected medicinal plants were collected from Gilgit-Baltistan, Northern areas of Pakistan. The possible benefits of plants based medicines have led towards the exploitation of natural sources involves a intricate approach combining botanical phytochemical studies.

The selected medicinal plants *Echinops niveus* Wall. ex Wall*., Iris lactea* Pall., *Lactuca orientalis* (Boiss.) Boiss*., Polygonum affine* D. Don., *Rhodiola imbricata* Edgew., *Salix planifolia* Pursh, *Saxifraga flagellaris* Willd., *Sophora alopecuroides* L. revealed substantial amount of phenolic and flavonoid contents and significant antioxidant potential in extracts made in six different solvents with decreasing polarity (methanol > ethanol > ethyl acetate > water> chloroform >n-hexane). Significant correlation was observed between phenolic and flavonoid contents and antioxidant activity of the plants. Antimicrobial activity (somewhat weakly or moderately)was detected in all species. Alpha-amylase inhibition was also observed significantly in the ethanol and methanol extracts as they possess remarkable phenolic and flavonoid compounds. Cytotoxic activity was evaluated in all extracts which confirmed the presence of toxic compounds in these species. However, some species displayed more toxicity in their Ethyl aceate and n-hexane extracts as compared to their ethanol and methanol extracts.

Among all extracts methanol seeds extract of *L. orientalis* showed highest phenolic contents (95.76±3.71 GAE/g) and methanol extract of *S. flagellaris* had the highest concentration of flavonoid contents (85.69 mg QE/g). FTIR analysis confirmed the presence of different functional groups such as phenols carboxyl and phenolics in selected plants. *S. alopecuroides* seed methanol extract showed best DPPH scavenging potential (82% at 250 mg/mL). Best total antioxidant capacity and total reducing power was determined in methanol extract of *S. alopecuroides* seed (90.60 ± 2.55 µg E/mg) and (94.44 ± 1.38 µg E/mg) respectively. Moreover, Disc-diffusion method showed significant antibacterial and antifungal activities in polar extracts.

The best cytotoxity and anticancer activity was found for *S. flagellaris* n-hexane extract (LD50 37.54±1.54 µg/mL) and 85 % inhibition in metabolic activity of PC3 cells. *R.imbricata* ethanolic extract showed highest protein kinase inhibition (27±1.6mm). Furthermore, highest alpha-amylase Inhibition potential was found in *L.orientalis* methanolic extract (78.8 %). Scanning Electron Microscopy was used as an identification tool and reported for the first time for seeds of *L. orientalis* and *S. alopecuroides*. It was concluded that selected medicinal plants used in the present study may provide a potential source for the development of future medicines. These plants needs to be explored further for the identification and isolation of bioactive compounds to develop leads for new drugs to treat different ailments.

3.10 Future Perspectives

- The Northern Areas of Pakistan including Gilgit and Deosai National park are rich in medicinal plants, some of them are near to extinction. This national heritage of Pakistan has still been under explored that could lead to play a significant role in moderan day medicines.
- Further exploration and experimentation (identification and isolation of bioactive compounds) can lead to a major break through in medicines.
- It is recommended to evaluate their biological and pharmacological potentials through *in-vivo* studies using animal models.

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Scanning electron microscopy of *Sophora alopecuroides* L. seeds and their cytotoxic, antimicrobial, antioxidant, and enzyme inhibition potentials

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Abstract

Sophora alopecuroides L. is a highly medicinal plant. The aim of the current study was to determine the phytochemical screening, pharmacological potentials and application of scanning electron microscope (SEM) of *S. alopecuroides* (SA) seeds. To achieve this purpose, six different solvents were used to prepare SA seed extracts. Phytochemical and antioxidant activities were determined calorimetrically. To investigate the antidiabetic activity, α-amylase inhibition assay was determined. Brine shrimp assay was used to determine cytotoxicity potential. Anti-leishmanial potential was confirmed using MTT assay. Disc-diffusion method was used to detect protein kinase inhibitory, antibacterial and antifungal activities and showed significant results. SEM analysis was used as an identification tool. Considerable amount of phenolic and flavonoid contents were identified in methanol extract (SASM) (93.76 ± 2.71 GAE/mg) and (77 ± 3.60 QE/mg). Highest DPPH scavenging potential (82%) was reported for SASM. Significant total antioxidant capacity (90.60 ± 1.55 alpha amylase enzyme [AAE]/mg) and total reducing power (94.44 ± 1.38 AAE/mg) were determined for LOSM. Highest α-amylase inhibition was reported in SASM (78.20 ± 1.58%). Highest LD_{50} of brine shrimp was found for n-hexane extract (SASH) 13.03 μg/ml. All extracts showed strong anti-leishmanial activity except SASH. The seeds of SA were seen to be oblong to obovate, projections, wavy slightly straight, anticlinal wall was raised with apex acuminate. In conclusion, our experimental findings highly support the ethnomedicinal and biological potentials of the SA seeds. Moreover, SA seeds need to be explored for identification and isolation of bioactive compounds. In future, we recommend further in vivo toxicity assays and clinical efficacies to further evaluate its different biomedical properties.

KEYWORDS
anti-leishmania, antioxidant, enzyme inhibition assays, scanning electron microscopy, *Sophora alopecuroides* L., antimicrobial

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RESEARCH ARTICLE

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Antimicrobial, cytotoxic, antioxidants, enzyme inhibition activities, and scanning electron microscopy of *Lactuca orientalis* (Boiss.) Boiss. seeds

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Abstract

Lactuca orientalis (Boiss.) Boiss. is one of the most frequently used ethnomedicinal plant. This research study was designed to decipher the phytochemical screening, pharmacological potential and implementation of scanning electron microscope (SEM). Six different solvents were used to prepare *L. orientalis* (LO) seed extracts. Phytochemical and antioxidant activities were determined calorimetrically. To investigate antidiabetic, α-amylase inhibition assay was performed. Brine shrimp assay was performed for cytotoxicity and anti-leishmanial via MTT assay. Disc-diffusion assay was performed to detect protein kinase inhibitory, antibacterial and antifungal activities. SEM was used as identification tool. Significant amount of phenolic and flavonoid content were identified in methanol extract (LOSM) (95.76 ± 3.71 GAE/mg) and (77 ± 3.60 QE/mg). Highest DPPH scavenging potential (82%) was reported for LOSM. Significant total antioxidant capacity (90.60 ± 1.55 AAE/mg) and total reducing power (94.44 ± 1.38 AAE/mg) were determined for LOSM. Highest α-amylase inhibition was found in LOSM (78.20 ± 1.58%). The highest LD_{50} of brine shrimp was found for n-Hexane extract (LOSH) 13.03 μg/ml. All extracts showed strong anti-leishmanial activity except LOSH. *L. orientalis* seeds showed significant protein kinase inhibition, antibacterial and antifungal activities. The seeds of *L. orientalis* were seen to be oblong to obovate, projections, wavy slightly straight, anticlinal wall was raised with apex acuminate. The outer-periclinal wall convex with fine texture. In conclusion, our findings scientifically support ethnomedicinal and biological potentials of *L. orientalis* seeds. In future, *L. orientalis* seeds need to be explored for identification and isolation of bioactive compounds. The results obtained necessitate further in vivo studies to evaluate their pharmacological potentials.

Key points

- An extensive phytochemical potential study on the seeds of novel medicinal plant *Lactuca orientalis* (Boiss.) Boiss. was reported.
- Scanning electron microscopy was used as an identification tool and reported for the first time for seeds of *L. orientalis*.

