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In Vivo Protein Profiling & Estimation of Plant Secondary Metabolites, Phylogenetic Analysis of Plant Leaves for Antifungal Activity

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Abstract: ***The present research work involved to evaluated the phytochemical screening on different plant leaf extracts, protein profiling and phylogenetic analysis of leaf extracts which is Psidium guajava, Syzygium cumini, Aegle marmelos, Nyctanthes arbor-tristis, Callistemon lanceolatus, Citrus limonum, Combretum indicum, Calliandra haematocephala, Polyalthia longifolia were used fresh leaves of the plants and analysed the phylogenetic analysis between all plant leaf which having antifungal activity against plant pathogens .According to study it was found that several plant leaf contain different bands of different molecular weight which is Nyctanthes arbortristis 80KDa, 72KDa ,70KDa, 63KDa, 61KDa, 53KDa, 50KDa, 41KDa, 38KDa, 37KDa. Aegle marmelos 72KDa, 70KDa, 64KDa, 54KDa, 54KDa, 40KDa, 36KDa, 35KDa. Polyalthia longifolia 75KDa, 70KDa, 44KDa, 41KDa, 35KDa. Psidium guajava 75KDa, 70KDa, 54KDa, 53KDa, 45KDa, 40KDa, 35KDa.The protein profiling of the different leaf extracts Psidium guajava, Syzygium cumini, Aegle marmelos, Nyctanthes arbor-tristis, Callistemon lanceolatus, Citrus limonum, Combretum indicum, and Calliandra haematocephala, Polyalthia longifolia gave an idea that proteins and peptides present in the leaves. The proteins also may act as antifungal agents. Thus, after the protein profiling was done, based on this the UPGMA (Un-weighted paired group method with arithmetic average) software was used to identify the evolutionary presence of plant. The evolutionary relationship has thus been depicted between the different trees.***
Keywords: Medicinal plant, Protein profiling, Molecular weight, Phylogenetic analysis, SDS-polyacrylamide gel electrophoresis.

I. INTRODUCTION

Different parts of plants such as leaf, root, stem, bark contain different medicinal compounds. Different parts such as leaves have been selected for phytochemical screening to identify the different class of secondary metabolites. Plant leaf contains alkaloids, phenols and polyphenols, so many metabolites which work as defence system in plants as well as for human health. All kind of medicinal plants, herbs, roadside plant contain natural plant proteins and peptides which provides antimicrobial agents. According to the world health organization (WHO) medicinal plants would be the best source to obtain a variety of drugs. Medicinal plants are used by 80% of the world population for their basic health needs[1]. Phytochemical screening is an important step which leads to the isolations of new and novel compounds. Phytochemical(from the Greek world Phyto meaning plant are biological active, naturally accruing chemical compounds founds in plants ,which provide health benefits for human further than those attributed to macronutrients and micronutrients[2]. Round more than 4000 phytochemical have been catalogued and are classified by protective function, physical characteristics and chemical characteristic.

II. METHODS

1) *Plant Materials:* Fresh leaves of different plant *Psidium guajava, Syzygium cumini, Aegle marmelos, Nyctanthes arbor-tristis, Callistemon lanceolatus, Citrus limonum, Combretum indicum,* and *Calliandra haematocephala, Polyalthia longifolia* were collected from the various regions of Noida and Greater noida, India. The leaves were initially washed with distilled water and dried on paper in the laboratory for 24hrs.
2) *Estimation Of Phenol In Plant Leaf Extracts:* 0.5gm leaf sample, added into 5ml of 80% ethanol & homogenization in pestle & motor. Centrifuge for 15min & remove the supernatant in a beaker. Added 2.5ml of 80% ethanol in the residue & centrifuge it and remove the supernatant in the same beaker. Let it evaporate until complete evaporation. Add 5ml of distilled water & take 20 µl samples for O.D. Gallic acid stock used as standard, weight 0.1gm of Gallic acid & adds 4ml ethanol in it and dilute with 40ml distilled water.

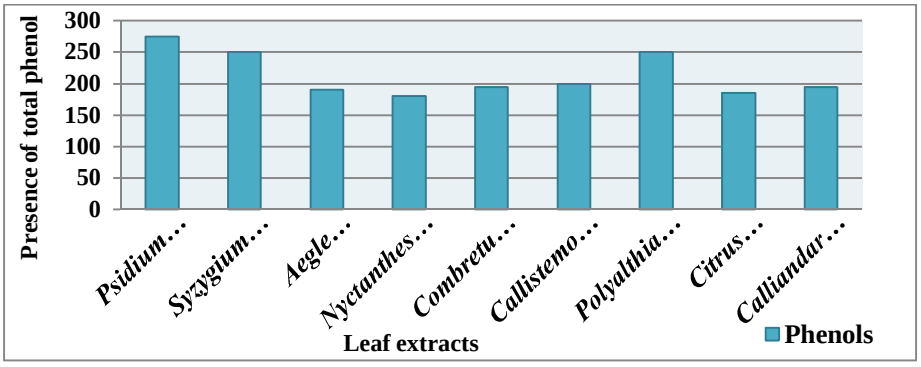
3) *Estimation of Alkaloids In Plant Leaf Extracts:* 1gm of leaf powder & added 10ml of methanol. Kept it for 1-2 days covered in bottle at 50C. Removed the supernatant & centrifuge it for 10 minute. Take the supernatant in a beaker & leave it for complete evaporation. Take O.D.the unknown sample. For standard –Atropine (1mg/10ml D/W) & Bromo cresol green (BCG) solution (pH 4.7) – 69.8mg BCG and 3ml of 2N NaOH & 5ml d/w& make final volume 1000ml. Phosphate buffer (pH 4.7) -2Msodium phosphate to 0.2M citric acid.

4) *Analysis Of Protein Profile Of Leaf By SDS–PAGE:* SDS electrophoresis was carried out according to the modified method of Laemmeli (1970). For extraction 1gm of fresh leaf weight and used10ml phosphate buffer, added PEB (Protein extraction buffer) crushed in pestle motor, made a semi liquid form, leave for overnight next day centrifuge for 10-15 min at 10,000rpm and collect the protein sample in clear appendrope tube used for protein profiling. Separating gel(12%)- 40%Acrylamide Bisaclamide solution (1.5ml),1M Separating gel(2ml), 10%SDS(0.5ml), Distilled water(made volume 5ml),10%APS(100ul),TEMED(10ul).Stacking gel (4%)- 40%AcrylamideBisaclamide solution (0.5ml),1M stacking gel(0.6ml), 10%SDS(0.5ml),Distilled water(made volume 5ml),10% APS(100ul), TEMED(10ul). Prepared the sample by vortexing 10µl of protein extract and 10ul 2x loading buffer and centrifuge. Expose the protein sample for a few seconds at 100^{o}C, vortex and centrifuge the sample, loaded 35µl of samples in to the lanes, Run gel at 100 volts until the bands reach ¾ the length of the gel. Remove the gel from between the plates and washed twice with distilled water, for 5min. Added the gel stain as to cover the gel completely and keep at room temperature overnight with shaking. Decant the stain and rinse with distaining solution.

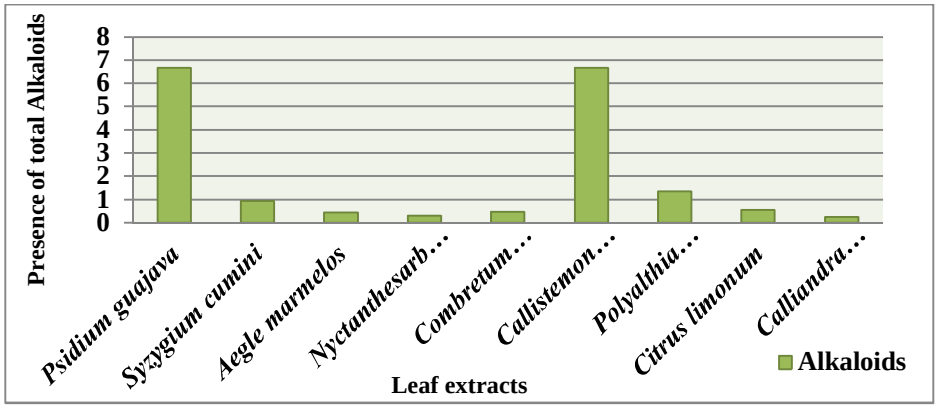
III. RESULTS

Total phenols were observed in plant leaves which is present in higher conc. that is *Polyalthia longifolia* present in (275mg/g tissue), *Psidium guajava* (250mg/g tissue), *Syzygium cumini* (250mg/g tissue), *Nyctanthes arbortristis*(215mg/g tissue). Phenol conc.present in all plant leaf extracts in higher amount which is toxic to all plant pathogens and which is responsible to show maximum antifungal activity against *Alternaria alternata, Aspergillus niger, Aspergillus flavus, Alternaria solani, Penicillium chrysogenum, Colletotrichum capsici, Alternari pori.*

1) *Graph:* Presence of Phenols in different plant leaf extracts.



2) *Graph:* Presence of Alkaloid in different plant leaf extracts.



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Alkaloids were present in rich conc. in plant leaf *Psidium guajava* & *Callistemon lanceolatus* present in rich amount than in *Polyalthia longifolia* present in maximum amount compare to other plant extracts but in *Calliandra haemetocephala* present in very least amount and in *Nyctanthes arbortristis.*

Fig3.3-SDS running gel plate photo of different plant leaf for protein profiling showing different protein band which is present in plant leaf.

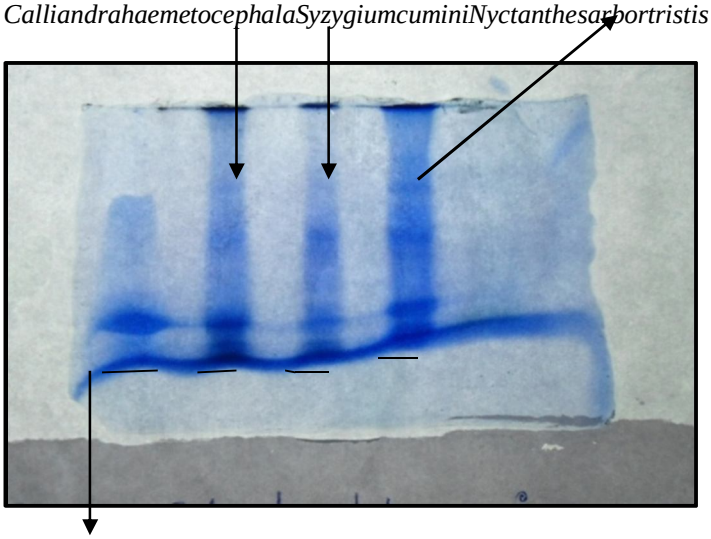


Fig3.4-SDS running gel plate photo of different plant leaf for protein profiling showing different protein band which is present in plant leaf.

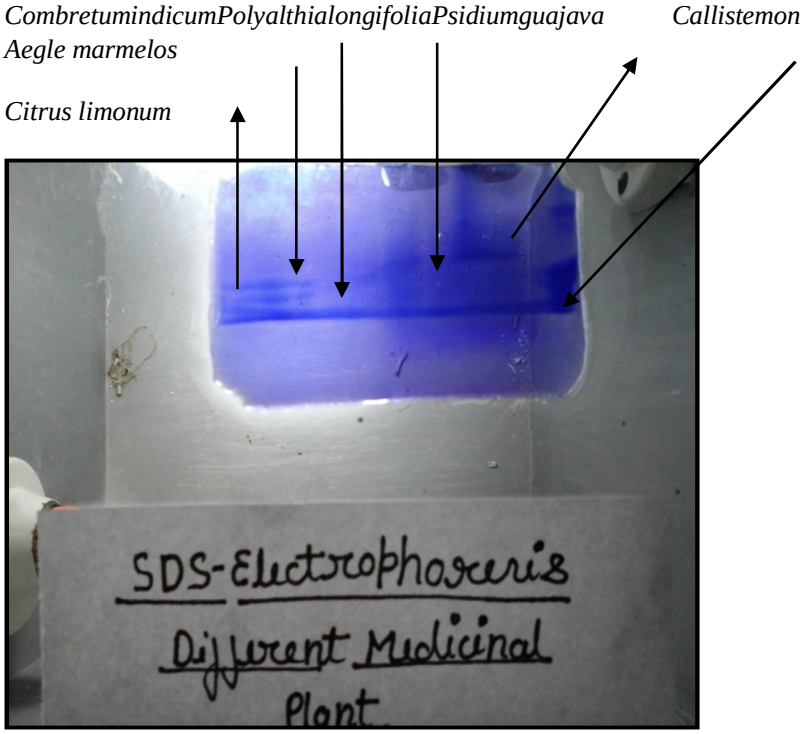
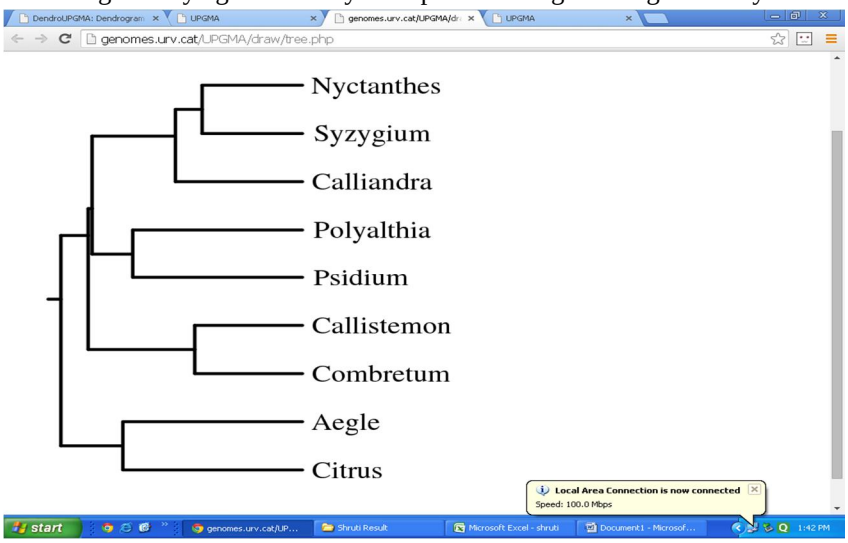


Table3.5 -Molecular weight of different plant leaf extracts.

Nyctanthes arbortristis	*Calliandra haemetocephala*	*Aegle marmelos*	*Callistemon lanceolatus*	*Combretum indicum*	*Citrus limonum*	*Polyalthia longifolia*	*Psidium guajava*	*Syzygium cumini*
80KD	72KD	72KD	70KD	70KD	56KD	75KD	75KD	61KD
72KD	61KD	70KD	63KD	55KD	43KD	70KD	70KD	53KD
70KD	45KD	64KD	50KD	42KD	34KD	44KD	54KD	41KD
63KD	40KD	54KD	38KD	37KD	35KD	41KD	53KD	38KD
61KD	38KD	53KD	34KD	35KD		35KD	45KD	37KD
53KD		40KD					40KD	
50KD		36KD					35KD	
41KD		35KD						
38KD								
37KD								

The above table depicts the different soluble proteins found in different tree leaf extracts which could be involved in providing antifungal activity. It was observed that plants having higher antifungal activity showed presence of similar type of peptides i.e.had similar M. Wt. proteins as seen in above table.

Fig3.6-Phylogenetic analysis of plants having antifungal activity.



The protein profiling of the leaf extract gave an idea into the proteins and peptides present in the leaves. The proteins also may act as antifungal agents. Thus, after the protein profiling was done, based on this the UPGMA software was used to identify the evolutionary presence of plant. The evolutionary relationship has thus been depicted between the different trees.

IV. DISCUSSION

Phenol present in higher conc. in *Polyalthia longifolia* (275mg/g tissue), *Psidium guajava* (250mg/g tissue), *Syzygium cumini* (250mg/g tissue), *Nyctanthes arbortristis* (215mg/g tissue). Alkaloids were present in *Psidium guajava* in rich amounts and in *Callistemon lanceolatus* also present in rich amount than in *Polyalthia longifolia* present in maximum amount compare to other plant extracts but in *Calliandra haemetocephala* present in very least amount and in *Nyctanthes arbortristis.*

Finally to analyse the protein polymorphism in the different tree leaves, SDS PAGE was used and a protein profile was obtained for all the tree leaf extracts. The difference in the protein or peptide types could also be the reason for antifungal activity of the trees like *Polyalthia longifolia, Psidium guajava* and *Nyctanthes arbortristis.* The protein fingerprint which was thus obtained was then used to analyse the Phylogenetic relationship between the different flowering trees.

V. CONCLUSIONS

This shows that the relative antifungal activity is to a high extent related to the concentration of phenolic and alkaloids present in the leaves of the trees. The phenolic thus act as the major antimicrobial principle.

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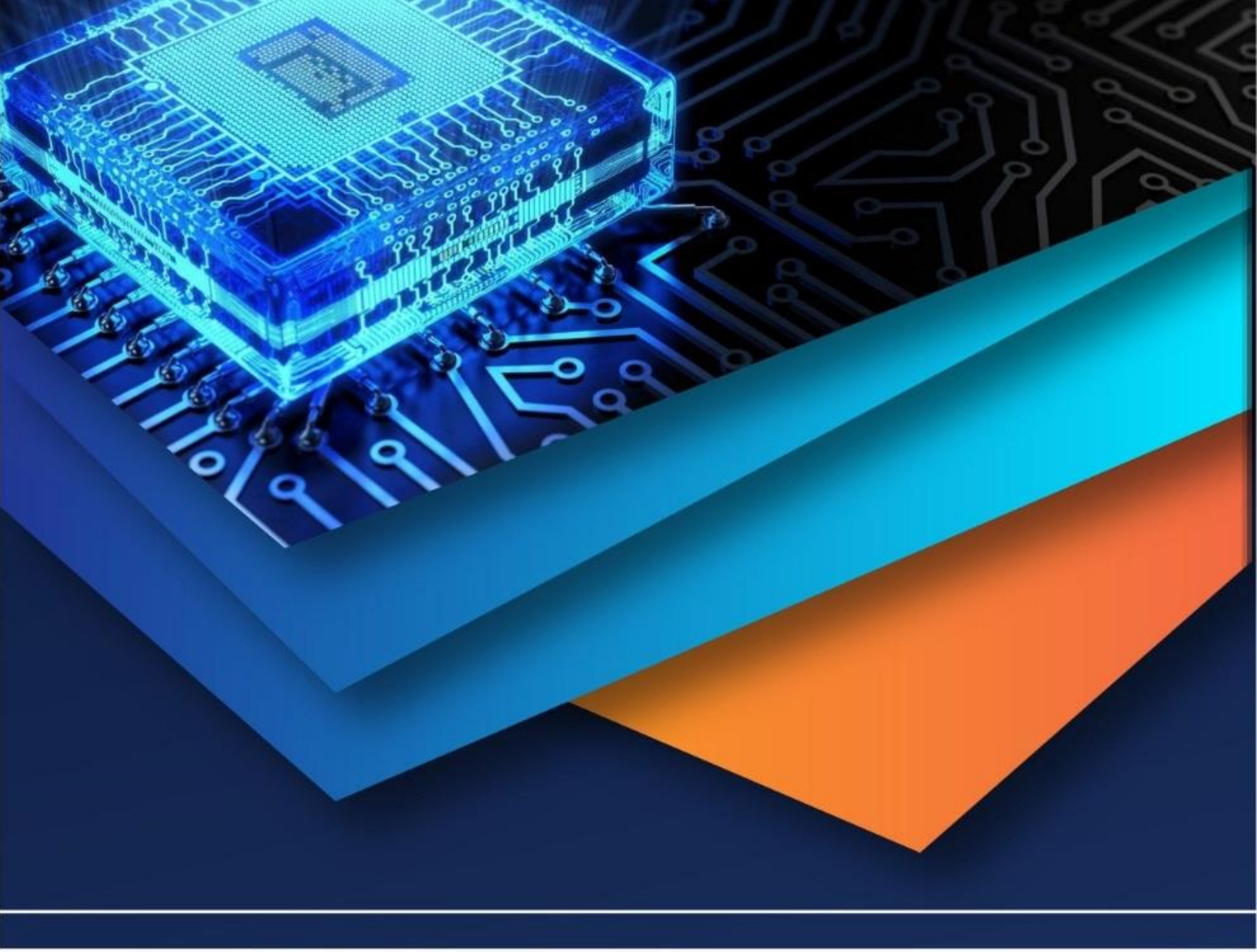

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