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Phytochemical Analysis of Medicinal Plant Extracts and its Growth Promoting Performance on *Amaranthus Gangeticus* (L.) and *Amaranthus Aritis* (L.) Lettuces.

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Abstract*— Investigation was carried out on the phytochemical and growth performance of ethanol extracts of three different plants and their phytotoxic effect on the growth of *Amaranthus gangeticus* (L.) and *Amaranthus aritis* (L.). The phytochemical analyses indicated the presence of bioactive substances such as alkaloids, carbohydrates, Flavonoids, Phenolic Compounds, Proteins and Amino acid, Terpenoids, Tannins, Saponins, Glycosides and Reducing Sugars in the ethanolic extracts of selective plants. The allelochemicals were of higher concentrations in the ethanolic extract of the plant *Pungamia pinnata* than other plants. The ethanolic extract was found to be more effective since the reduction of the germination percentage of both the lettuce was observed in *Ocimum tenuiflorum* (93.7%, 87.5%). But the moderate germination percentage was noticed in the ethanolic extracts of *P. pinnata* (74.4%, 60.1%) treated both the lettuces. the least germination percentage was noticed in the control category for both lettuces (7.0%, 27.0%) respectively. Likewise the germination index and total water content of both lettuces increases when treated the ethanolic extracts of plant extracts. The root and shoot length and also the fresh and dry weight was observed according to the effects of ethanolic extracts of plants like *P. pinnata, A. indica* and *O. tenuiflorum*. Thus the results of this study suggest that, all the ethanolic extracts were have the great growth performance of *A. gangeticus* and *A. aritis.

***Keywords*—Phytochemical screening, Germination percentage, Plant extracts, Lettuces.**

I. Introduction

Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization. Nowadays many intensive field vegetable production systems are not sustainable due to causing severe environmental damage. Agriculture involves the growing, processing, and distribution of food and its byproducts by way of intensive plant cultivation and animal husbandry in and around cities in order to feed the local populations [1, 2, 3].

Considering that high yields are central to a sustainable food supply given a finite land resource in urban areas [3] creating the motivation for innovative agricultural practices such as hydroponics and organic farming is thus imperative if food supply in cities is to be maintained. Among the benefits of urban agriculture include increased access to healthy and affordable produce for urban residents, while creating less pollution from transportation and waste products [4]. On the other hand, organic farming is a system aimed at producing food with minimal harm to ecosystems, animals, or humans [5, 6]. Organic farming or agriculture matches or even exceeds yields from conventional farming. While nonorganic methods result in slightly higher yields in developed areas, organic methods result in slightly higher yields in developing areas [7].

In 2019, lettuce production reached 29,134,653 tons in the world from which about 547,590 tons were produced in Iran [8]. Considering the important and valuable nutritional role of lettuce due to its daily consumption and, on the other hand, the excessive application of chemical fertilizers with negative impacts on human health and the environment; there is an effort to find alternative methods for reducing the chemical fertilizers input in lettuce production areas. The use of chemical fertilizers is widespread throughout the world, leading to soil degradation and environmental pollution. Therefore, the global approach to establishing a sustainable agricultural system has changed with the employment of new management methods. Considering this, it is important to pay attention to the biological and integrated systems, especially biofertilizers, to meet the plant nutritional requirements and reduce the consumption of chemical fertilizers [9]. Bio-fertilizers (bio-stimulants) release their content to make it slowly available to the plants and simultaneously improve soil quality [10]. Therefore, the use of bio-fertilizers has advantages such as removing toxic substances and improving the physicochemical properties of soil [11].

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This scenario sets a challenge to develop eco-friendly innovative methods that increase agricultural yields, with minimum input [12, 13] by adopting sustainable production practices that could reduce or substitute the use of chemical inputs like chemical fertilizers and pesticides with natural or biological substances. The development of natural substances promoting plant growth called plant biostimulants and water-holding polymer gels has received increased attention [14, 15].

Tulsi (*Ocimum tenuiflorum* L.) is an aromatic shrub belonging to the Lamiaceae family of basil (tribe ocimeae). It originated in north-central India and is now cultivated as a native species in the eastern tropics of the world [16]. Tulsi is said to improve the appearance of the skin, the sweetness of the voice, and the development of intelligence, and stamina [17, 18]. Leaves, fruits, essential oils of tulsi exert anti-tussive, antioxidant, antimicrobial, radioprotective, antihypertensive, and immuno modulatory effects [19, 20, 21, 22, 23]. Tulsi also used for the plant fertilizer for the growth of lettuces, this study expel the growth performance of *Amaranthus gangeticus* and *Amaranthus aritis*. In tulsi, biologically active compounds have been isolated from the leaves including ursolic acid, apigenin and luteolin that activates the cell mediated immune response and therefore, creates an enhanced response to any future challenges occurred by disease organisms.

The neem plant (*Azadirachta indica* A. Juss) is a tree that is common in the Northern parts and sparsely distributed in the Northwest Region of Cameroon. It is used as a remedy for several pathologies, amongst which we have gastric ulcers which is our area of interest. Neem has several botanical benefits, that's why, this plant leaf used as plant fertilizer for the growth of

A. gangeticus and *A. aritis*. The neem leaves and fruit both exceedingly bitter are known to possess fungicidal, and nematicidal properties [24]. Besides, it is known to induce some toxic effects; neem preparations fed to laying hens have been reported by Gowda et al [25] to significantly reduce the content of haemoglobin, erythrocyte count and packed cell volume.

Pongamia pinnata (Linn.) is frequently notable as karanja, kanji, Indian beech is an adaptable tree and has been recognized in different system of traditional medicines. The plant is used for anti-inflammatory, anti-spasmodic, anti-hyperglycaemics, anti-lipidoxidative, anti-diarrhoeal, anti-ulcer, antioxidant etc. In Indian sanskriti their seeds were used for skin aliments and anthelmintic properties [26, 27].

Further, plant growth promotion is affected by producing phyto-hormones such as auxin or cytokinin, or by producing the enzyme 1-aminocyclopropane-1- carboxylate (ACC) deaminase, which lowers plant ethylene levels. the medicinal plants will enhance the growth promoting factors in the lettuces.

Lettuce, the most commonly consumed salad vegetable, is an annual winter crop which can be found in the market during whole year [28]. Since its vegetation period is short and market demand is constant, lettuce production increases each year. On the other hand, cultivation practices especially fertilization and pest management must be performed carefully because lettuce is a leaf vegetable and consumed uncooked. This study was conducted to observe the growth of lettuce with three different plant extracts.

II. Matereials And Methods

Source of Plant Materials: Harvesting and identification of plant leaves: The fresh leaves of Ocimum tenuiflorum (Tulsi), Azhadirachta indica (Neem) and Pongamia pinnata (Pungam) were harvested from different villages around the city Sankarankovil, Tenksi, Tamilnadu, India.

Plant material drying of leaves: The collected leaves were cleaned and dried in a shade, on mats placed on a flat surface. The dried leaves were pulverized to get fine powder and stored in airtight containers before extraction.

Extraction of leaf powder: In this process, 1500g of the coarsely powdered crude plant was put in a stoppered container with 1.5L of distilled water and water was allowed to stand at room temperature for a period of 48 hours with frequent agitation until the soluble matter has dissolved. The mixture was then strained, the marc (the damp solid material) was pressed, and the combined liquids were clarified by filtration using Whatman paper and collected the supernatant. This filtrate was evaporated and the extract collected for further study.

Phytochemical screening: Several research papers have proven the presence of diverse functional groups in the Neem, Tulsi and Pungam plants. In the scope of our research, were carried out according to the methods described by Trease and Evans [29] of the crude ethanol extracts of leaves for the identification of phytochemicals like alkaloids, carbohydrates, Flavonoids, Phenolic Compounds, Proteins and Amino acid, Terpenoids, Tannins, Saponins, Glycosides and Reducing Sugars.

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Study Site and Research Design: This study was carried out in PG and Research Department of Zoology, Pasumpon Muthuramalinga Thevar College, Melaneelithanallur, Sankarankovil, Tenkasi, Tamilnadu, India. The study was an experimental in field design done in the Research laboratory field.

A. Growth Assessments: The germination percentage (GP) was calculated according to the International Seed Testing Association (ISTA) method:

GP = number of normally germinated seeds/total number of seeds sown × 100 The germination index (GI) was calculated using the following formula:

$$GI = 6(Gt/Tt),$$

where Gt is the number of seeds germinated on day t, and Tt is the number of days [43].

The plants were carefully removed from the field, and the roots were washed using tap water to eliminate the residue from the growth substrate. The number of leaves and root and shoot lengths of 42-day-old plants were measured.

The tissue water content (TWC) was calculated according to the formula of Black and Pritchard [44]:

$$TWC = (Wf - Wd)/W * f\,100.$$

Biomass accumulation was calculated as fresh (Wf) and dry weight (Wd). The dry weight (Wd) was assessed after 72 h of drying at room temperature [30].

B. Statistical analysis: The significance of differences between the means of the treatments was evaluated by one way analysis of variance at the significance level of 5%. The statistical software Excel (Excel Inc., 2003) and SPSS version 11.0 (SPSS Inc., 2003) were used for the analysis.

III. Results And Discussion

The phytochemical analysis of *O. tenuiflorum* ethanol extract showed the presence of alkaloids, carbohydrates, Phenolic Compounds, Proteins and Amino acid, Tannins, Saponins, Glycosides and Reducing Sugars except flavonoids and terpanoids. Phytochemical screening of the *A. indica* ethanol leaf extract showed the presence of alkaloids, carbohydrates, Phenolic Compounds, Proteins and Amino acid, Terpenoids, Tannins, Saponins, Glycosides and Reducing Sugars except flavonoids.

These results obtained are similar to those report in earlier studies but different from that found by Dash *et al.* [31] who found alkaloids, saponins, glycosides and reducing sugars [32]. The phytochemical analysis of *A. indica* extract had earlier been reported [33].

Phytochemical screening of the stem bark extract of *A. indica* in the present study also revealed presence of terpenes and glycosides. The same in *P. pinnata* showed the presence of alkaloids, carbohydrates, Phenolic Compounds, Proteins and Amino acid, Tannins, terpanoids, Saponins, Glycosides and Reducing Sugars except flavonoids. Arote *et al.* [34] mentioned their studies have no proteins, but in our study the phytochemical with some amount of proteins which is present, that is useful for the growth of lettuces.

Growth performance of the two different lettuce *Amaranthus gangeticus* and *Amaranthus aritis* were calculated and tabulated. In table 1, the root and shoot length of the different lettuce with various plant extracts at Melaneelithanallur field. Here, the maximum root length was observed in *A. aritis* when treated with *Pungamia pinnata* (10.4) where as in the minimum root length was observed in the control treated for both lettuce. The shoot length was highest in *A. aritis* (13.6) followed by *A. gangeticus* (11.7) when treated with *P. pinnata*. But the lowest shoot length was noticed both lettuces when treated with *Azhadirachta indica* (11.0 and 7.5 respectively) other than control category. The leaf size also maximum in *P. pinnata* (13.6) treated lettuce *A. aritis* but minimum in control (4.1) followed by *A. indica* (5.8) treated *A. gangeticus* (Figure 1). Based on our results the shoot and root length of the both lettuce was highly observed in *P. pinnata* followed by *A. indica* and *O. tenuiflorum*. It is because of the highly rich phytochemical compounds like primary metabolites present in the above said plants. Sarma and Venkatesh [35] studied the phyto-chemistry of aqueous and methanolic leaves extracts of *P. pinnata* and revealed similar finding of alkaloids, tannins, flavonoids, glycosides and sterols. In present findings, sterols, proteins, glycosides and reducing sugars were detected in aqueous extracts whereas anthraquinones was detected in methanolic extract. Maestrini *et al.* [36] studied that Medicago spp. contains saponins and prosapogenins have in vitro inhibiting effects against sheep gastrointestinal strongyle eggs, although with a different level of efficacy. Saponins affect the cell wall integrity interacts with the collagen of the cuticle of the nematodes where by the cell will lose electrolytes and chemicals leading to the flushing of the parasite from gastrointestinal tracts [37].

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Table 1: The root, shoot length and leaf size of lettuce *Amaranthus gangeticus* and *Amaranthus aritis* with various plant extracts at Melaneelithanallur field.

Plant extracts	*Amaranthus gangeticus*			*Amaranthus aritis*		
	Root length	Shoot length	Leaf size (l x b)	Root length	Shoot length	Leaf size (l x b)
Pungamia pinnata	5.42	11.7	5.8	10.4	13.6	13.2
Ocimum tenuiflorum	9.7	11.8	7.0	7.8	9.0	6.3
Azadirachta indica	7.6	11.0	8.7	7.5	8.4	5.8
Control	3.5	5.3	3.4	4.6	4.0	4.1

Note: Data were analyzed statistically at the 5% (p<0.5) level significance

In table 2, fresh and dry weight of the lettuce *A. gangetuicus* and *A. aritis* where treated with different plant extracts. Usually all the plants or lettuce dry weight is lower when compared to the fresh weight of the same. Here also the dry weight of lettuce was lower than the fresh weight of the lettuce. The uppermost fresh (56.0) and dry weight (25.3) was noticed in *A. gangeticus* than in *A. aritis* the moderate fresh (55.6) and dry weight (10.7) was observed when treated with *O. tenuiflorum* and control category respectively. This indicates the humidity of the environment is highly responsible for the dry weight after two days of the lettuce was settled. But the colour and the nature of both lettuce is not changing even after taking the dry conditions. The result was further supported by the work of Arshad *et al.* [38] who reported that water and methanol extracts of *Withania somnifera* markedly suppressed the germination, root and shoot growth of *Parthenium hysterophorus*.

Table 2: Fresh and dry weight of lettuce *Amaranthus gangeticus* and *Amaranthus aritis* with various plant extracts at Melaneelithanallur field.

Plant extracts	*Amaranthus gangeticus*		*Amaranthus aritis*	
	Fresh weight	Dry weight	Fresh weight	Dry weight
Pungamia pinnata	12.3	14.9	42.5	20.8
Ocimum tenuiflorum	54.5	26.4	55.6	16.4
Azadirachta indica	56.0	26.0	46.8	16.0
Control	9.0	5.3	23.3	10.7

Note: Data were analyzed statistically at the 5% (p<0.5) level significance

In table 3, that explains about the growth performance of both lettuces. The total water content was maximum in *A. gangeticus* (74.4) and in *A. aritis* (60.1) when treated with *P. pinnata* followed by *O. tenuiflorum* (63.9, 59.6) and *A. indica* (13.8, 48.1) in both lettuce respectively. The growth performance like germination percentage was highest in *O. tenuiflorum* (93.75%, 87.5%) in both lettuce respectively (Figure 3). Among the naturally occurring plant growth enhancers, Pungam has attained enormous attention because it is considered to be rich in a variety of natural plant growth regulators such as zeatin which belongs to class of cytokinins and thus can be used as a source of cytokinins.

Table 3: Growth performance of lettuce *Amaranthus gangeticus* and *Amaranthus aritis* with various plant extracts at Melaneelithanallur field.

Plant extracts	*Amaranthus gangeticus*			*Amaranthus aritis*		
	TWC	G %	GI	TWC	G %	GI
Pungamia pinnata	74.4	50	2.5	60.1	75	3.75
Ocimum tenuiflorum	63.9	93.75	4.68	59.6	87.5	4.37
Azadirachta indica	13.8	68.75	3.43	48.1	56.25	2.81
Control	1.9	7	0.31	14.7	27	1.25

Note: Data were analyzed statistically at the 5% (p<0.5) level significance

It is also enriched with various macro-nutrients such as phosphorous and potassium along with micro-nutrients [39]. The inhibition of shoot growth may be due to a decrease in photosynthesis; it upsets mineral nutrition and water balance, changes hormonal status and affects membrane structure and permeability [40, 41]. The inhibition of root growth may be due to a decrease in calcium in root tips, leading to a decrease in cell division or cell elongation [42, 43, 44]. The lowest germination percentage was noticed in control (7%, 27%) for both lettuce respectively.

The germination % was highly observed in *A. gangeticus* followed by *A. aritis*. Most importantly the *O. tenuiflorum* induces the growth of the lettuces in 93.75%. in recent researches the plant extracts improve the growth of lettuce and the total water content was also increased. The phytotoxic effects of the methanol extracts and water extract were dependent on concentration of the extracts. This agreed with the work of Lovett [45] who reported that biological activities of receiver plants to allelochemicals are concentration dependent.

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