Lupeol a Potential Growth Regulator: In vitro Physiochemical Response of *Physalis peruviana* Shoot Tip Culture

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DECLARATION

I hereby declare that the work presented in this thesis is my own effort, except where otherwise acknowledged, and that the thesis is my own composition. No part of this thesis has been previously published or presented for any other degree or certificate.

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DEDICATION

This work is earnestly dedicated to my esteemed and affectionate parents, and brothers, whose unwavering support, unwavering motivation, invaluable guidance, and constant remembrance in their prayers have been instrumental in shaping my journey.

Rida-Tul-Haya

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LIST OF ABBREVIATIONS

DMSO	Di-methyl-sulfoxide
DPPH	2,2-diphenyl -1-picrylhydrazile
DW	Dry weight
FC	Folin ciocalteu
FW	Fresh weight
GAE	Gallic acid equaivalent
IAA	Indole-3-acetic acid
LSD	Least significance difference
NAA	Naphthalene acetic acid
PGRs	Plant growth regulators
POD	Peroxidase
ROS	Reactive oxygen species
SD	Standard deviation
SE	Somatic embryogenesis
SOD	Superoxide dismutase
TAC	Total antioxidant capacity
TFC	Total flavonoid content
TPC	Total phenolic content
TRP	Total reducing power

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ABSTRACT

In vitro techniques for the propagation of plants have emerged as a pivotal tool in facilitating the rapid multiplication of cultivars possessing desirable traits, as well as the generation of robust and pathogen-free plants. Lupeol, a plant triterpenoid has gained attention for its pharmacological properties, yet its impact on plant physiology remains a subject of growing fascination. An in vitro study was conducted to assess the impact of lupeol as a growth regulator on *Physalis peruviana* (cape gooseberry). The application of lupeol resulted in a substantial enhancement of the morphological characteristics observed in plants cultivated under varying concentrations (1 µM, 5 µM, and 10 µM). Lupeol increased shoot length by 44%, 56%, and 83% and root length by 46%, 100%, and 135% at 1µM, 5µM, and 10 µM, respectively. Fresh and dry weights also enhanced in the presence of lupeol compared to control groups. An alteration in the phytochemical composition of plants accounted for the varying physiological responses. The phenolic and flavonoid content increased with increasing concentration of lupeol. The free radical scavenging activity enhanced when lupeol was applied as compared to positive controls. The total antioxidant response increased by 4.1% at 1 µM, 8.9% at 5 µM, and 54% at 10 µM in shoots and in roots decreased by 1% at 1µM, enhanced by 21% and 50% at 5µM and 10 µM of lupeol. Improvement in total reducing power was observed at different concentrations of lupeol. However, significant variation in antioxidant enzyme activity (SOD and POD) was recorded compared to control. This study shows the significance of lupeol as a promising growth regulator and as a protective agent against oxidative damage in plants.

CHAPTER 1

1 INTRODUCTION AND REVIEW OF LITERATURE

1.1 Plant Tissue Culture

Plant tissue culture is a fundamental area of study within the realm of plant biotechnology, which contributes to the knowledge of plant growth and its development at cellular level. Tissue culture refers to the controlled development of certain parts (cells, tissues, or organs) in a sterile artificial media, with precise regulation of external factors such as light, heat, and humidity (Thorpe, 2007).

Gottlieb Haberlandt presented to the German Academy of Science his findings on plant tissue culture by successfully separating photosynthetic leaf cells for the first time in 1902 (G Haberlandt, 1902). Haberlandt's contributions encompassed the proposition of generating embryos from vegetative cells, as well as the introduction of the concept of totipotency. Consequently, he is widely recognized as a seminal figure in the field of Plant Tissue Culture, earning him the title of the "Father of Plant Tissue Culture" (Sudheer et al., 2022).

Plant tissue culture and associated biotechnology techniques advanced significantly during the 1940s and 1960s. In order to evaluate the caliber, of cellular development, esteemed scientists endeavored to comprehend the behavior of cells as they proliferated on diverse nutrient-rich substrates derived from coconut water, protein hydrolysates, and a growth medium that had been modified via the inclusion of many vitamins and minerals (Thorpe, 2007).

1.2 Plant Tissue Culture Fundamentals

Cultivation of plant tissues or organs in a controlled laboratory environment, specifically on synthetic growth media, while maintaining sterility and regulated circumstances, is referred to as plant cell culture. The foundation of this technique principally relies upon the principle of totipotency in cells of plant, as proposed by G. Haberlandt (1902). Totipotency elucidates the capacity of an individual cell to manifest the complete genome during the process of cell division. While plant cells possess totipotent potential, their capacity to undergo metabolic, growth, and developmental changes is equally crucial for the rejuvenation of the whole plant (Thorpe, 2007). The media utilized for plant tissue culture contains every nutrient that is needed

for plant normal growth and progression. For a solid medium, it predominantly comprises growth regulators, vitamins, various organic constituents, a carbon source, gelling agents, as well as macronutrients and micronutrients (Murashige & Skoog, 1962). In vitro vegetative growth of numerous plant species is widely performed by using MS (Murashige and Skoog) media. The media pH is particularly significant since it influences both plant development and the function of PGRs. The pH is predetermined between range of 5.4 and 5.8. Cultivation can be performed using both solid and liquid medium. The explant's initial reaction is highly persuaded with the media's chemical makeup, notably the presence of plant hormones and the nitrogen availability. Plant growth regulators (PGRs) are responsible for the regulation of plant cell or tissue development within a culture medium. PGRs that are commonly utilized for a broad number of applications include auxins, cytokinins, as well as gibberellins. The selection of hormones used is primarily influenced by factors such as the specific plant species, the grown tissue or organ, and the desired objectives of the experiment (Ting, 1982). Plant growth regulators that are predominantly employed in tissue culture include auxins as well as cytokinins, wherein the relative proportions of these regulators dictate the specific type of culture that is established or revitalized. An elevated concentration of auxin often facilitates the formation of roots, whereas a heightened concentration of cytokinin promotes the rejuvenation of shoots. The creation of a callus, which refers to a mass of undifferentiated cells, is a consequence of achieving equilibrium between auxin and cytokinin.

Plant tissue culture approaches facilitate the cultivation of whole plants, organs, tissues, or cells in a regulated and sterile environment within a laboratory setting. In tissue culture energy, essential nutrients and water needed for development of plants or explants is supplies by basal media. Furthermore, the utilization of monitored incubating environments facilitates the provision of optimized light and temperature settings, hence enhancing the promotion of growth. The manipulation with in the plant development can be acquired with the application of plant growth regulators, which may include natural phytohormones or synthetic analogues, administered at specific periods of growth or maturation (Phillips & Garda, 2019).

1.3 Plant Tissue Culture Techniques and Applications

Plant tissue culture encompasses many methodologies that are categorised according to the specific organ or tissue used as an explant, the regenerative pathway employed, the composition

of the culture media, and other relevant factors. Meristem culture, callus culture, protoplast culture, organ culture, embryo culture, root culture, among others, represent notable tissue culture techniques.

1.3.1 Meristem Culture

Meristem culture is a significant technique within the field of plant tissue culture that has attained considerable recognition as well as widespread use to regenerate plants or their respective tissues and organs. Meristem culture refers to the sterile cultivation of the apical meristem on nutrient-rich media, with the aim of regenerating tissue, organs, or complete plants in a sterile environment. Meristem culture demonstrates superior efficacy compared to other culture techniques in the elimination of viral and pathogenic agents. Plants often experience viral or pathogenic infections that can be inherited between generations. However, the propagation of virus- or pathogen-free plants can be achieved through the application of meristem culture techniques (Lakhera et al., 2018).

Plant micropropagation using meristem culture is an extensively used technique in the area. Micropropagation is a highly prevalent technique in tissue culture that is extensively used for the abrupt proliferation of many plant species. For the multiplication of haploid plants, meristem culture is a significant technique. Haploid plants based on anther or pollen culture exhibit sterility until they undergo the process of becoming homozygous diploid. The propagation of haploid plants can be achieved by the utilization of meristem or shoot-tip culture techniques (Lakhera et al., 2018).

1.3.2 Micropropagation

Micropropagation, also known as the extensive propagation of plants, has emerged as a significant technique for the propagation of medicinal and crop plants, serving many industrial and non-industrial objectives (Gosal et al., 2010 & El-Esawi, 2016). The process of micropropagation has demonstrated efficacy in the cultivation of Tylophora indica, a perennial climbing vine known for its production of tylophorine, an alkaloid compound. There could be distinct phases of micropropagation (Torres, 1989).

Phase 0: Donor plant preparation

It is possible to utilize any kind of plant tissue through in vitro techniques. To improve the probability of achieving favorable outcomes, it is advisable to cultivate the mother plant under ideal ex vitro conditions, hence mitigating the risk of contamination within the in vitro culture (Cassells & Doyle, 2006).

Phase 1: Inauguration phase

In this specific phase, the explant is subjected to surface sterilization and thereafter placed into a nutritional media. It is normally recommended to employ a synergistic combination of bactericidal and fungicidal products. The assortment of products is contingent upon the nature of the explant to be inserted. The process of surface sterilization of explants via chemical treatments is a crucial procedure for eliminating contaminants and minimizing potential harm to plant cells (Husain & Anis, 2009), Sodium hypochlorite (Tilkat et al., 2009 & Marana et al., 2009), calcium hypochlorite(Garcia et al., 1999), mercuric chloride (HgCl2) (Husain & Anis, 2009) and ethanol are the most frequently used disinfectants. The incubation of cultures is carried out in a growth room, and the choice of light or darkness as the mechanism of propagation determines the specific conditions for incubation (Saini & Jaiwal, 2002).

Phase 2: Multiplication stage

Enhancing the quantity of propagates is the purpose of this phase (Saini & Jaiwal, 2002). Through repetitive subcultures, propagates undergo multiplication until the intended (or predetermined) quantity of plants is obtained.

Phase 3: Rooting phase

The rooting procedure might occur simultaneously within the analogous culture material that is utilized for explant proliferation. To stimulate the process of root formation and promote the establishment of strong root systems, it may be essential to modify the composition of the growth medium. This can involve making adjustments to the nutritional content also the makeup of growth regulators in certain instances (Hussain et al., 2012).

Phase 4: Acclimatization

In this step, the in vitro plants are extracted and subjected to a hardening process. The process of transitioning from elevated to moderate moisture and from low to elevated intensity of light results in a gradual increase in hardening. Subsequently, the next step is to transfer the plants to a suitable substrate, such as sand, peat, compost, or other suitable materials, and subject to a gradual hardening process within a controlled greenhouse environment (Hussain et al., 2012).

1.3.3 Organ Culture

The practice of organ culture involves the cultivation of organs or plant components using a synthetic medium or a culture obtained from an insulated medium. Explant sources in plant tissue culture encompass various plant parts, including the stalk (for shoot tip cultures), the leaf (for leaf cultures), the root (for root tip cultures), the flower (for anther, ovary, or ovule cultures), and the root (for root tip cultures). Embryo culture, shoot tip, meristematic culture, isolated root, and nodal culture of distinct lateral buds represent the quintessential forms of organ culture employed in the realm of in vitro plant propagation.

The implementation of in vitro organ culture and chemical metabolic analysis has been effectively established in a broader spectrum of plant species, including Fritillaria unibracteata. The swift development of plants might be acquired by the utilization of miniature bulb scraps in organ culture. By subjecting these cuttings to a period of culture in MS medium, cultured bulbs can be obtained. Notably, the pace of development, microelement content, and alkaloid levels in cultured bulbs have been observed to exhibit a significant increase, reaching values that are 30-50 times higher compared to non-cultured bulbs. Consequently, the utilization of organ culture has emerged as a pivotal approach in the augmentation of alkaloid content within plants (Gulzar et al., 2020).

1.3.4 Somatic Embryogenesis and Synthetic Seeds

Somatic embryogenesis, as described by Hofmann. (2014), refers to an innovative in vitro methodology that enables the generation of undulating embryos from either a solitary or a cluster of somatic cells. During this experimental methodology, the explant is introduced onto a growth medium that is provided with particular plant growth regulators (PGRs), incorporating diverse arrangements and dosages. After a few days of inoculation, the cells of the explant begin to dedifferentiate into a mass of cells, known as a callus. Alternatively, in some instances, the cells may bypass the callus formation stage and directly initiate the development of an embryo. The

induction of dedifferentiation, a transformative process from nondividing (somatic) to dividing cells, is triggered by stress within the microenvironment of the medium. This stress encompasses various elements such as light, nutrition, oxygen, as well as hormones (Gulzar et al., 2019). The onset of environmental stress triggers a complex signaling cascade, whereby various molecular pathways are altered, leading to stimulation and inhibition of numerous genes (Fehér, 2015).

The successful development of a somatically regenerated embryo into a fully formed plant is of utmost importance, as not all embryos generated through this process have the ability to reach maturity. Numerous plant species exhibit a lack of successful establishment into a complete plant entity, despite the presence of somatic embryos that display normal and healthy characteristics. It represents a significant constraint across the SE plant multiplication (Isah, 2016). The somatic embryos, once developed, must be moved to the ground with care to ensure their continued viability and field establishment. The conversion occurs when the newly implanted embryos become mature plants. Synthetic seed technology has been proven to greatly simplify the aforementioned operations. Synthetic seeds are created by enclosing an embryo or other propagating material (such as an axillary bud, cell aggregate, or shoot bud) in a medium containing nutrients and surrounding it with a light covering of gelling substance (potassium alginate, carrageenan, sodium pectate, or sodium alignate are some examples) (Tonk et al., 2016, & Haque & Ghosh, 2014). The utilization of synthetic seed technology serves as the fast and effective technique for rejuvenating the plants, particularly those that do not naturally propagate through conventional seed production (Rihan et al., 2017).

1.3.5 Haploid Culture

Haploids are widely embraced by plant breeders due to their potential for rapid duplication, facilitating the expeditious attainment of homozygosity across all loci within a concise timeframe (Basu et al., 2011). The expression of recessive genes occurs within haploid organisms, and it is possible for any gene that plays a significant role in crop productivity or immunity against pathogens (Basavaraju, 2011) or any other characteristics to be appeared in haploid organisms (Basu et al., 2011). Haploid species are being efficiently produced, subsequently propagated by tissue culture techniques, and subsequently utilized to enhance agricultural productivity. Anther, pollen, ovary, and ovule cultures were used to create in vitro haploids. (Basu et al., 2011). Haploidy, a technique that has been successfully implemented in various plant species, has

yielded promising results. Notable examples of plants include sunflower, asparagus, rice, maize, tobacco, barley, wheat, potato, barley, wheat, and brassica (Bajaj, 1990).

1.3.6 Culture of Hairy Roots and Genocidal Modification

Hairy root culture refers to the cultivation of plant tissues that have been infected by the gram-negative soil bacterium *Agrobacterium rhizogenes*, resulting in the development of distinctive root structures (Tepfer & Casse-Delbart, 1987). Transformed root culture, alternatively referred to as transgenic root culture, serves as a valuable tool for investigating plant biological functions, generating vital phytochemicals and recombinant proteins. Additionally, this technique facilitates the in-depth examination of genes involved in these processes (Georgiev et al., 2007). During this experimental procedure, the first step is to infect the bruise explants initially with Agrobacterium rhizogenes, then transfer them to solid media containing antibiotics, such as cefotaxime, for a limited duration. The utilization of hairy root culture exhibits an extraordinary attribute in ensuring the expansion of biomass on a broader spectrum, maintaining the continuous generation of secondary metabolites, displaying responsiveness to external stimuli, and releasing metabolites into the culture media.

The phenomenon of incorporating supplementary bacterial genes into a culture by means of the bacteria being attached to the roots is referred to as genetically altered root cultures (Sheludko & Gerasymenko, 2013). The initial study demonstrated the development of a rapid and prolific hairy-root culture system, which yielded elevated concentrations of medically significant isoflavones. This breakthrough was attained with the help genetic manipulation of Trifolium pratense using Agrobacterium rhizogenes. The knowledge about the biochemical process underlying T-DNA transfer within genetically engineered roots has significantly advanced the field of metabolic engineering as well as facilitated the synthesis of recombinant proteins within the studied plant species (Ochoa-Villarreal et al., 2016). This methodology holds significant importance in the tissue culture field because of its demonstrated efficacy foforfficiently producing intended plant metabolic byproducts and facilitating the expression of foreign proteins on a large scale, all while remaining economically viable. Recent studies have documented a notable augmentation in the formation of phenolic compounds, flavonoids, as well as antioxidants within transgenic Lactuca serriola harry roots. (El-Esawi et al., 2017).

1.4. *Physalis peruviana* and its Importance

1.4.1 Botanical Description and Economic Importance

Physalis peruviana L., a perennial medicinal plant, is commonly referred to as the cape gooseberry or golden berry. It is member of the Solanaceae family. It is indigenous to tropical regions of Peru, as well as various tropical and temperate regions across the globe. Delicious orange fruits of the cape gooseberry plant are smooth berries that resemble little, yellow tomatoes. There are many names for this plant around the world. In English-speaking nations, this plant's name is frequently referred to as the "golden berry," while in Colombia, it is known as "uchuva." South Africa recognizes it as the "cape gooseberry," whereas Ecuadorian locals identify it as "uvilla." In India, it goes by the name "ras bhari," while in Peru, it is referred to as "aguaymanto." And in Venezuela, it is recognized as "topotopo" (Erkaya et al., 2012). It is an upright, perennial verdurous plant with semi-shrub characteristics, capable of reaching a height of 0.9 meters in regions with subtropical climates. The fruit, which weighs about 4 to 5 grams, is shielded by an enlarged calyx that has undergone accretion. Its outer layer possesses a vibrant yellow hue, elegantly encasing the fruit within (Mayorga et al., 2001). Nearly 30,622 ha worldwide are used for growing the golden berry fruit, and this area produces 162,386 t of output (FAOSTAT 2013).

Given its worth, the plant is quickly becoming a crucial crop in the manufacturing of functional foods, and it represents an emerging market with rising economic significance. (Ramadan & Moersel, 2007). *P. peruviana* plants can produce 300 fruits per plant and can produce 20 to 33 tons of fruit per hectare when they are well-tended (Ramadan, 2011). The fruit's great production and extensive shelf life may increase demand for it globally. While the knowledge of *P. peruviana* has existed for many years, the exploration of its potential for extensive farming has only been initiated in recent times. especially in light of the plant's recent discovery of its unique storage, industrial, and nutritional properties, which make it suited for a variety of food and medicine purposes (Ramadan, 2011). The cultivation *of P. peruviana* is undertaken for industrial purposes in South Africa, and the fruit is canned and utilized for both domestic and international markets. Additionally, Egypt, Gabon, and other countries in Central Africa are among those where the plant is farmed on a large scale. (Hassan et al., 2017).

1.4.2 Applications in Pharmaceutical and Nutraceutical Industries

The nutraceutical benefits of this fruit facilitate its application in formulations (Bazalar et al., 2019) particularly in the context of addressing non- transmissible illnesses and the development of nutritional foods (Nocetti et al., 2020). These fruits are an excellent supply of beneficial and bioactive compounds for health. These include phytosterols, vitanolids, carotenoids (specifically α-carotene, β-carotene, and cryptoxanthene) (Hassan et al., 2017), phenolic substances, physalins, ascorbic acid, as well as vitamins A, C, and B-complex, along with essential minerals such as iron and phosphorus. A plethora of metabolic processes are exhibited by these substances, encompassing antitumor, anti-inflammatory (Bazalar Pereda et al., 2019), immunostimulatory, microbicidal, insect repellent, cytotoxic, as well as antimicrobial properties (Nocetti et al., 2020). *Physalis peruviana* contains anolide, which has anti-adipogenic properties (Kumagai et al., 2021). Moreover, there has been speculation regarding the potential of anolide as a therapeutic agent for individulas with COPD (chronic obstructive pulmonary disease) (Yang et al., 2020).

The efficacy of this botanical specimen in the therapeutic management of cancer has exhibited promising outcomes (Hassan et al., 2017 & Yu et al., 2021), and colorectal adenocarcinoma development (Megahed et al., 2019). According to Badr and Naeem's research (2019), use of *P. peruviana* fruit prevented from cancer causing effects of aflatoxins as well as the neurotoxicity caused by cadmium (Badr & Naeem, 2019, Abdel Moneim et al., 2014). Fruits possess a rich array of essential minerals, including Fe, Mn, Zn, Na, Mg, K, Ca, P, S, Al, B, and Cu (Karasakal, 2021).

For centuries, the traditional medicinal practices have harnessed the therapeutic potential of *P. peruviana*, employing it for medication of various ailments like tumor, cancer of the blood, malaria, respiratory disorders, hepatitis, skin infections, and arthritis (Wu et al., 2009). There are a lot of other alleged medicinal qualities of the plant. They consist of the removal of intestinal parasites, amoebas, albumin from the kidneys, as well as their usage as antiasthmatics, antiseptics, optic nerve strengtheners, and treatments for throat infections (Arun & Asha, 2007). Additionally, it has been proposed that *P. peruviana* lowers cholesterol and has anti-ulcer properties (Arun & Asha, 2007).

The investigation into the factors governing the development of *P. peruviana* L. was motivated by the considerable presence of bioactive compounds within the berries (Karasawa & Mohan,

2018), which additionally serve as a rich reservoir of vital vitamins and minerals (Y.-M. Wu & Kanamori, 2005).

1.4.3 Propagation Methods

The optimal technique for the normal reproduction of *P. peruviana* involves the utilization of sexual reproduction, whereby plants are propagated either in their indigenous habitat or within restrained environments, employing seeds as the primary means of propagation. Environmental factors like humidity, temperature, light, and oxygen are necessary for successful seed emergence. Seed germination is adversely affected by variations in any of these parameters. The process of sexual reproduction in the plants exhibits certain limitations on the generation of offspring, despite the *P. peruviana* exhibiting a prolific production of seeds for each berry, which are known for their favorable germination potential. The plant's vulnerability to ailments and insect infestations, coupled with its limited production of secondary metabolites, accounts for this phenomenon. (Oliveira et al., 2016).

Asexual reproduction, using grafting and micropropagation, is another technique for *P. peruviana*. Asexual in vitro propagation is an alternate technique for producing pathogen-free, genetically homogeneous, and wholesome plantlets quickly (Chaves et al., 2005).

1.5 Plant Growth Regulators

All plants have their natural hormones that help them adapt and thrive in their environment. These hormones control everything from their growth and development to their shape and function. Hormones, like little messengers, travel through plants, finding their way to specific cells to trigger responses. They are produced in several plant segments, including the buds, leaves, as well as roots (Rademacher, 2015). Hormones exert a profound influence on various aspects of plant physiology, including cell division, cell expansion, morphology and activity, as well as the plant's reaction to various external stressors. (Ferguson & Grafton-Cardwell, 2014). Depending on the cells/tissues they act on the plant's stage of development, the proportional amount of hormones compared to others, the availability and uptake of nutrients also of water as well as the weather/environmental conditions, they can have a wide range of effects and reactions (Ferguson & Grafton-Cardwell, 2014).

Hormones have been investigated since the 1930s to enhance agricultural growth as well as output (Rademacher, 2015). The intricate interplay of hormones in plants exerts a profound influence on the modulation of various biological functions (Ferguson & Grafton-Cardwell, 2014). Since these hormones are only found in trace amounts in plant tissue, isolating, identifying, and extracting enough of them for lab testing presents significant difficulties (Rademacher, 2015). The use of synthetic hormones has been found to effectively regulate numerous biological functions, including the intricate mechanisms involved in the growth and differentiation of diverse plant components including roots, stems, leaves, flowers, and fruits (Flasiński & Hąc-Wydro, 2014). The aforementioned breakthrough has opened the route for the progression and manufacturing of synthetic hormone products, commonly referred to as plant growth regulators (PGRs).

The utilization of PGRs has been demonstrated to augment the process of germination, as well as the subsequent growth and development of plants, through their ability to exert influence over the intricate hormonal activity within the plant. (Hopkins & H¨uner, 2009). There are several variables, such as plant type, stimulant, dose, timing, growth stage, and application location, that influence how plants respond to PGRs (Mitchell, 1942).

PGRs have been extensively employed in the fields of cultivation, viticulture, and gardening with the aim of augmenting agricultural output and facilitating the harvesting process. For instance, they have proven effective in preventing premature fruit detachment, expediting the maturation and developing stages, and providing other similar benefits (Harms & Oplinger, 1988) and to enhance growth under unfavorable or adverse situations such as instances of inadequate soil reproduction, disease outbreaks, or limited periods for development (Rademacher, 2015).

PGRs are categorized into five primary classes, such as auxins, gibberellins, cytokinins, ethylene a well as abscisic acid. In recent times, a fascinating revelation has emerged in the field of plant biology, shedding light on the regulatory mechanisms governing phytohormonal activities. The discovery of novel PGRs, namely brassinosteroids and jasmonates, has captivated researchers and opened up new avenues for exploration. These remarkable compounds have demonstrated their ability to modulate the intricate interplay of phytohormones, unraveling the complex web of

plant growth regulation. (Rademacher, 2015). Altogether these hormones govern all aspects of plant life.

1.6 Lupeol

The triterpene molecule lupeol has been found in a broad range of plants and has been the focus of numerous studies. These plants include both edible ones, like mangoes, and therapeutic ones, such as the well-known Aloe Vera. The prospective therapeutic applications and pharmacological activities of this biologically active molecule, with its distinctive chemical structure and unique features, have attracted considerable attention from the scientific community. Lupeol is a member of the triterpenoid family, which includes a wide variety of chemicals found throughout plants.

These are the chemical properties of lupeol:

The chemical formula under consideration is $C_{30}H_{50}O$.

The molecular weight of the compound under consideration is determined to be 426.72 grams per mole.

Alternative Designations: Fagarasterol, Clerodol

Structure: Lupane is a fascinating compound characterized by the substitution of a hydroxyl group for a hydrogen atom at the 3-beta position. This substitution endows lupane with a unique pentacyclic triterpenoid structure, adding to its scientific intrigue. The molecular structure under examination exhibits a singular five-membered ring, acquiring an envelope conformation, alongside four six-membered rings, each acquiring chair conformations. The rings exhibit a cohesive arrangement, specifically in a trans conformation (Corrêa et al., 2009).

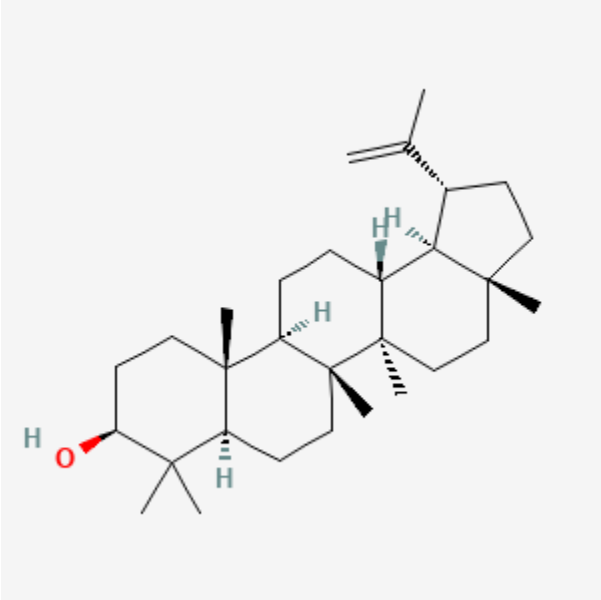

Figure 1.1 Structure of Lupeol (PubChem)

According to Saleem, (2009) the melting point of the substance under consideration is recorded to be within the range of 215-216 °C. The physical appearance of the substance under consideration is that of a solid with a white crystalline structure when maintained at standard room temperature, as documented by Corrêa et al. in 2009.

Solubility: The compound exhibits solubility in various organic solvents such as chloroform, ether, and ethanol, owing to its hydrophobic characteristics. However, it does not dissolve in water due to its aversion to aqueous environments.

According to Corrêa et al. (2009), the stability of the structure is deemed to be relatively robust under normal circumstances. These properties encompass its solubility, reactivity, and stability, which collectively shape its behavior and potential applications.

The ecological significance and evolutionary importance of this compound are defined by its widespread occurrence in many botanical sources. The pentacyclic triterpene skeleton is defined by the fusion of five rings, providing the molecule with exceptional stability and rigidity. The presence of lupeol in a broad spectrum of plants, like fruit and medicinal plants, underscores its widespread distribution and ecological significance. Numerous scientific inquiries have been

undertaken to examine the diverse characteristics of this chemical, with a specific emphasis on its possible medicinal effectiveness. Numerous inquiries have been conducted to explore different facets of its pharmacological characteristics, revealing insights into its abilities to mitigate inflammation, inhibit cell proliferation, and regulate hyperglycemia. Through a rigorous analysis of its modes of action, scientists have endeavored to decipher the complex molecular pathways through which this chemical elicits its advantageous outcomes. The diligent pursuit of scientific research has yielded an increasing amount of empirical data, highlighting the considerable potential of this substance as a beneficial therapeutic agent within the field of medicine.

1.6.1 Natural Sources of Lupeol in Plants

Lupeol, a bioactive compound, has been found in numerous medicinal plants involving American ginseng, Shea butter plant, Tamarindus indica, Allanblackia monticola, Himatanthus sucuuba, Celastrus paniculatus, Zanthoxylum riedelianum, Leptadenia hastata, Crataeva nurvala, Bombax ceiba, and Sebastiania adenophora. These plants are traditionally used by indigenous populations in North America, Latin America, Japan, China, Africa, and the Caribbean islands. Additionally, lupeol has been detected in common food sources including olive, fig, mango, strawberry, red grapes, Japanese Pear, white cabbage, pepper, cucumber, tomato, carrot, Cajanus cajan, Capsicum annuum, Coccinia grandis, and Cucumis sativa. (Saleem, 2009b). Table 1 displays a comprehensive compilation of plant species that have been documented to possess substantial amounts of lupeol.

Table 1.1 List of plants containing lupeol

S.no	Plants	Scientific name	Content	Reference
Medicinal plants				
1	Elm plant	*Ulmus spp.*	880 lg/g bark	(Nguemfo et al., 2009)
2	Bitter root	*Apocynum cannabinum*		(Imam et al., 2007)
3	Aloe leaves	*Aloe vera L.*	280 lg/g dry leaf	(Nguemfo et al., 2009)

Vegetables				
4	Tea	*Camellia sinensis*	-	(Imam et al., 2007)
5	Cucumber	*Cucumis sativus*	-	(Imam et al., 2007)
6	Carrot	*Daucus carota*	-	(Imam et al., 2007)
7	Capsicum	*Capsicum annum*	-	(Imam et al., 2007)
8	Common pea	*Pisum sativum*	-	(Imam et al., 2007)
9	Tomato	*Lycopersicon esculentum*	-	(Imam et al., 2007)
Fruits				
10	Grapes	*Vitis vinifera*		(Imam et al., 2007)
11	Mango fruit	*Mangifera indica L.*	180 lg/g	(Nguemfo et al., 2009)
12	Japanese pear	*Pyrus pyrifolia*	175 lg/g twig bar	(Nguemfo et al., 2009)
13	Olive fruit	*Olea europa L.*	3 lg/g of fruit	(Nguemfo et al., 2009)
14	Date palm	*Poenix dactylifera*	-	(Imam et al., 2007)
15	Ginseng oil	-	15.2 mg/100g	(Nguemfo et al., 2009)
16	Common fig	*Ficus carica*	-	(Imam et al., 2007)
17	Guava	*Psidium guajava*	-	(Imam et al., 2007)

18	Mango sps (peel like banana	*Mangifera pajang Kosterm*	> 300 (µg/ml)	(S. Ahmad et al., 2015)
19	White mulberry	*Morus alba*	-	(Imam et al., 2007)

1.6.2 Role of Lupeol in Plants

According to (Szakiel et al., 2012) lupeol, a naturally occurring triterpenoid molecule, can be found in its unadulterated form in a variety of plant species, most frequently within resins or waxes. Moreover, lupeol presumes a crucial and indispensable role as a precursor for the biosynthesis of various specialized triterpenoid metabolites. These metabolites, renowned for their paramount importance, actively participate in the intricate mechanisms governing plant defense and development.

It has been proposed that lupeol's presence in the cuticular wax of the castor bean plant (Ricinus communis) serves a physiological purpose by protecting the plant from the deleterious effects of dehydration (Guhling et al., 2006).

It has been determined that Lotus japonicus, a legume plant species, need lupeol for the complex process of nodule production. Its capacity to control the expression of the ENOD40 gene, that performs a crucial function in nodule formation within legumes, is largely responsible for its central role in this process. The OSC3 gene was identified to be the sole determinant in the production of lupeol in L. japonicus roots and nodules (Delis et al., 2011). By regulating the expression of the ENOD40 gene, lupeol is essential for the establishment of a productive symbiotic connection between nitrogen-fixing bacteria and the plant that results in the creation of nodules (Delis et al., 2011).

Another interesting fact is that betulinic acid, a triterpenoid chemical with potent anticancer effects, could be synthesized from lupeol (Jin et al., 2019 & Cunha et al., 2021) trees are the only ones in which this fascinating natural chemical is known to concentrate in the bark tissues (Cárdenas et al., 2019). Numerous preclinical investigations have demonstrated the promising potential of betulinic acid for use an antitumor agent (Jiang et al., 2021 & Oliveira-Costa et al., 2022).

1.6.3 Medicinal Properties of Lupeol

Numerous edible fruits, vegetables, and medicinal plants contain lupeol, a naturally occurring triterpenoid molecule. Over the past decade, numerous studies have been conducted in order to learn more about lupeol's pharmacological effects. These studies have provided substantial evidence to support the diverse therapeutic benefits of lupeol, including its notable anticancer, antioxidant, anti-inflammatory, and antibacterial properties (Table 1.2).

Table 1.2 Medicinal properties of lupeol

Effect	**Cell lines**	**Activity**	**Reference**
Anti-oxidant	Rat hepatocytes	Restored levels of antioxidant enzyme, lowered lipid peroxidation, inhibited ROS production, and prevented mitochondrial depolarization.	(Kumari & Kakkar, 2012)
Apoptosis	Human, HCC cell lines SMMC7721 and HepG2.	HCC cell lines SMMC7721 and HepG2 were induced to undergo apoptosis.	(He et al., 2011)
Apoptosis	human colorectal cancer (CRC) cells	Enhanced apoptosis in CRC cells that have Wnt/β-catenin signaling that is always on	(Tarapore et al., 2013)

Anti-cancer	Human, MDA-MB-231 breast cancer cells	Reduced TNBC proliferation via the PI3K/mTOR pathway-induced autophagy, and inhibited TNBC metastasis via EMT by autophagy-mediated Twist1 downregulation.	(Zhang et al., 2022)
Anti-cancer	human cervical carcinoma (HeLa) cells	Induced S-phase cell cycle arrest, decreased S-phase Cyclins and CDKs, and elevated the expression of cyclin-dependent kinase inhibitors, p21 at transcription and translation.	(Prasad et al., 2018)
Anti-cancer	Human OS cells	Repressed the tumor by regulating the expression of an oncogene miR-212-3p, which targeted HMGA2	(Zhong et al., 2020)
Anti-inflammatory	neonatal rat cardiomyocytes (NRCMs)	Inhibited nuclear translocation of NF-B p65 and decreased inflammatory cytokines.	(Li et al., 2022)
apoptosis	Human, in-vitro 2D cell line models (HEp-2,	Increased the expression of p53 which in turn induced the expression of	(Bhattacharyya et al., 2017)

	UPCI:SCC-131)	Bax and turned on the apoptosis pathway.	
Anti-inflammatory	Mice	Neuroinflammation was prevented by inhibiting activated glial cells and inflammatory mediators. Reduced Aβ and beta-secretase-1 (BACE-1) expression and improved memory and cognitive function.	(R. Ahmad et al., 2020)
Anti-inflammatory	Human skin in-vitro	Downregulated NF-kappaB expression in keratinocytes and upregulated matrix metalloproteinase (MMP)-2, indicating anti-inflammatory activity.	(Pereira Beserra et al., 2018)
Anti-cancer	human non-small cell lung cancer (NSCLC)	Reduced EGFR and STAT3 activity and induced apoptosis in NSCLC cells.	(Min et al., 2019)

1.7 Significance of Lupeol in Plant Tissue Culture

At present, it is important to acknowledge the notable dearth of comprehensive and substantial data pertaining to the exogenous administration of lupeol within the realm of plant tissue culture. The presence of this research void underscores the critical need for conducting extensive investigations that seek to offer a comprehensive comprehension of the potential consequences

and ramifications of this specific compound on the complex mechanisms of in vitro plant growth and development. A recent study was conducted to investigate the potential of lupeol in alleviating the adverse impacts of salt stress on Brassica nigra (Zia et al., 2022).

1.8 Aims and Objectives

- To investigate the role of lupeol as a potential growth regulator in *Physalis peruviana* shoot tip culture.
- To study the impact of lupeol on morphological characteristics of *Physalis peruviana* plant.
- To identify the enzyme antioxidant potential of *Physalis peruviana* in presence of lupeol.
- To study the physiological and biochemical responses of *Physalis peruviana* plant.

CHAPTER 2

2 MATERIALS AND METHODS

2.1 Chemicals and Equipment

Ethanol, hydrochloric acid (HCl), methanol, plant growth regulators, mercuric chloride ($HgCl_2$), twin twenty, sodium hydroxide (NaOH), and distilled water were the chemicals utilized during this study. Equipment includes blades, filter paper, forceps, pH meter, autoclave, spirit lamp, laminar flow cabinet, and glassware (like measuring cylinder, glass beakers, Erlenmeyer flasks and petri dishes).

2.2 Media Preparation:

In this study, the basal MS media developed by Murashige and Skoog in 1962 was utilized for the purpose of in-vitro germination of seeds. The media was supplemented with a 3% concentration of sucrose, and no further growth regulators were included. The media pH was modified to 5.68 using 0.1 M NaOH and 0.1 M HCl solutions. To facilitate solidification, gelrite (0.7%) was introduced and then dissolved with the use of heat. The media was dispensed into conical flasks with a volume of 100 mL, with each flask receiving 30 mL of media. The media underwent autoclaving for a duration of 20 minutes at 121°C and a pressure of 15 psi.

2.3 Seed Sterilization and Preparation of Explants:

The seeds of *Physalis peruviana* L. were subjected to a treatment involving the use of detergent tween-20 for 2 minutes. Subsequently, the seeds were wholly cleansed by using tap water, followed by a process involving the use of 70% ethanol for a period of 5-10 minutes. Following the washing process, the seeds underwent a meticulous surface sterilization procedure lasting a duration of 5 minutes, utilizing a solution containing 0.1% mercuric chloride (HgCl2). To ensure the complete elimination of any residual HgCl2, a meticulous process of triple washing with sterile water was employed subsequent to the surface sterilization of the seeds. Per flask, six seeds were inoculated. The shoot tips were carefully excised from seedlings that had been germinated in vitro. These excised shoot tips were subsequently employed as explants for further experimentation.

2.4 Inoculation and Culture Condition:

The shoot tips of the in vitro germinated seedlings were carefully removed, with a length ranging from 0.5 to 1.0 cm. The explants were employed in their natural state, without undergoing surface sterilization, and subsequently introduced into a culture medium known as MS basal medium. This medium was enriched with varying concentrations of plant growth regulators (lupeol, IAA and NAA), thereby facilitating the growth and development of the explants. Four replicates for each of three different concentrations of lupeol were used; 1µM,5µM and 10µM. Four replicates for one concentration of Indole acetic acid (IAA) hormone were utilized as positive control; 1µM. And for one concentration of Naphthalene acetic acid (NAA) hormone four replicates were also employed as positive control.

In each flask, six explants were allocated for each concentration and a total of three flasks were designated for each concentration. At first, the flasks were subjected to a controlled environment within a growth, maintaining a temperature of 25 °C, while being shielded from any external light sources. After the initiation of seed germination, the flasks were transferred to an alternating dark and light cycle, specifically an 8-hour dark period followed by a 16-hour light period. The light source used for this cycle consisted of white LEDs with a wavelength range of 380-780 nm, emitting a photosynthetic photon flux density (PPFD) of 15 mol/m2/s.).

2.5 Morphological Characteristics:

The plants were harvested at the end of a 29-day period, and the conventional method was used to measure the root length, shoot length. Number of leaves were also calculated.

2.6 Fresh and Dry Weight of Roots and Shoots:

The plants' shoots and roots were cut off and split up. And with the help of an electronic measuring balance their fresh weight was measured. Following that, plants were placed in petri plates in an incubator at 37°C for 3 days to allow them to dry at low temperature. By using the measuring balance, roots and shoots were weighed after drying.

2.7 Determination of Antioxidant Enzymes Activities:

Fresh shoots and roots were harvested, followed by a thorough cleansing using distilled water. Subsequently, the shoots and roots were individually crushed in a phosphate buffer solution with a pH of 7.0. The resulting mixture was then transferred to separate eppendorf tubes. To assess the specific enzyme activities, the eppendorf tubes were centrifuged at a speed of 10000

revolutions per minute for 10 minutes. Then for a variety of enzymatic tests the supernatant was obtained and utilized including;

2.7.1 Superoxide Dismutase (SOD):

The procedure employed by Ullah et al. (2019) was utilized for the assessment of enzymatic superoxide dismutase (SOD) activity with regards to its antioxidant properties. The experiment required the following chemical agents:

EDTA at a concentration of 1 mM, methionine at a concentration of 130 mM, NBT at a concentration of 0.75 mM, and riboflavin at a concentration of 0.02 mM.

The reaction mixture of 200 µL was generated by combining 20 µL of EDTA, 20 µL of methionine, 20 µL of NBT, 78 µL of phosphate buffer, 2 µL of riboflavin, and 60 µL of enzyme extract on a 96 well plate. All components were uniformly combined, with the exception of the enzyme extract, in order to create the blank solution. Following an incubation period of 7 minutes in the presence of fluorescent light, thoptical density of the mixture was quantified with the help of a micro-plate reader set at 560nm. To determine the level of enzymatic activity, the subsequent calculation was employed:

A = ELC

In this context, we denote A as the sample absorbance, C as the enzyme concentration (expressed in nM/min/mg FW), E as the extinction coefficient (measured in 6.39 mM-1 cm-1), and L as the length of the wall (0.25 cm).

2.7.2 Per-oxidase Activity (POD):

By employing (LM Lagrimini, 1990)) method the POD activity of *Physalis peruviana* fresh shoot and root extracts was determined.

The following chemicals were utilized;

guaiacol 100mM, dH_2O, H_2O_2 27.5 mM, and KH_2PO_4 buffer 50 mM.

By mixing 40 µL KH_2PO_4 buffer, 20µL guaiacol, 20µL of each sample, 100µl dH_2O, 20 µL of 27.5 mM H_2O_2 in 96 well plate reaction mixture of 200 µL was made. All the chemicals were mixed in the same quantity except enzyme extract to prepare the control. Enzymatic activity was quantified by measuring absorbance at 470 nm with the help of a micro-plate reader. The formula given was utilized to check the enzymatic activity.

A = ELC

In this context, the variables are denoted as follows:

A represents the sample's absorbance, C represents the enzyme concentration measured in nM/min/mg FW, E represents the extinction coefficient (expressed as 6.39 mM-1 cm-1), and L represents the length of the wall, which is 0.25 centimeters.

2.8 Biological and Biochemical Analysis of Roots and Shoots

The dried roots and shoots were ground using a pestle and mortar, afterwards transferred to Eppendorf tubes. The crushed roots and shoots were weighed again, and 1 mL of DMSO was applied to each tube. These test samples were then submitted to several biological assays such as;

2.9 Phytochemical Screening:

Total phenolic and total flavonoid contents of Physalis peruviana root and shoot test samples were determined for the phytochemical analysis.

2.9.1 Estimation of Total Phenolic Contents (TPC)

The methodology given by (Astill et al., 2001) was employed to identify the TPC of test samples. To each well of the 96 well plate, 20 µL of each of the test samples, blank and standard, i.e. sample, DMSO, and Gallic acid, were carefully placed. Following that, 90 µL of 10 times diluted freshly made F_C (Folin- Ciocalteu) reagent was combined with these test samples, standard, and blank. After incubating the plate for 5 minutes, 90 µL of Na_2CO_3 (6%) solution was added to the reaction mixture. After 1 hour of incubation, the optical density was calculated at 650 nm by using micro-plate reader.

At various concentrations for the standard (gallic acid), a calibration curve was plotted, and the total phenolic content of the test samples was assessed using the value generated by that curve (R2 value). As µg Gallic acid equivalent per mg extract (µg GAE/mg extract) the TPC was estimated and the assay was repeated three times.

2.9.2 Determination of Total Flavonoid Contents (TFC):

The methodology proposed by (Almajano et al., 2008) was used to ascertain the overall flavonoid content of the experimental samples. The first step involved dispensing 20 µL of the test sample, standard (Quercetin 4mg/mL), and blank into the 96 well microplate, afterwards adding 10 µL of a 10% solution of aluminium chloride. After the introduction of 10 µL of a 1 M potassium acetate solution, the volume was uplifted to 200 µL with the addition of 160 µL of distilled water. Following a 30-minute incubation period, the samples absorbance was assessed at 415 nm by using a microplate reader (Bioteck, USA).

The calibration curve of the standard was developed utilizing Quercetin at different final concentrations. The estimation of the total flavonoid content in the test samples was conducted using an equation acquired by the calibration curve (R2 value). The assay was performed in triplicate to ensure accuracy and reliability of the results. The values were determined in micrograms Quercetin equivalent per milligram of extract (µg QE/mg extract).

2.10 Antioxidant Assays:

Various antioxidant assays were performed to assess the antioxidant capacities of *Physalis peruviana* roots and shoots, including;

2.10.1 DPPH Free Radical Scavenging Assay:

The methodology employed by Clarke et al. (2013) with minimal alterations was utilized to assess the antioxidant efficacy of the experimental sample against DPPH. The experimental procedure consisted of transferring 10 µL of the sample, standard (ascorbic acid), and blank into the wells of a 96-well microplate using a pipette. Subsequently, 190 µL of DPPH was transferred into each well and the contents were stirred. The reaction mixture was incubated for a duration of one hour at a temperature of 37°C, while being shielded from light. The optical density at 515 nm of the samples was determined by employing a microplate reader.

% inhibition of the test sample was calculated by employing the formula given below:

Test sample percent inhibition = (1 - A / B) * 100

Here:

A = DPPH solution's O.D. with sample.

B = O.D. of the negative control, which contains the reagent but not the sample.

2.10.2 Evaluation of Total Antioxidant Capacity (TAC):

The total antioxidant activity of shoots and roots of *Physalis peruviana* was assessed with the help the method given by Clarke et al. (2013), with minor alterations.

The procedure was initiated by transferring 100 µL of stock solution/test samples into Eppendorf tubes, then 900 µL of TAC reagent (50mL) (1.63 mL H_2SO_4, 0.218 g $NaH_2PO_4.H_2O$, 0.347 g $(NH_4)_6Mo_7O_{24}.4H_2O$) was added. The resulting mixture was thoroughly mixed. Then the incubation of tubes was carried out at 95°C for 90 minutes in water bath. After incubating the reaction mixtures, they were subsequently chilled to the surrounding temperature. Subsequently, 200 µL of an evenly mixed solution were introduced into a 96 well plate. The optical density at 630 nm was calculated with the help of a microplate reader.

2.10.3 Total Reducing Power Assay (TRP):

The methodology described by Jafri et al.(2017) was utilized to assess the overall reduction potential of the experimental samples.

In order to conduct the total reducing power assay, 100 µL of the test materials were placed in Eppendorf tubes and combined with 200 µL of phosphate buffer (0.2 M, pH 6.6) and 250 µL of a 1% potassium ferricyanide solution. Subsequently, the reaction solutions were incubated at 50°C for 20 minutes. After incubation, the reaction solution was acidified by adding 200 µL of a 10% solution of trichloroacetic acid. The mixtures obtained were centrifugated for ten minutes at a speed of 3000 revolutions per minute. A volume of 150 µL from the supernatant of each centrifuged mixture was carefully moved onto a microplate. Subsequently, 50 µL of a 0.1% ferric chloride solution was added to each well of the microplate and well mixed.

The measurement of optical density was conducted with the help of a microplate reader set at 630 nm. The results were expressed in units of micrograms of ascorbic acid equivalent per milligram of extract (µg AAE/mg extract).

2.10.4 Determination of ABTS Radical Scavenging Activity

The ABTS reagent was produced by combining 7 mM of ABTS with 2.45 mM of potassium persulphate in a 1:1 ratio (Wang et al., 2009). The reagent was subjected to a period of darkness lasting 12-18 hours at ambient temperature, after which it was subsequently diluted with methanol in a ratio of 1:2. The optical density was measured and subsequently diluted until the optical density at 540 nm reached a value of 0.7 ± 0.02. In a 96-well microtiter plate, 10 µl of samples were reacted with 100 µl of ABTS reagent for the assay. After incubating the reaction solutions for ten minutes at ambient temperature in the dark, the optical density was obtained at 540 nm.

2.10.5 Evaluation of Metal Chelating Activity

The experiment was conducted in a 96-well plate, by using the methodology given by Wang et al. (2009). Each well was filled with 100 µl of each sample, then 50 µl of a 2 mM FeCl2 solution was added. For 10 minutes, the plate was incubated in the dark. Following that, 20 µl of 5 mM ferrozine was poured to each well and incubated for 5-10 minutes. The optical density was calculated against EDTA as a positive control at 562 nm. MC activity was determined by

MC ability%

[(Absorbance of control – absorbance of sample)/ absorbance of control)] *100

2.11 Statistical Analysis

The layout of the experiment was entirely random. For each concentration, four flasks were prepared. The findings are presented in the form of mean ± SD (standard deviation). The biochemical and antioxidant assays were conducted in triplicate. The statistical method of analysis of variance (ANOVA) was employed to determine the differences between means, and the least significant difference (LSD) test was utilized for further analysis. The significance level was set at $p<0.05$

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CHAPTER 3

3 RESULTS

3.1 Morphological Characteristics

3.1.1 Root Length

The application of lupeol significantly augmented the root length of *Physalis peruviana* plants relative to the control. In contrast to the positive control of 1µM NAA, the inclusion of lupeol caused a noticeable improvement in root length. Particularly, a notable 18% increase in root length was found at a concentration of 1µM, followed by a substantial 64% rise at 5µM. Furthermore, at a concentration of 10µM, the root length exhibited a remarkable increase of up to 91% (Fig 3.1). In contrast to the negative control, an increase of 46% was detected at 1 µM, an increase of 102% was recorded at 5 µM, and an increase of 135% was detected 10 µM concentration (Table 3.1).

3.1.2 Shoot Length

Presence of lupeol increased (13.0, 14.1, 16.5 cm) the shoot length of *P. peruviana* at 1, 5, and, 10 µM respectively as compared to positive control 5 µM IAA (1.6 cm). Comparatively, the presence of lupeol also increased shoot length as compared to positive control NAA (10.3 cm), where 26% increase was observed at 1 µM, 36% increase was observed at 5µM while at 10 µM of lupeol, 60% increase was observed in shoot length. Comparison of lupeol with negative control (9.0cm) showed that 44% increase in shoot length occurred at 1 µM, 56% increase occurred at 5 µM, while at 10 µM 83% increase in shoot length was observed (Fig 3.1).

Table 3.1 **Length of roots and shoots and number of leaves of *P. peruviana* in presence of IAA, NAA, and lupeol.** Values represent means± standard errors from triplicates. The alphabetes after each value indicates significant difference in values at $p<0.05$.

CONCENTRATIONS (µM)	ROOT LENGTH (cm)	SHOOT LENGTH (cm)	NUMBER OF LEAVES

Negative Control	3.93±0.152e	9.03±0.1527d	12±0.6e
5µM IAA	Callus on the base	1.6±0.3605e	3±0.15f
1µM NAA	4.86±0.251d	10.33±1.258c	15±0.75d
1µM Lupeol	5.7±0.556c	13.03±0.642bc	18±0.9c
5µM Lupeol	7.93±0.208b	14.1±0.624b	22±1.1b
10µM Lupeol	9.22±0.351a	16.53±1.001z	28±1.4a

3.1.3 Number of Leaves

The number of leaves of *Physalis peruviana* plants exhibited an increase when subjected to varying doses of lupeol, relative to the control. When compared to the positive control IAA, the application of lupeol at 1 µM, 5 µM, and 10 µM resulted in a substantial enhancement in the number of leaves (18, 22, and 28, respectively). An augmentation in leaf count was also noted compared to positive control (15). The number of leaves exhibited a significant increase at various doses of lupeol, as compared to the negative control (Table 3.1).

3.2 Fresh Weight and Dry Weight of Roots and Shoots

A substantial increase in FW and DW of *P. peruviana* roots was observed between control and plants grown at different concentrations of lupeol. In comparison to positive control NAA (1.004 g), at 1µM concentration of lupeol 25% increase in FW was observed, 57% increase was observed at 5 µM, while at 10 µM up to 1.24 times increase in FW of roots was observed (Table 3.2). The presence of lupeol also increased (1.26, 1.58, 2.25 g) FW of roots in comparison to negative control (0.58 g).

DW of roots enhanced in lupeol-treated plants relative to control. An increase in DW was observed as compared to positive control NAA (0.054g) where a maximum increase was observed at 10 µM (0.142g) concentration of lupeol while minimum increase was observed at 1µM (0.073g) respectively. In comparison to negative control (0.028g) DW was also increased at 1 µM, 5 µM, and 10 µM of lupeol.

Physalis peruviana shoots exhibited an increase in FW with the increase in the concentration of lupeol in contrast to positive control IAA (1.64 g), where 2.97 mg FW was observed at 1 µM, 3.54mg was observed at 5 µM while 5.74 mg FW was observed at 10 µM. In comparison to

positive control NAA (2.45 g), 21% increase in FW was observed at 1 µM, 45% increase was observed at 5 µM while at 10 µM FW was increased by 1.35 times. Comparison of lupeol with negative control (1.09 g) also showed an increase in FW (Table 3.2).

DW of shoots of lupeol-treated plants also increased in comparison to the control. In comparison to positive control NAA (0.139 g) and positive control IAA (0.117 g) DW was increased, 0.161 mg DW was observed at 1 µM concentration of lupeol, 0.218mg was observed at 5µM and 0.323mg was observed at 10µM (Table 3.2). DW was also enhance relative to the negative control (0.064 g).

Table 3.2 Fresh weight and dry weight of roots and shoots of *Physalis peruviana* plants under IAA, NAA, and lupeol. Values represent means ± standard errors from triplicates. The alphabetes after each value indicate significant difference in values at $p<0.05$.

CONCENTRATIONS (µM)	ROOTS FW(g)	ROOTS DW(g)	SHOOTS FW(g)	SHOOTS DW(g)
Negative control	0.586±0.02e	0.028±0.0014e	1.098±0.05f	0.064±0.003f
5µM IAA	-	-	1.64±0.08e	0.117±0.002e
1µM NAA	1.004±0.05d	0.054±0.0027d	2.97±0.12c	0.139±0.006d
1µM lupeol	1.267±0.06c	0.073±0.0036c	2.476±0.14d	0.161±0.008c
5µM lupeol	1.586±0.08b	0.094±0.0047b	3.548±0.17b	0.218±0.10b
10 µM lupeol	2.254±0.11a	0.142±0.0071a	5.74±0.28a	0.323±0.01a

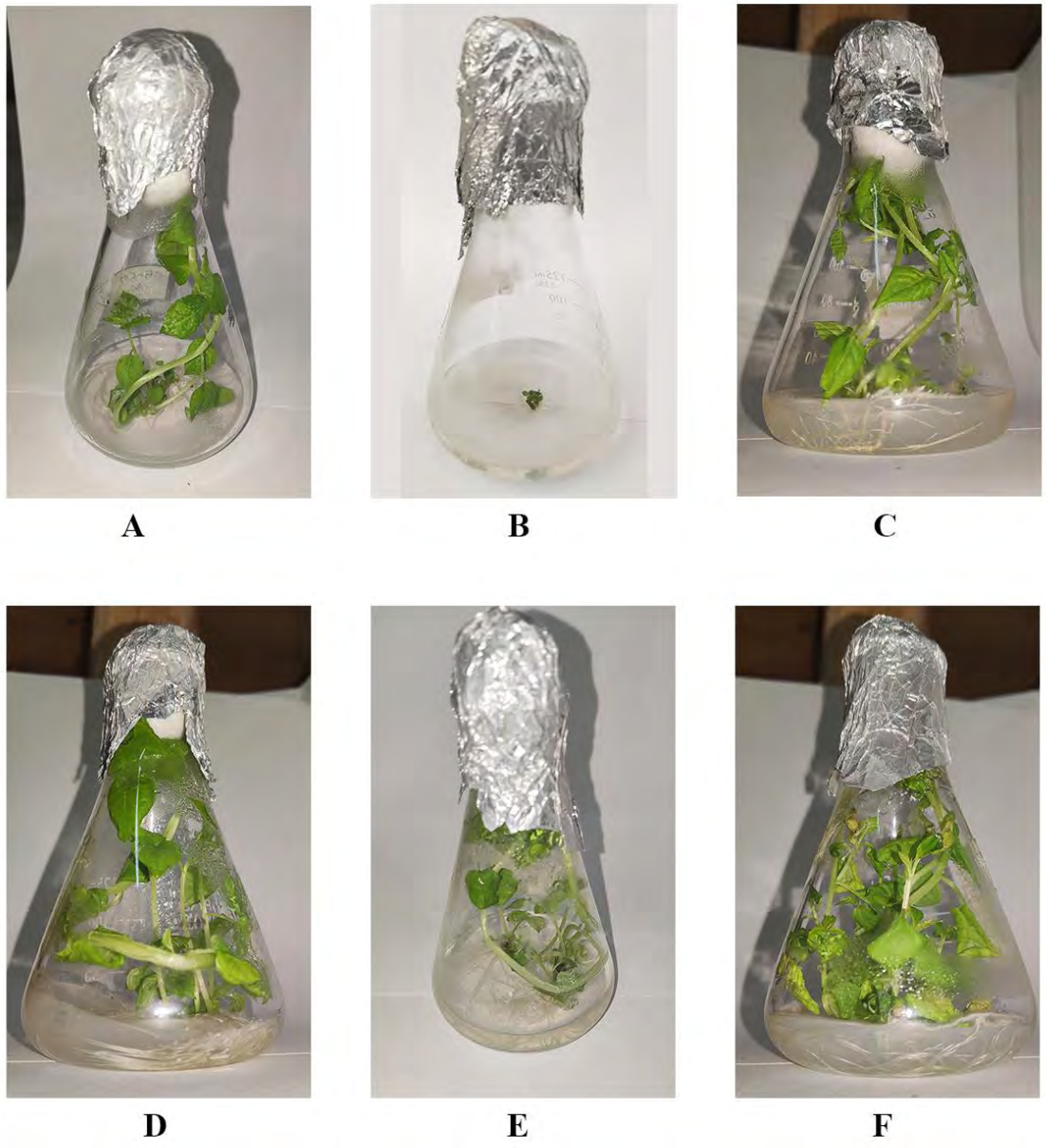

Figure 3.1 Growth of *Physalis peruviana* Shoot Tip Culture in presence of Negative control(**A**), 5µM IAA(**B**), 1µM NAA (**C**), 1µM Lupeol (**D**), 5µM Lupeol (**E**), and 10µM Lupeol (**F**).

Figure 3.2 Impact of IAA, NAA, and different concentrations of lupeol on shoot length, root length and number of leaves of *Physalis peruviana*. Negative control(**a**), 5µM IAA(**b**), 1µM NAA (**c**), 1µM Lupeol (**d**), 5µM Lupeol (**e**), 10µM Lupeol (**f**).

3.3.1 Total Phenolic Content

Roots

Total phenolic contents were found to increase in roots of *P. peruviana* grown under lupeol, (15.08, 14.68, and 16.64 µgGAE/mg D.W). Minimum content was observed at 5 µM which was 14.68 and maximum at 10 µM which was 16.64 µgGAE/mg D.W in comparison to control. As compared to a positive control NAA (14.68 µgGAE/mg D.W), phenolic content was higher at 1 µM and 10 µM which was 15.08 and 16.64 g µGAE/mg D.W as shown in Fig 3.2. In contrast to the negative control (blank) reduction in TPC was only observed at 5 µM in plants grown in the presence of lupeol as shown in figure 3.3.

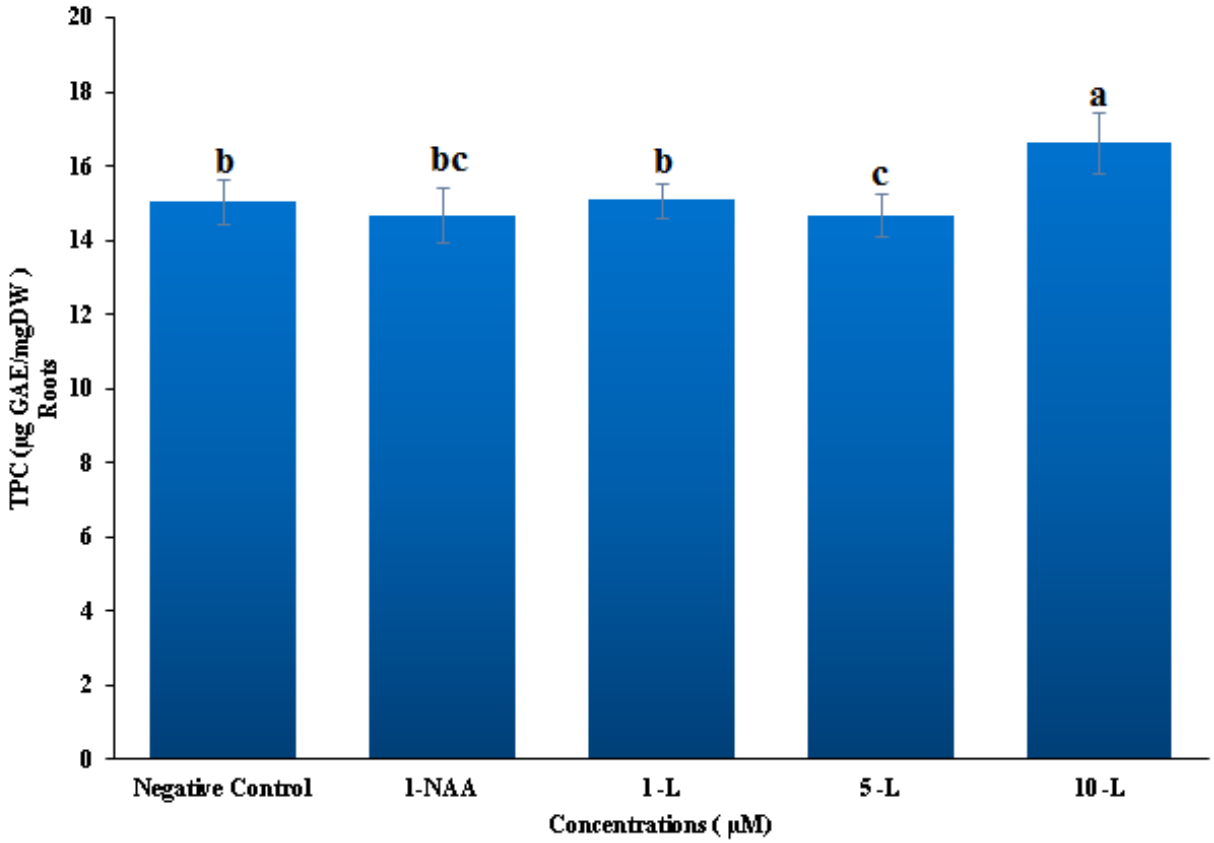

Figure 3.3 Impact of lupeol (L) and NAA on total phenolic content of *P. peruviana* roots. Values depict means ± standard errors from triplicates. The small letters on each value indicate significant difference among mean values at $p<0.05$.

Shoots

Due to presence of lupeol, total phenolic content in the shoots of *P. peruviana* was found to be increased in comparison to the control, with a minimum of 21.63 and a maximum of 28.50 µgGAE/mg D.W at 1 and 5 µM of lupeol, respectively. Compared to the positive control 1 µM NAA, lupeol significantly increased the TPC, which was 28.50 µgGAE/mg D.W at 5µM and 24.19 µgGAE/mg D.W at 10µM (Fig 3.3). Lupeol decreased the TPC at 1µM which was 21.63 µgGAE/mg D.W in comparison to positive control IAA (23.65 µgGAE/mg D.W). Comparing lupeol to a negative control revealed an increase in TPC content. However, at 10µM of lupeol, the TPC decreased by approximately 14%, resulting in a TPC of 24.19 µg GAE/mg D.W. in comparison to 5 µM which was 28.50 µgGAE/mg D.W (Fig 3.4).

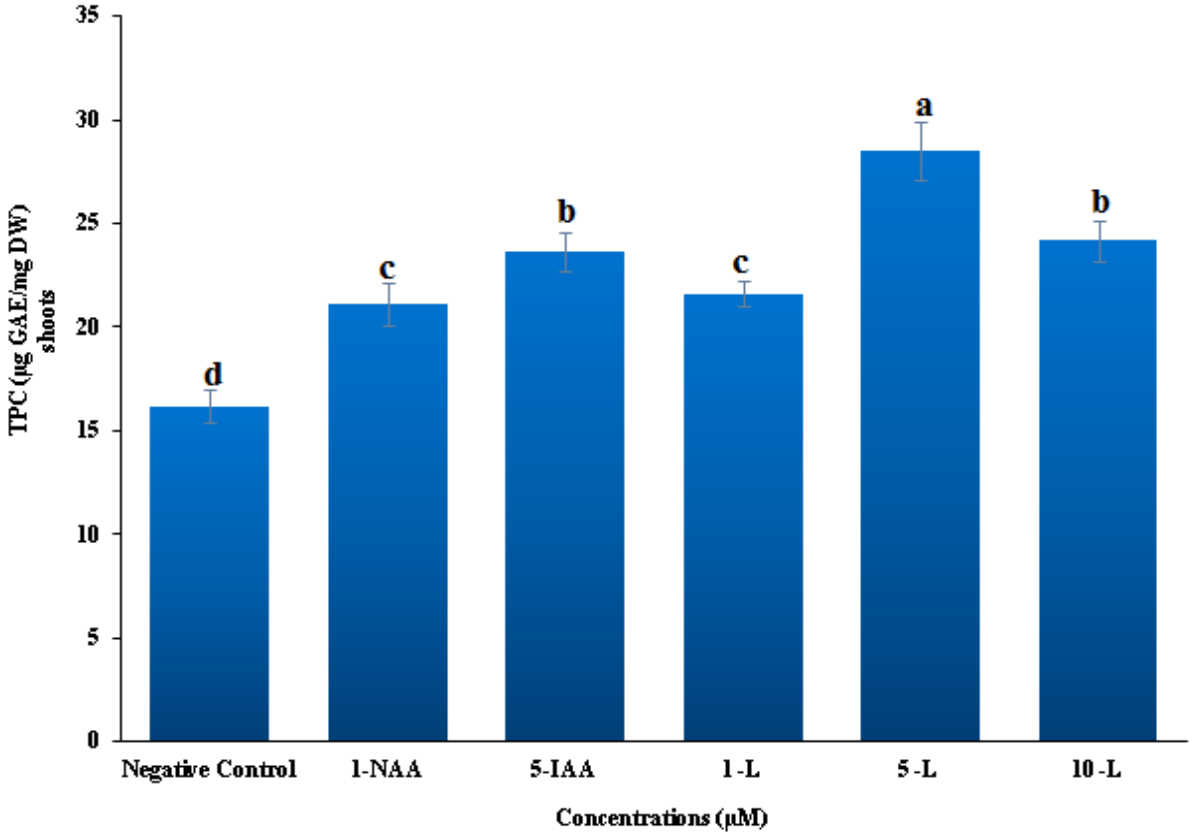

Figure 3.4 Impact of lupeol (L), NAA, and IAAon total phenolic content of *P. peruviana* roots. Values represent means ± standard errors from triplicates. The small letters on each value indicate significant difference among mean values at $p<0.05$.

3.3.2 Total Flavonoid Content

Roots

The roots of *Physalis peruviana* plants exhibited an increase in total flavonoid content when cultivated in the presence of lupeol. The concentration of lupeol at 1µM resulted in a total flavonoid content of 6.07 µgQE/mg D.W, while at 5 µM it was 6.49 µgQE/mg D.W, and at 10 µM it reached 7.67 µgQE/mg D.W. In comparison to negative control, the positive control NAA yielded a total flavonoid content of 5.20 µgQE/mg D.W. The administration of lupeol led to a noteworthy augmentation in the overall flavonoid content when compared to the negative control (Fig 3.5).

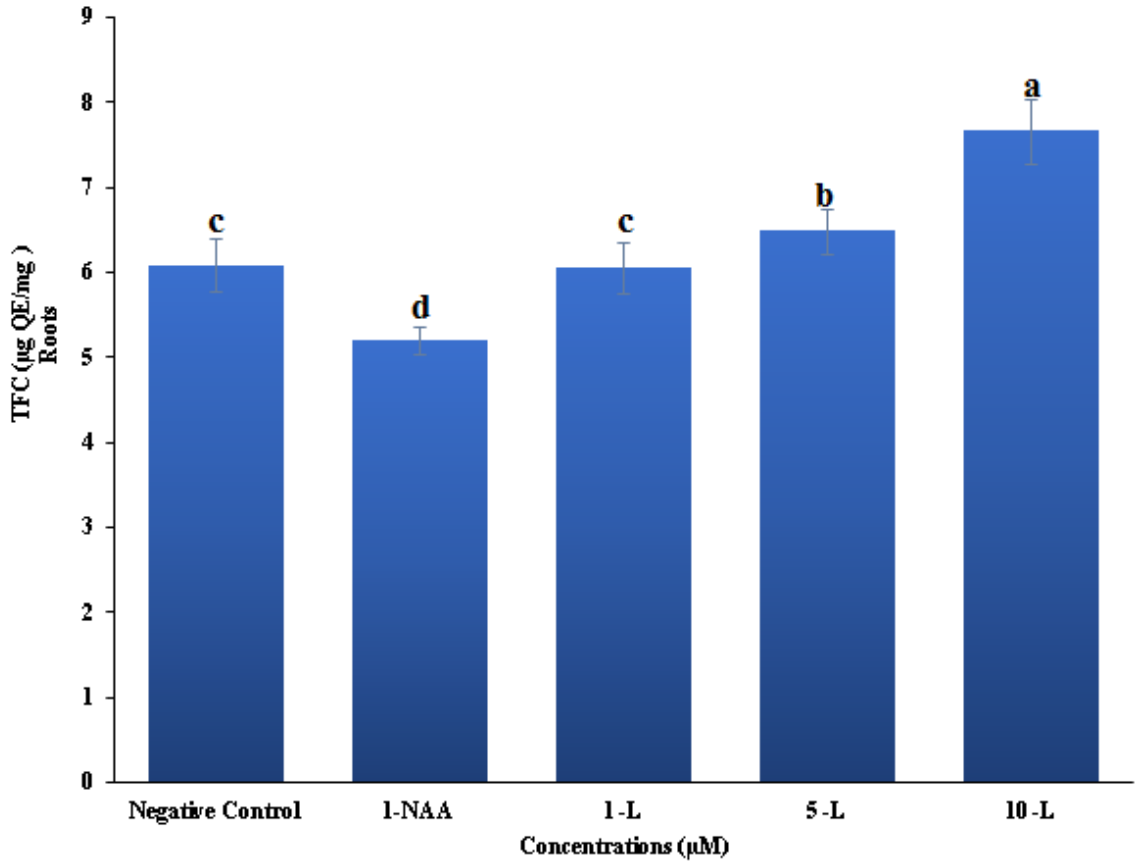

Figure 3.5 Lupeol (L) and NAA impact on total flavonoid content of *P. peruviana* roots.Values represent means ± standard errors from triplicates. The small letters on each value indicate significant difference among mean values at $p<0.05$.

Shoots

Total flavonoid content in P. peruviana shoots exhibited a significantly greater quantity compared to the control with minimum value of 8.23 gQE/mg D.W and maximum value of 14.46 gQE/mg D.W at 1 µM and 10 µM of lupeol, respectively. Compared to the positive control 5 µM IAA(8.18 µgQE/mg D.W), lupeol significantly increased the Total flavonoid content, which was10.23 µgQE/mg D.W at 5µM and 14.46 µgQE/mg D.W at 10µM (Fig 3.6). Lupeol decreased the TFC at 1µM which was 8.23 µgQE/mg D.W in comparison to positive control NAA (9.70

µgQE/mg D.W). TFC content increased when lupeol was compared to negative control (7.16 gQE/mg D.W).

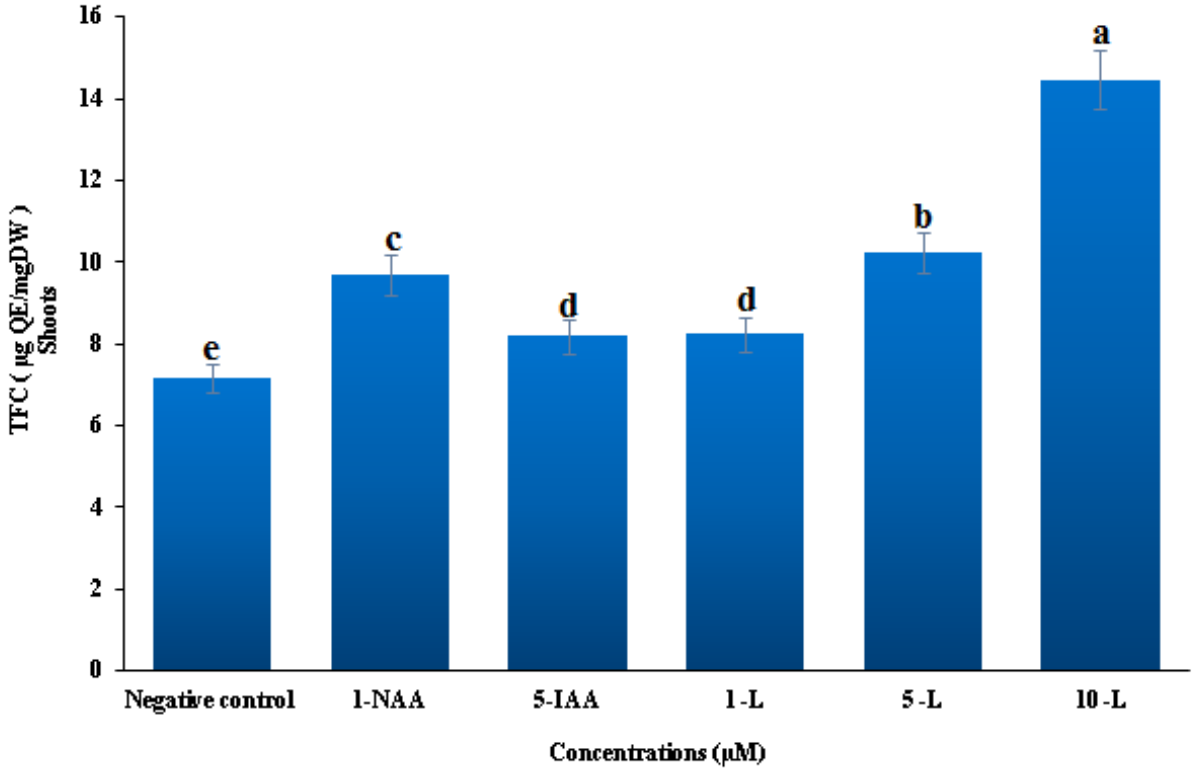

Figure 3.*6* Lupeol(L), NAA and IAA impact on total flavonoid content of P. peruviana shoots. Values represent means ± standard errors from triplicates. The small letters on each value indicate significant difference among mean values at $p<0.05$

3.4 Antioxidant Assays

3.4.1 DPPH

Roots

In the presence of lupeol, the DPPH scavenging activity in *P. peruviana* plant roots was increased relative to the control, which was 20.65% at 1 µM, 22.93% at 5 µM, and 24.95% at 10 µM of lupeol, respectively. The utilization of lupeol resulted in a noticeable enhancement in DPPH-scavenging activity across all concentrations (Fig 3.7). The maximum activity was calculated at a 10 µM, which exhibited a 24.95% in comparison to the positive control of 1 µM

NAA (15.23%). The application of lupeol led to a notable enhancement in DPPH activity when compared to the negative control (19.73%).

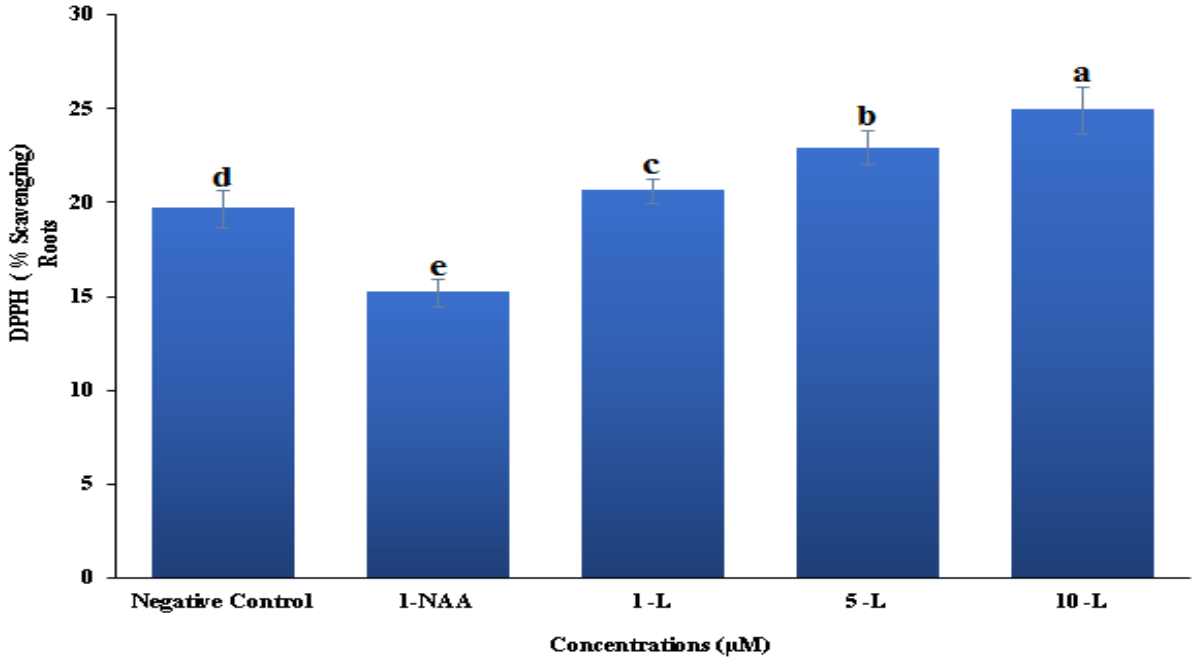

Figure 3.7 DPPH free radical scavenging activity of P. peruviana roots under the impact of lupeol (L) and NAA. Values represent means ± standard errors from triplicates. The small letters on each value indicate significant difference among mean values at $p<0.05$.

Shoots

In the shoots of *Physalis peruviana* plants grown in the presence of lupeol, DPPH scavenging activity decreased at 1 µM and 5 µM, which was 38.03% and 38.11% in comparison of positive controls. However, an increase in DPPH scavenging activity was observed only at 10 µM, which was 46.34% as compared to positive control NAA and positive control IAA, which were 40.59% and 39.46%, respectively. In contrast to the negative control (35.69%), DPPH activity was found to be significantly increased when lupeol was present as shown in figure 3.8.

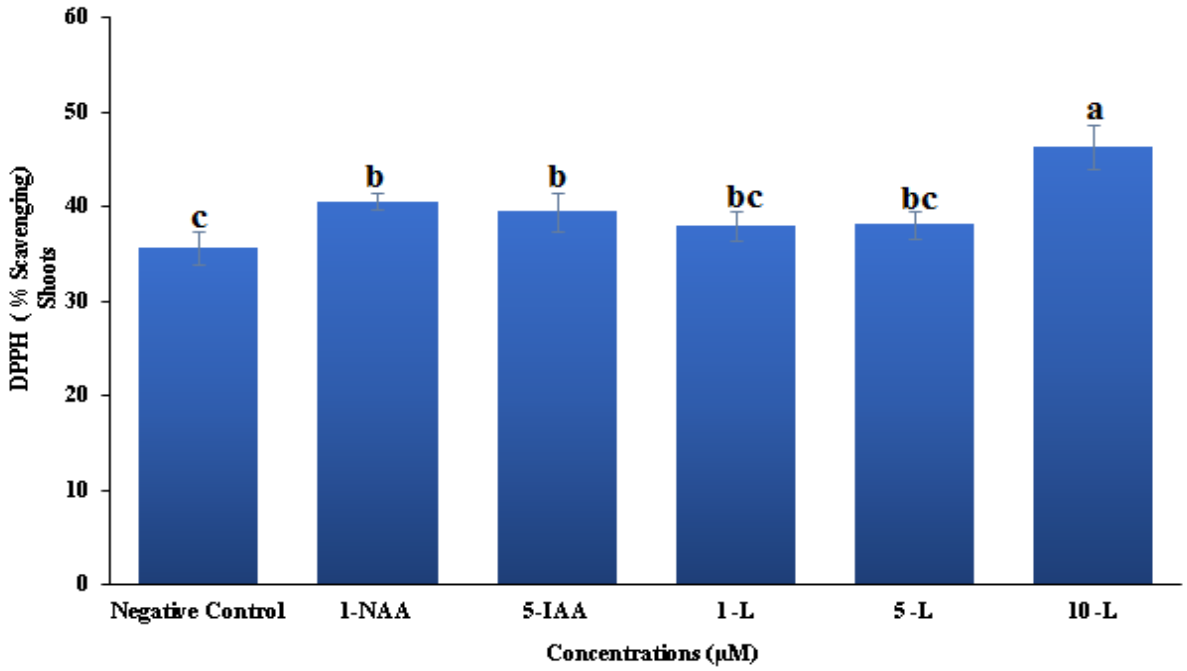

Figure 3.*8* DPPH activity of P. peruviana shoots against lupeol (L), NAA, and IAA. Values represent means ± standard errors from triplicates. The small letters on each value indicate significant difference among mean values at $p<0.05$.

3.4.2 ABTS

Roots

Considerable alteration in the activity of ABTS was detected in the roots of Physalis peruviana plants under the influence of lupeol as compared to the control. In comparison to positive control 1µM NAA (16.25%), ABTS activity increased at different concentrations of lupeol, which was 18.41% at 1 µM, 27.16% at 5 µM, while at 10 µM ABTS activity was 30.5%, respectively (Fig 3.9). In comparison to the negative control (22.41%), the ABTS activity exhibited a decrease of 18.41% at a concentration of 1 µM of lupeol. However, a notable rise in ABTS activity was calculated at higher concentrations of 5 µM and 10 µM of lupeol.

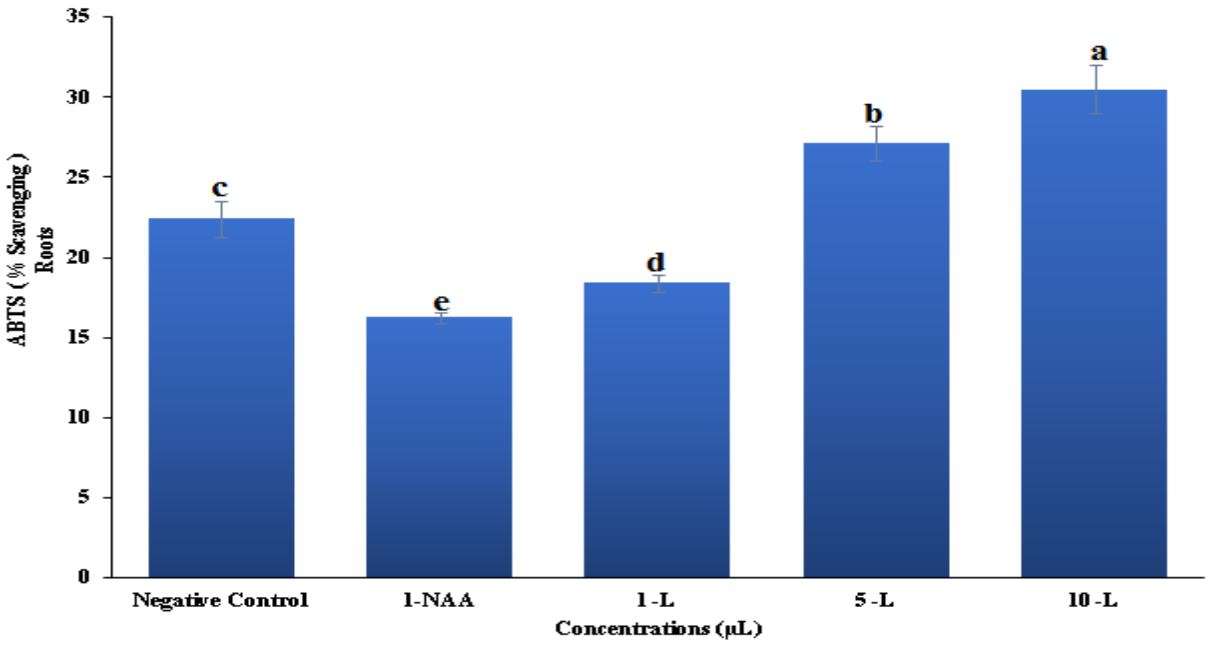

Figure 3.9 ABTS free radical scavenging activity of *P. peruviana* roots against lupeol (L) and NAA. Values represent means ± standard errors from triplicates. The small letters on each value indicate significant difference among mean values at $p<0.05$.

Shoots

ABTS activity in shoots of lupeol-treated *P. peruviana* plants decreased at 1 µM as compared to positive control NAA (27.33%) and positive control IAA (20.91%) which was 19.33%. However, at higher concentrations of lupeol ABTS activity was increased significantly, which was 28.5% at 5 µM and 39.75% at 10 µM. In comparison to the negative control (30.58%), ABTS activity decreased at 1 µM (19.33%) and 5 µM (28.5%) while ABTS activity increased at 10 µM which was 39.75% respectively as shown in figure 3.10.

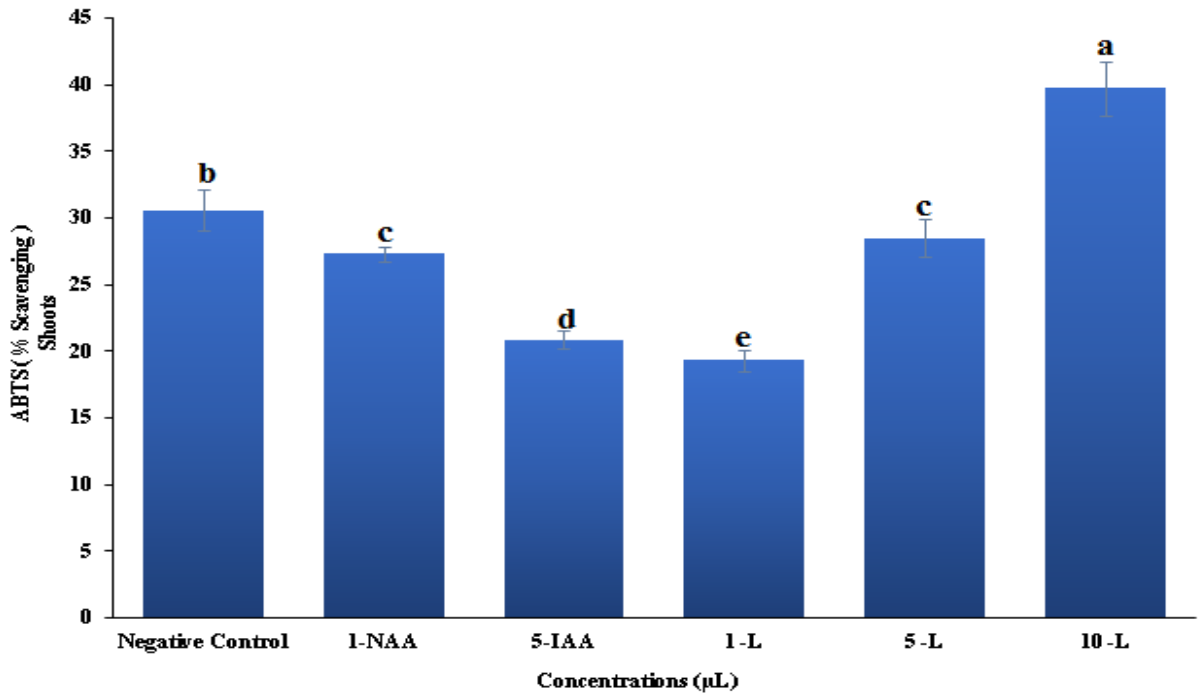

Figure 3.10 ABTS radical scavenging activity of *P. peruviana* shoots under impact of lupeol (L), NAA, and IAA. Values represent means ± standard errors from triplicates. The small letters on each value indicate significant difference among mean values at $p<0.05$.

3.4.3 Metal Chelating

Roots

A significant enhancement in metal chelating activity was noticed in the roots of P. peruviana plants when treated with lupeol, in comparison to the positive control NAA (43.25%), which was 44.25% at 1 µM, 47% at 5 µM and at 10 µM it was 65.62%. As compared to the negative control (49.87%), MC activity was observed to decrease at 1µM and 5 µM, while at 10µM (65.62%) of lupeol, MC activity significantly increased (Figure 3.11).

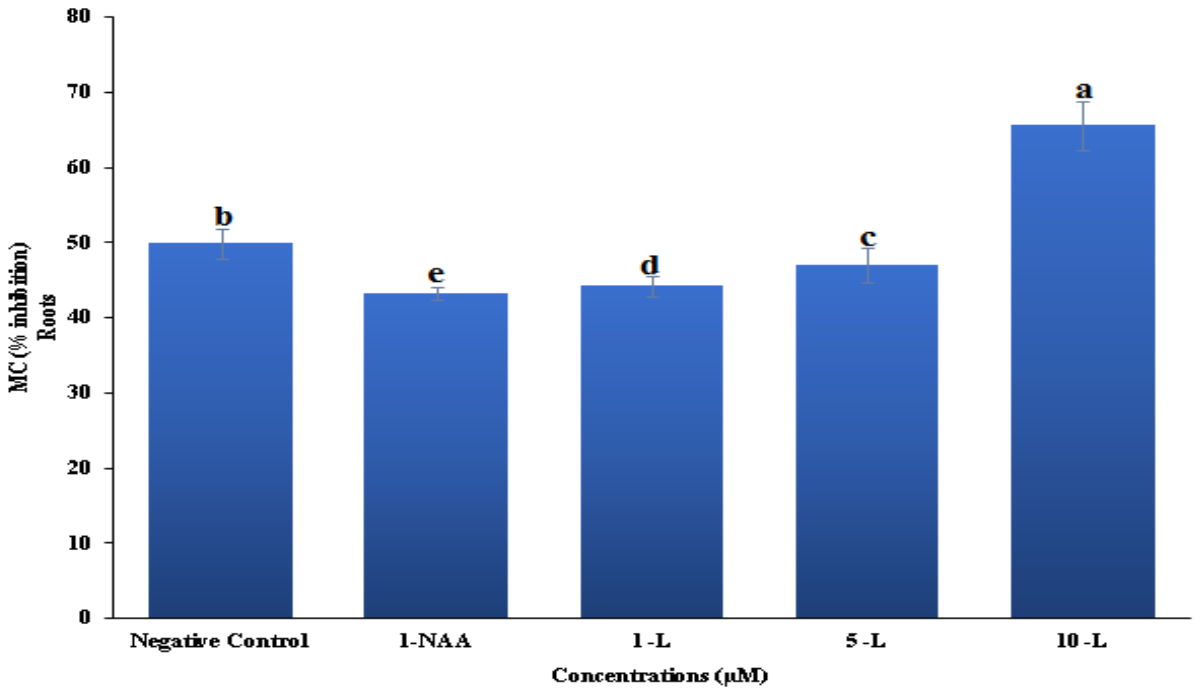

Figure 3.11 Impact of lupeol (L) and NAA on metal chelating activity of *P. peruviana* roots. Values represent means ± standard errors from triplicates. The small letters on each value indicate significant difference among mean values at $p<0.05$.

Shoots

In shoots of *P. peruviana* plants MC activity increased at 1µM, 5µM, and 10 µM of lupeol in comparison to positive control NAA (33%), which was 38.62%, 44.87%, and 54%, respectively. MC activity also increased in comparison to positive control IAA (30.37%). However, MC activity was observed to decrease as compared to negative control (39%) at 1µM and 5 µM of lupeol, while at 10 µM significant increase (54%) was observed as shown in figure 3.12.

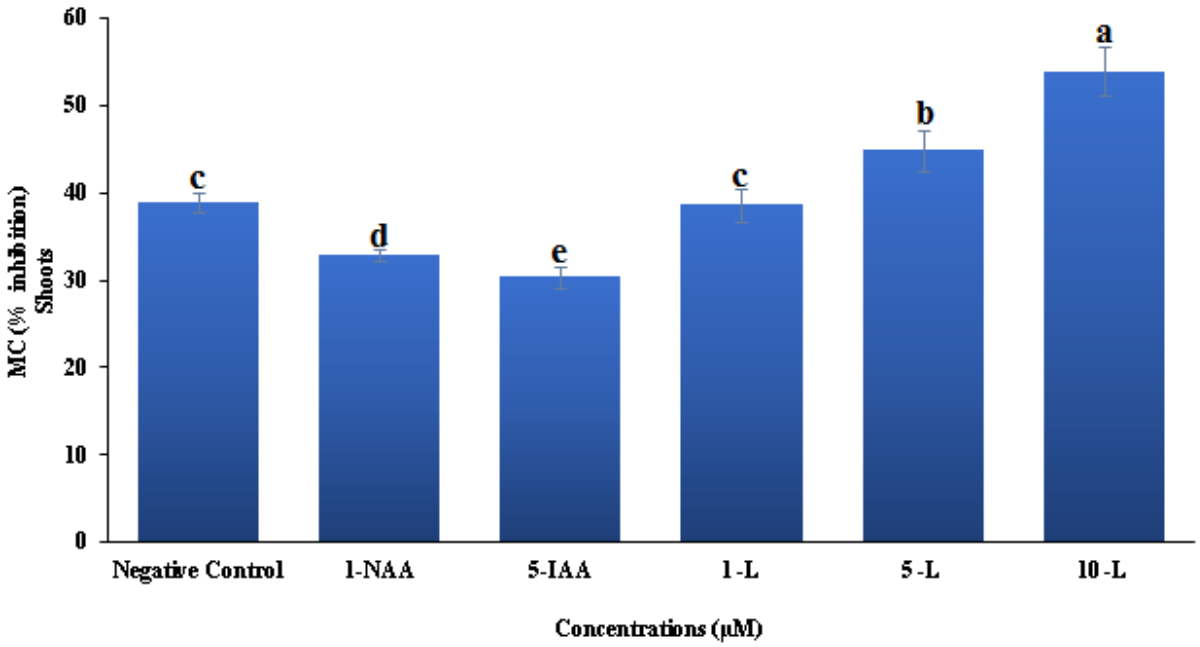

Figure 3.12 Impact of lupeol (L), NAA, and IAA on metal chelating activity of *P. peruviana* shoots. Values represent means ± standard errors from triplicates. The small letters on each value indicate significant difference among mean values at $p<0.05$.

3.5 Total Antioxidant Capacity (TAC)

Roots

An elevation in the overall antioxidant capacity was noted in the roots of plants cultivated in the presence of lupeol, in comparison to the positive control NAA (28.33 µgAAE/mg D.W). Specifically, at a concentration of 1 µM, the total antioxidant capacity (TAC) was measured at 29.80 µgAAE/mg D.W, while at 5 µM, it increased to 36.69 µgAAE/mg D.W. Furthermore, a TAC of 45.31 µgAAE/mg D.W was recorded at 10 µM of lupeol. In contrast to the negative control (30.15 µgAAE/mg D.W), the TA capacity exhibited a decrease at 1 µM (29.80 µgAAE/mg D.W), while an increase in TA capacity was observed at 5 µM and 10 µM compared to the negative control (Fig 3.13).

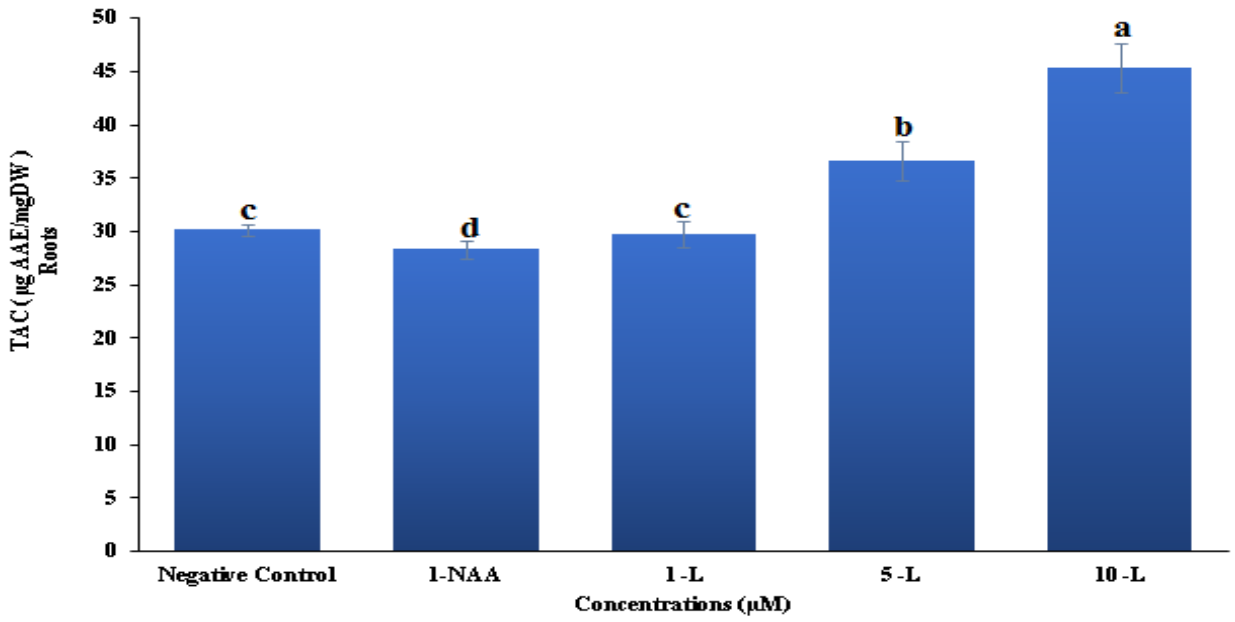

Figure 3.13 Total antioxidant capacity of *P. peruviana* roots against lupeol (L) and NAA. Values represent means ± standard errors from triplicates. The small letters on each value indicate significant difference among mean values at $p<0.05$.

Shoots

TAC decreased at 1 µM and 5 µM which were 42.21 gAAE/mg D.W and 44.19 gAAE/mg D.W. in the shoots of *Physalis peruviana* plants cultivated in the presence of lupeol in comparison of NAA and IAA. Nevertheless, a notable rise in overall antioxidant capacity was noted at a concentration of 10 µM, reaching a value of 62.73 µgAAE/mg D.W. This value surpasses the antioxidant capacities of the positive controls NAA and IAA, which were 49.02 µgAAE/mg D.W and 39.71 µgAAE/mg D.W, respectively. In contrast to the negative control, which demonstrated a total antioxidant capacity of 40.57 µgAAE/mg D.W, the application of lupeol led to a noteworthy augmentation in total antioxidant capacity (Fig 3.14).

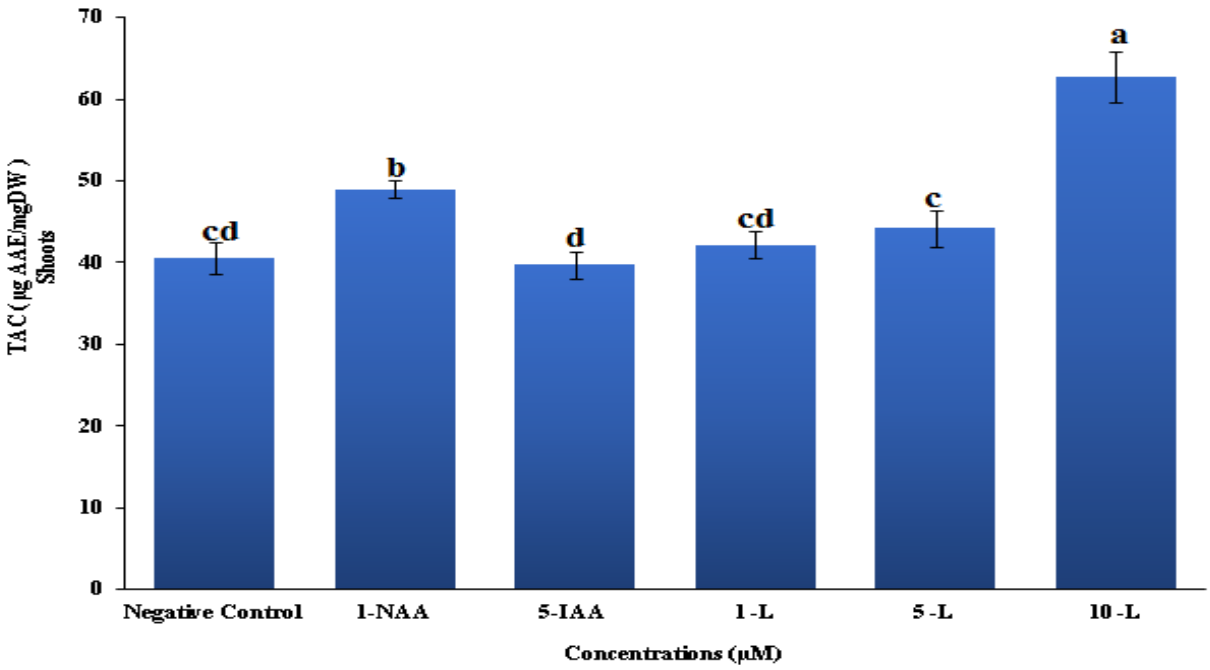

Figure 3.14 Total antioxidant capacity of *P. peruviana* shoots against lupeol (L), NAA, and IAA Values represent means ± standard errors from triplicates. The small letters on each value indicate significant difference among mean values at $p<0.05$.

3.6 Total Reducing Power (TRP)

Roots

A notable enhancement in total reducing power was observed in the roots of *P. peruviana* plants cultivated under the influence of lupeol, in comparison to the positive control NAA (8.52 µgAAE/mg D.W). The minimum reducing power was seen at a concentration of 1 µM of lupeol, yielding a measurement of 9.65 µgAAE/mgD.W. Conversely, the maximum reducing power was recorded at a dosage of 10 µM, resulting in a measurement of 28.71 µgAAE/mgD.W. The treatment of lupeol caused a noticeable increase in TRP levels at 5 µM and 10 µM of lupeol, relative to the negative control (10.28 µgAAE/mg D.W) as shown in figure 3.15.

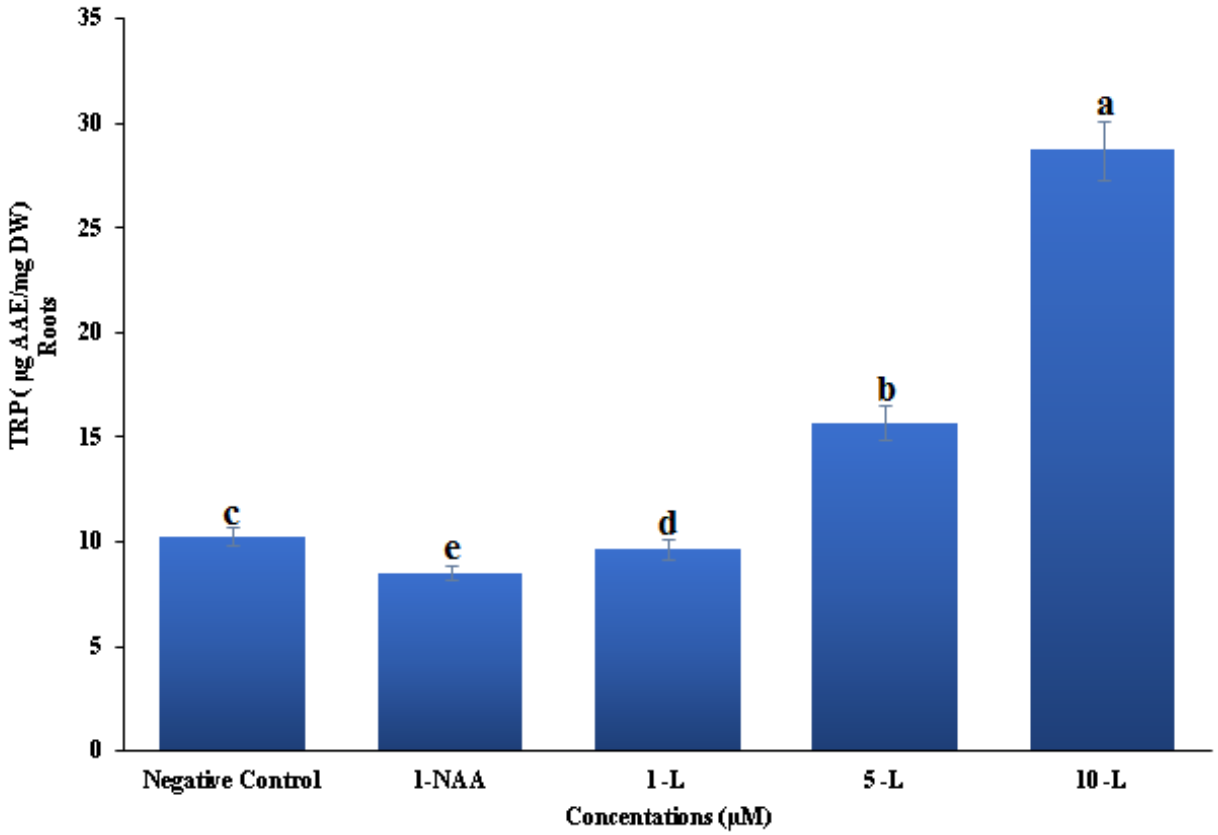

Figure 3.15 Total reducing power of *P. peruviana* roots against lupeol (L) and NAA. Values represent means ± standard errors from triplicates. The small letters on each value indicate significant difference among mean values at $p<0.05$.

Shoots

Shoots of *physalis peruviana* plants exhibited a decrease in TRP levels at a concentration of 1µM (17.73 µgAAE/mg D.W) when treated with lupeol relative to control. However, a rise in TRP levels was recorded at 5 µM (25.24 µgAAE/mg D.W) and 10 µM of lupeol (27.62 µgAAE/mg D.W) in comparison to the positive control NAA (19.80 µgAAE/mg D.W) and IAA (19.02 µgAAE/mg D.W). Application of lupeol led to a significant elevation in TRP levels in comparison to the negative control (18.37 24 µgAAE/mg D.W) as shown in figure 3.16.

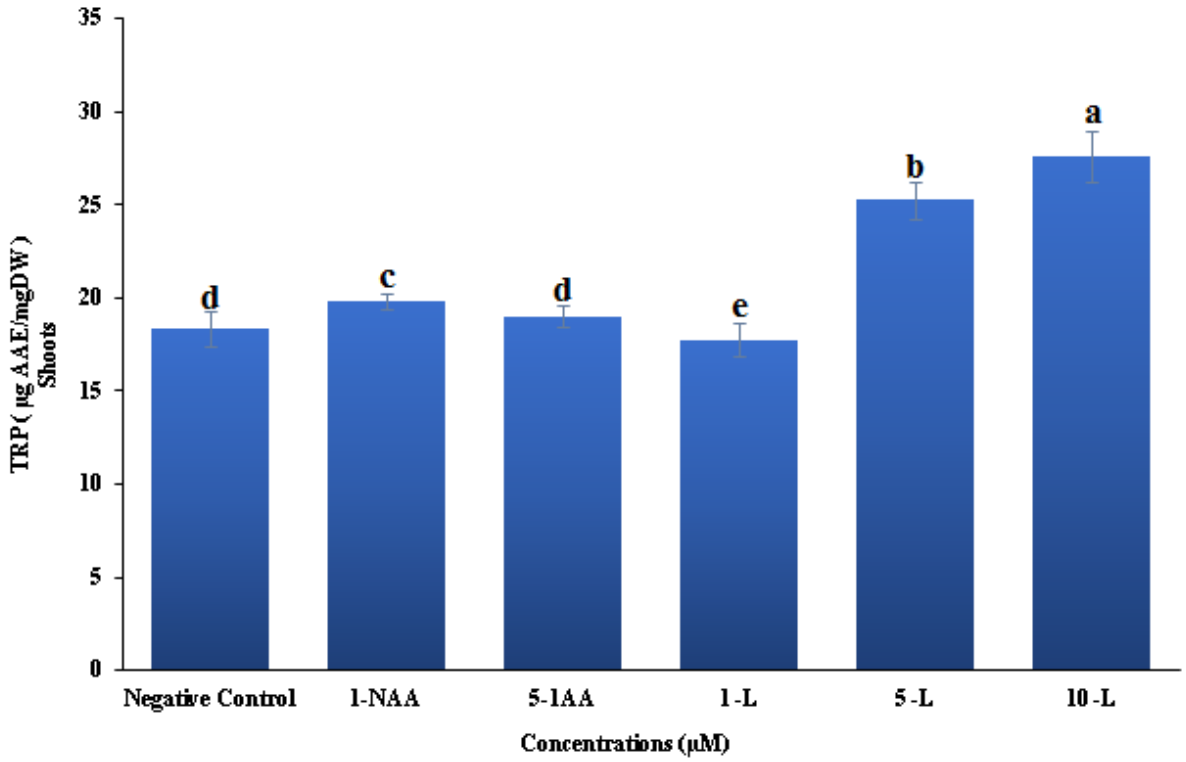

Figure 3.16 Total reducing power of *P. peruviana* shoots against NAA, IAA, and lupeol (L). Values represent means ± standard errors from triplicates. The small letters on each value indicate significant difference among mean values at $p<0.05$.

3.7 Determination of Antioxidant Enzymes Activities

3.7.1 Superoxide Dismutase (SOD)

Roots

A rise in the enzymatic activity of Superoxide dismutase (SOD) was noted when lupeol was present, in comparison to the positive control NAA (0.08 nM/min/mgFW), in the roots of the *Physalis peruviana* plant. The lowest activity was detected at 1 µM of lupeol, yielding an activity rate of 0.087 nM/min/mgFW. Conversely, the concentration of 10 µM exhibited the greatest amount of activity, leading to an activity rate of 0.10 nM/min/mgFW. Plants cultured under different amounts of lupeol exhibited a reduction in SOD activity (0.08, 0.09, and 0.104

nM/min/mgFW), in comparison to the negative control (0.107 nM/min/mgFW), as depicted in figure 3.17.

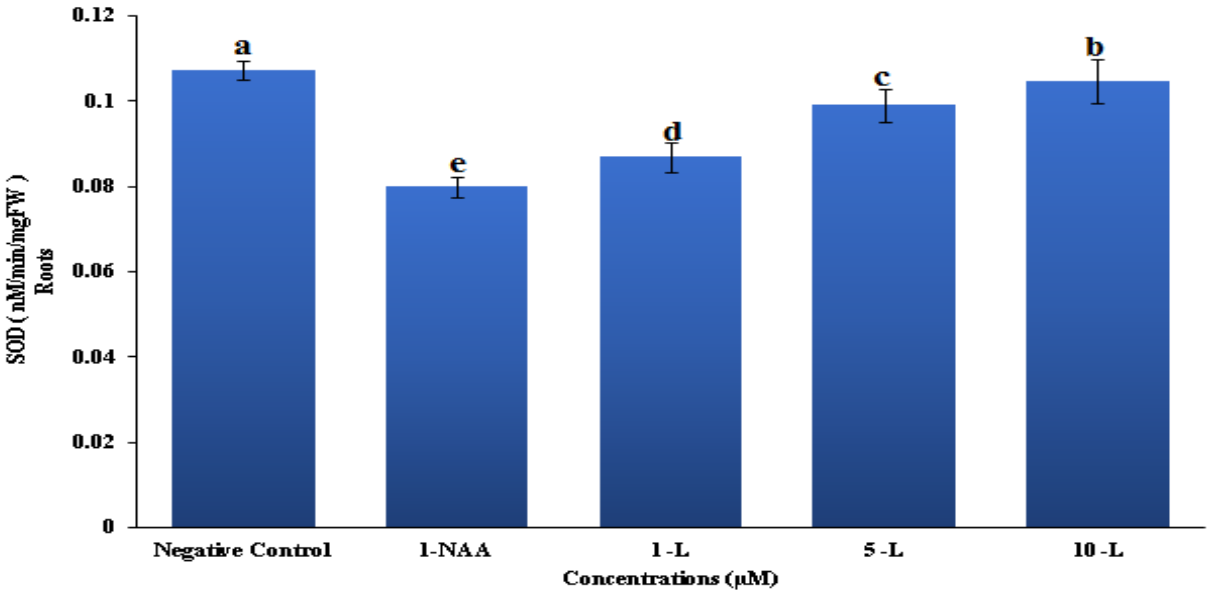

Figure 3.17 Activity of superoxide dismutase of *P. peruviana* roots against NAA and lupeol (L)**.** Values represent means ± standard errors from triplicates. The small letters on each value indicate significant difference among mean values at $p<0.05$.

Shoots

Superoxide dismutase activity was decreased (0.0864 nM/min/mgFW) at 1 µM concentration of lupeol as compared to positive control 5µM IAA (0.0889 10 nM/min/mgFW) and positive control 1 µM NAA (0.0883 10 nM/min/mgFW) in shoots of *Physalis peruviana*. However, an increase in superoxide dismutase activity was observed at higher concentrations of lupeol in comparison to positive control where 0.0952 nM/min/mgFW value was observed at 5 µM and 0.1256 nM/min/mgFW was observed at 10 µM of lupeol respectively. While in comparison to negative control (0.111 nM/min/mgFW), superoxide dismutase activity decreased at 1 µM and 5µM of lupeol while at 10 µM SOD activity slightly increased (0.1256 nM/min/mgFW) as depicted in figure 3.18.

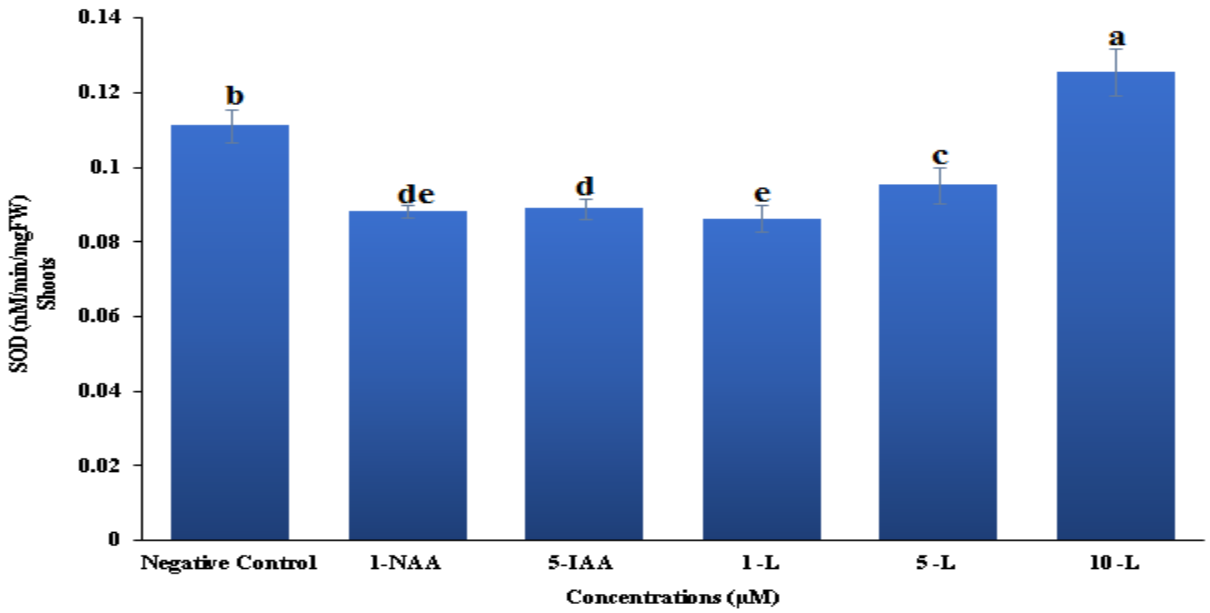

Figure 3.18 Activity of superoxide dismutase of *P. peruviana* shoots against NAA, IAA, and lupeol (L). Values represent means ± standard errors from triplicates. The small letters on each value indicate significant difference among mean values at $p<0.05$.

3.7.2 Peroxidase (POD)

Roots

The presence of lupeol resulted in an increase peroxidase (POD) activity in roots. The minimum POD activity was recorded at 1 µM of lupeol, measuring 2.308 nM/min/mgFW (Fig 3.19). Conversely, the maximum POD activity was detected at 10 µM, measuring 2.504 nM/min/mgFW as compared to positive control NAA (1.049 nM/min/mgFW). The POD activity exhibited an increase at various concentrations of lupeol (2.308, 2.494, and 2.504 nM/min/mgFW), when compared to the negative control (1.07 nM/min/mgFW).

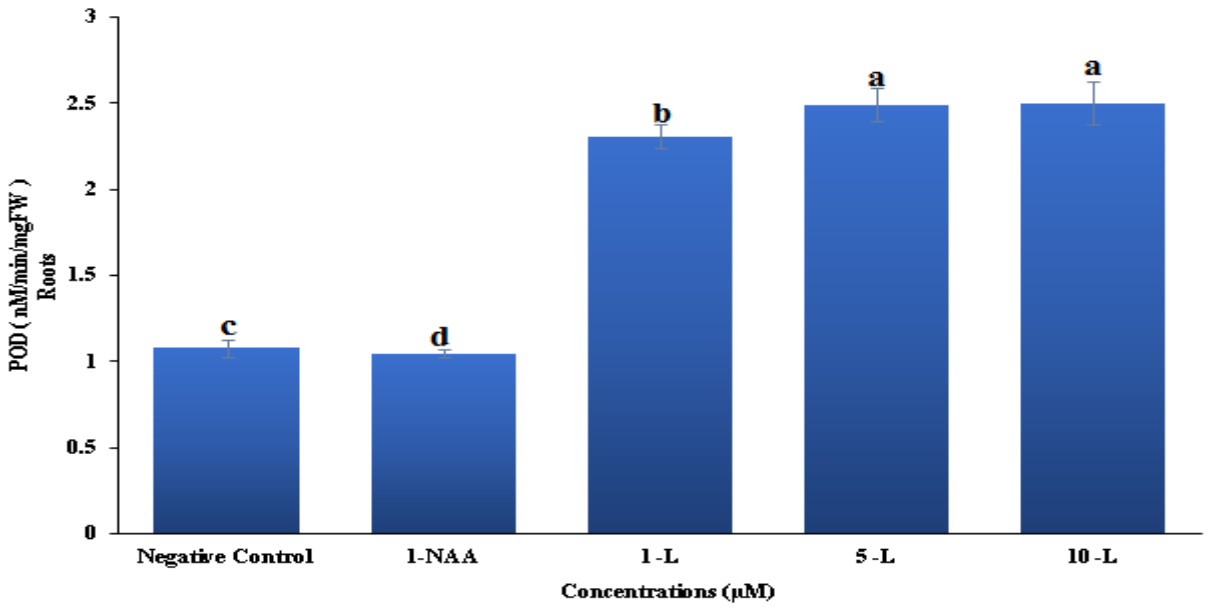

Figure 3.19 Peroxidase activity of *Physalis peruviana* roots against NAA and lupeol (L). Values represent means ± standard errors from triplicates. The small letters on each value indicate significant difference among mean values at $p<0.05$.

Shoots

In shoots POD activity was found to increase in the presence of lupeol where minimum POD activity was observed at 1 µM which was 0.862 nM/min/mgFW and maximum POD activity was observed at 10 µM which was 1.624 nM/min/mgFW as compared to positive controls NAA (0.677 nM/min/mgFW) and IAA (0.644 nM/min/mgFW). POD activity was higher at different concentrations of lupeol (0.862, 1.372, and 1.625 nM/min/mgFW) in comparison to the negative control (0.733 nM/min/mgFW) as shown in figure 3.20.

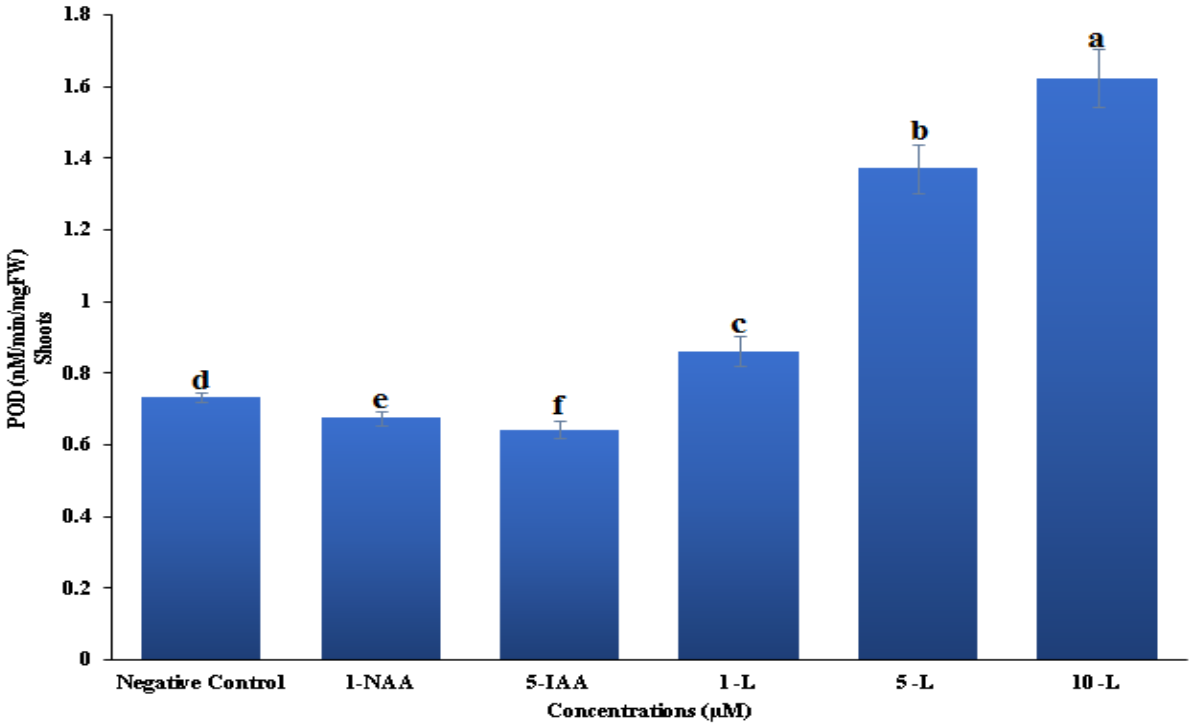

Figure 3.20 Peroxidase activity of *Physalis peruviana* shoots against NAA, IAA and lupeol (L). Values represent means ± standard errors from triplicates. The small letters on each value indicate significant difference among mean values at $p<0.05$.

CHAPTER 4

4 DISCUSSION

4.1 Discussion

The present investigation demonstrated that lupeol exhibited a favorable influence on the growth characteristics of *Physalis peruviana*. Lupeol acted as a growth stimulant, since it resulted in a notable enhancement of growth metrics as compared to the control group. 10 µM lupeol was the optimal concentration for better growth of plants. The root length and shoot length increased in lupeol-treated plants as compared to control groups (NAA, IAA, and blank). Increase in lupeol concentration enhanced the root and shoot lengths. At 10 µM of lupeol, 135% rise in root length and 83% in shoot length was observed. Fresh weight of roots increased up to 125% while dry weight enhanced up to 4 times at 10 µM of lupeol. A comparable pattern was noted in the measurements of fresh and dried weight of shoots. The findings align with a prior investigation conducted by Zia et al. (2022), which shown a favorable influence of lupeol on growth parameters under conditions of salt-induced stress. One possible explanation for this phenomenon is that triterpenoids are known to have significant involvement in several physiological and developmental mechanisms within the plant kingdom. The potential mechanisms underpinning this phenomenon may be attributed to the interaction between lupeol and plant growth regulators, including auxins and cytokinins, which are recognized for their ability to regulate several physiological processes in plants. Triterpenoids possess a vital function in the intricate defense mechanisms employed by plants to protect themselves against various threats, as highlighted by Cárdenas et al. (2019). Moreover, these remarkable compounds are not limited to defense alone, but also actively participate in the intricate processes governing plant development, as reported by (Szakiel et al., 2012).

The phenolic contents of both shoots and roots exhibited a significant increase, with shoots experiencing a remarkable 49% increment and roots showing a more modest 10% rise at 10 µM of lupeol. Similarly, the flavonoid content in both shoots and roots demonstrated an upregulation, with shoots displaying a striking 100% enhancement and roots exhibiting a more moderate 24% increase. Phenolics, a type of non-enzymatic antioxidants, have gained significant recognition for their ability to defend plants against the accumulation of reactive oxygen species (ROS)

(Sharma et al., 2012). The results of present study demonstrate that plants treated with lupeol exhibited a higher accumulation of phenolic and flavonoid content in comparison with positive and negative controls. This suggests that lupeol application induces the production of secondary metabolites in plants as a response to abiotic stress (Javed et al., 2022). These results are consistent with Geneva et al. (2022) who reported higher phenolic and flavonoid contents in Physalis peruviana micro propagated plantlets when cultured in presence of 1 mg/ L of BAP and 1 mg/L of Zeatin.

The results of current investigation showed notable disparities in the antioxidant activity and overall reducing capacity of plants when exposed to varying concentrations of lupeol. The total reducing power (TRP) in control shoots of P. peruviana was measured to be 18.37 µgAAE/mgDW. The TRP exhibited an increase in the presence of 1 µNAA and 5 µM IAA. In case of lupeol, TRP exhibited a notable increase, reaching a level of 27.62 µgAAE/mgDW at a concentration of 10 µM. No statistically significant variation was observed in the levels of TRP, in both shoots and roots grown under the influence of 1µM concentration of lupeol, relative to the control group. Nevertheless, a progressive augmentation in the TRP was discerned as the concentrations of lupeol were systematically elevated. The study observed that in roots TRP exhibited a higher level at a concentration of 5 µM of lupeol, which further increased with the rise in concentration. The activity of total antioxidant capacity (TAC) in roots was found to be diminished in the presence of NAA. Similarly, when examining the effects of lupeol, TAC activity decreased at a concentration of 1µM. However, intriguingly, it exhibited a gradual upsurge as the concentration of lupeol was incrementally elevated. The introduction of indole-3-acetic acid (IAA) led to a reduction in the overall antioxidant capacity (TAC) activity observed in the shoots, as compared to the negative control. Conversely, the addition of NAA led to an enhancement of TAC activity. However, it is important to acknowledge that the utilization of lupeol on the botanical specimens yielded a notable augmentation in the TAC. This enhancement was particularly pronounced at a concentration of 10 µM, where the TAC activity reached its peak at 62.73 µgAAE/mgDW. The observed elevation in TRP (total radical trapping potential) and TAC (total antioxidant capacity) activity levels with increasing concentrations of lupeol suggests a potential dose-dependent relationship, wherein lupeol may have the ability to enhance the antioxidant activity of the plant. The elevated levels of secondary metabolites and antioxidants are intricately associated with the plant's defense mechanism, which is triggered in

response to various stressful conditions (Bartwal et al., 2013). These results are similar to Namiesnik et al. (2013) who found that antioxidant activity of Physalis peruviana water extract was higher than that of blue berries and cranberries

The presence of lupeol has been observed to induce changes in various antioxidant activities, including free radical scavenging activity as recorded by the DPPH assay, ABTS assay, and metal chelating ability. The utilization of NAA(1µM) and IAA (5µM) resulted in a notable enhancement of the free radical scavenging activity (DPPH) in the aerial parts of plants. Specifically, the application of NAA led to a 14% enhancement in free radical scavenging activity, while the application of IAA resulted in an 11% increase. The efficacy of lupeol in enhancing the activity was observed relative to the control groups. Notably, the activity exhibited a substantial rise of up to 31% at a concentration of 10 µM of lupeol. The DPPH scavenging activity of the roots exhibited a decrease in the presence of NAA, whereas the activity was observed to be upregulated in a similar fashion when lupeol was introduced. The findings from the ABTS and metal chelating assays provide additional evidence supporting the potential antioxidant properties of lupeol. At 10 µM of lupeol ABTS activity was induced by 36% and MC scavenging activity by 32% in roots of *Physalis peruviana* in comparison to control groups. An analogous pattern was noted in the shoots, wherein the ABTS activity exhibited an upregulation of 30% and the MC activity displayed an upregulation of 38%, respectively. The present study highlights the potential of lupeol in mitigating free radical-induced damage, as evidenced by the observed rise in DPPH, ABTS, and metal-chelating activities at higher concentrations of this compound. These results are corelated to previous study where free radical scavenging activity enhanced with increasing the ethanol extract concentration of *Physalis peruviana* fruit (El-Beltagi et al., 2019). Free radical scavengers possess the ability to directly neutralize peroxide radicals, thereby halting the progression of the chain reaction. The augmentation of antioxidant activity signifies a reduction in the generation of free radicals, achieved through the inhibition of free radical-generating enzymes or the enhancement of other antioxidant enzymes' activities and expressions (Zaeem et al., 2020).

Under typical conditions of plant growth, there exists a finely balanced state between the generation and elimination of ROS. The equilibrium can frequently be disrupted in the presence of abiotic or biotic stress factors (Gill and Tuteja, 2010). SOD is currently regarded as the

primary protective mechanism against the production of ROS (Kregel & Zhang, 2007). In the present study, the activity of antioxidant enzymes (SOD) in the roots of *P. peruviana* was observed to decrease by 25% when treated with NAA. In contrast to the negative control, the activity level of SOD exhibited a reduction of 18% at a concentration of 1 µM, 7% at 5 µM, and 2% at 10 µM in plants treated with lupeol. A comparable pattern was detected in shoot samples at concentrations of 1 and 5 µM of lupeol. However, at 10 µM of lupeol, a notable increase of 10% in SOD activity was observed. These results are consistent with Zia et al. (2022) where similar variations in SOD activity were recorded by increasing concentration of lupeol in *Brassica nigra* under salt stress.

The application of lupeol to plants resulted in a concentration-dependent rise in the activity of peroxidase (POD). In the roots of *P. peruviana*, 114% rise in POD activity was observed at a concentration of 1µM lupeol, followed by a 132% increase at 5µM, and a 133% increase at 10µM. In shoots POD activity was likewise augmented through the elevation of lupeol concentration. It is imperative to acknowledge that the activity is contingent upon the level of stress-inducing chemicals, the free radicals' formation, the accessibility of non-enzymatic antioxidants, and the physiology of the plant. These results are similar to a previous study where POD level was enhanced in *Physalis peruviana* seedlings under stress conditions (Roveda-Hoyos et al., 2019). Previous study has indicated a correlation between the activation of antioxidative enzymes and the presence of induced stress (Hanif et al., 2023). The purpose of antioxidative enzyme activation process is to alleviate the oxidative harm induced by reactive oxygen species (ROS) in plants (Husen et al., 2018). In order to mitigate the deleterious impacts, there is an elevation in the levels of antioxidative enzymes and molecules, which serve to counteract the presence of reactive oxygen species (Zafar et al., 2020).

4.2 Conclusion and future prospective

The study conducted on Physalis peruviana plants in the presence of lupeol has yielded substantial findings regarding the impact of this naturally occurring triterpene on morphological traits. The results of this research show the significant impact of lupeol on promoting growth, as demonstrated by the considerable increase in plant length and biomass traits across different concentrations.

The results of this study indicate that lupeol exhibits significant potential as a growth regulator for P. peruviana and potentially other plant species. The notable improvements observed in growth characteristics, biochemical profiles, and antioxidant parameters hold considerable implications for agricultural practices, potentially heralding advancements in crop productivity and overall plant well-being. The findings of current study carry significant ramifications for the fields of agriculture, plant biotechnology, and pharmacology. The examination of lupeol's potential antioxidant properties and its influence on growth parameters presents an intriguing opportunity for leveraging crop enhancement and the production of therapeutic natural products. Moreover, this study establishes a foundation for forthcoming investigations to further explore the physiological mechanisms that underlie the growth-enhancing properties of lupeol. Additionally, it paves the way for exploring the potential applications of lupeol in diverse areas of agriculture.

CHAPTER 5
5 REFERENCES

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