In vitro Antifungal Activity of Solvent Extracts of *Psidium guajava* and *Albizia amara* Leaves Against Selected Fungal Strains *Aspergillus flavus* and *Fusarium* Species

[1] Shyna R., [2] Dr. R. Dhivya
[1]PG Student, [2]Assistant Professor
PG and Research Department of Zoology, Nirmala College for Women, Coimbatore-18, Tamilnadu
Email – [2] dhivya.adu@gmail.com

Abstract: Recently traditional medicine is considered as an important source of potentially useful compounds for the development of chemotherapeutic agents. The present study was carried out to evaluate the antifungal properties of different solvent extracts of leaf of *Psidium guajava* and *Albizia amara* against selected fungal strains *Aspergillus flavus* and *Fusarium species*. The leaf powders were extracted with various solvents like ethanol, chlorofom and acetone. *In vitro* antifungal activity was determined by using agar well diffusion technique and percentage of zone of inhibition was measured in millimetres. Phytochemical analysis was carried out to find out the secondary metabolites present in the effective extracts. Among the three extracts of *A. amara*, maximum antifungal activity was exhibited by acetone extract against both tested fungal strains. Likewise among the three solvent extracts of *P. guajava*, ethanol extract displayed maximum resistance toward both fungal species tested. Acetone extract of *A. amara* and ethanol extract of *P. guajava* revealed the presence of various phytochemicals that might be reason for its antifungal activity.

Key Words: *Antifungal activity; Plant extracts; Solvents; Phytochemicals; Agar well diffusion.*

1. INTRODUCTION:

The fungi species are major disease causing agents on plants and can lose up to 90% agricultural yield. Many fungicides have been used to control the plant diseases. But due to indiscriminate use of synthetic fungicides, various important pathogens have developed resistance to many of the currently available fungicides and they also pollute soil and water[1]. Vey often the fungicide adversely affect on the non target organisms. Therefore, it is indeed necessary to search new antifungal compounds as an alternatives, safe, eco-friendly, cheap to synthetic fungicides from plants, since they produce different secondary metabolites which perform defensive role in plants and protect the plants from their invaders[2].

The antimicrobial activity has been screened because of their great medicinal relevance. With the recent years, infections have increased to a great extent and resistant against antibiotics, has become an ever increasing therapeutic problem[3]. Biocompounds of higher plants may give a new source of antimicrobial agents. There are many research groups that are now engaged in medicinal plants research. Plant extracts and essential oils have been investigated throughout the world for their antifungal activity against wide range of fungi[4].

Human infections particularly those involving microorganism i.e. bacteria, fungus, viruses; causes serious infections in tropical and subtropical countries of the world. In the recent times, multiple drug resistance in human pathogenic microorganism has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of such diseases[5]. Medicinal plants and its products have been used as medicines since the start of history. Many researchers have conducted research on the plant products to check their antimicrobial effects[6]. Healing with the help of plant is a age old practice. The oldest known method for healing is the use of plant. Using higher plants for treatment of diseases had started since the man started to live on this planet[7].

Phytochemical studies have attracted the attention of plant scientists due to the development of new and sophisticated techniques[8]. These techniques played a significant role in giving the solution to systematic problems on one hand and in the search for additional resources of raw materials for pharmaceutical industry on the other hand[9]. Plant produce a wide variety of chemical compounds, which can be sorted by their chemical class, bio synthetic origin and functional groups into primary and secondary metabolites[10]. Knowledge of the secondary metabolites of plants is desirable, not only for the discovery of therapeutic agents, but also because such information is valuable in disclosing new resources of such chemical substances[11]. The present study is conducted to study the antifungal activity of solvent extracts of *Psidium guajava* and *Albizia amara* against selected fungal strains *Aspergillus flavus* and *Fusarium species*.

2. MATERIALS AND METHODS:

Collections of test materials:

Leaves of *Psidium guajava* and *Albizia amara* were collected from Coimbatore locale and the specimens were identified; certified (BSI/SRC/5/23/2018/Tech/2476, BSI/SRC/5/23/2018/Tech/2475) respectively and the voucher specimen number were deposited at the Botanical Survey of India, Southern Circle, Coimbatore.

Preparation of leaf powder and extracts:

Fresh leaves of *Psidium guajava* and *Albizia amara* were collected, and air dried under shade. Dried leaves were powdered using an electric pulverizer. Fine powder was obtained by sieving. The powder was subjected to extraction[12,13]. Acetone extraction was followed by chloroform extraction and ethanol extraction so that the powders were subjected to extraction with solvents of increasing polarity. The leaf extracts thus obtained were concentrated by distillation and dried by evaporation in a water bath at 40°C. The residue thus obtained was stored in tightly closed glass vials in the refrigerator for further use. Antifungal activity of the leaves of *Psidium guajava* and *Albizia amara* was investigated.

Test Microorganism:

The fungal strains used were the clinical isolates obtained by culturing in the college laboratory. The fungal strains used were *Aspergillus flavus* and *Fusarium* sp.

Antifungal assay:

The activity of various solvent extracts of leaves of *Psidium guajava* and *Albizia amara* on selected fungal strains was assayed by agar well diffusion method. For this, method of Murray *et al* [14] later modified by Olurinola [15] was used. Antifungal susceptibility was tested on solid media in petriplates. For fungus Rose Bengal agar was used for developing surface colony growth.

Reagents - Rose Bengal Agar Medium:

One litre of Rose Bengal agar was prepared by dissolving 32.15 g of commercially available Rose Bengal agar powder (Hi media) in 1L distilled water and boiled to dissolve the medium completely. The medium was prepared and poured on to the petriplates and was left on sterile surface until the agar has solidified. The plates were swabbed (sterile cotton swabs) with 24 h old culture of fungal strains. Wells (10 mm diameter and about 2 cm apart) were made in each of these plates using sterile cork borer. Stock solution of each solvent extract viz., acetone, chloroform and ethanol was prepared at a concentration of 1 mg/ml. The concentrations viz., 10, 20 30 40 µl of different solvent extracts of the leaves of *Psidium guajava* and *Albizia amara* were added into the wells and allowed to diffuse at room temperature for 2h. Fluconosole was treated as positive antifungal control.

The plates were incubated at 37°C for 72 h for fungal pathogens. The antifungal activity was assayed by measuring the diameter of the inhibition zone formed around well[16]. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.

Statistical Analysis:

The antimicrobial data was interpreted by calculating standard deviation and mean of three replicates.

Phytochemical Analysis:

Preliminary phytochemical screening of leaf extract of selected plant was carried out using the standard procedures.

Test for Alkaloids

- **Mayer's test**[17]**:** 1 ml of extract was treated with a drop or two of Mayer's test reagent along the sides of test tube and observed for the formation of white or cream coloured precipitate.
- **Wagner's test**[18]**:** 1 ml of extract was treated with Wagner's reagent along the sides of the test tube and observed for the formation of reddish brown colour precipitate.
- **Hager's test**[19]**:** 1 ml of extract was treated with 1 or 2 ml of Hager's reagent and observed for the formation of prominent yellow precipitate.

Test for Tannins

- **Ferric chloride test**[20]**:** 0.5 g extract was stirred with about 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate, and observed for the blue-black, green or blue-green precipitate.

Test for Phenols

- **Ferric chloride test**[21]**:** The extract (50 mg) was dissolved in 5 ml of distilled water and treated with few drops of 5% ferric chloride and observed for the formation of dark green colour
- **Lead acetate test**[22,23]**:** The extract (50 mg) was dissolved in 5 ml of distilled water and 3 ml of 10% lead acetate solution was added and observed for the formation of bulky white precipitate.

Test for Flavonoids

- **NaOH test**[20]**:** 1 ml the extract was dissolved in water and filtered; to this 2 ml of the 10% aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids.
- **Lead acetate test**[22,23]**:** Fifty milligram of the extract was taken in a test tube and few drops of lead acetate solution was added to it and observed for yellow coloured precipitate.

Test for Sterols

- **Liebermann-Burchard test**[24]**:** The extract (50 mg) was dissolved in 2 ml of acetic anhydride. To this one or two drop of Conc. H_2SO_4 was added along the side of the test tube and observed for any colour change.

Test for Terpenoids

- **Liebermann-Burchard test**[25]**:** A little of extract (50 mg) was dissolved in ethanol. To it 1 ml of acetic anhydride was added followed by the addition of Conc. H_2SO_4. Change of colour from pink to violet indicates the presence of terpenoids.

Test for Saponins

- **Foam Test:** The extract (50 mg) or dry powder was diluted with distilled water and made up to 20 ml. The solution is vigorously shaken for 15 minutes and observed for the formation of 2 cm layer thick foam.

Test for Anthraquinones

- **Borntrager's test**[26]**:** Extract(0.2 g) to be tested was shaken with 10 ml of benzene and then filtered. Five ml of the 10% ammonia solution was added to the filtrate, shaken and observed for the appearance of a pink, red or violet colour.

Test for Proteins

- **Ninhydrin test**[27]**:** Three drops of ninhydrin solution (10 mg of ninhydrin in 200 ml of acetone) was added to 2 ml of extract and observed for the present of characteristic purple colour.
- **Biuret test**[27]**:** Two ml of extract was treated with one drop of 2% copper sulphate solution. To this 1 ml of 95% ethanol was added followed by excess of potassium hydroxide pellets and observed for the formation of pink ethanolic layer.

Test for Quinones

- **H_2SO_4 test**[23]**:** To 1 ml of extract, 1 ml of Conc. H_2SO_4 was added and observed for the formation of red colour.
- **HCl test**[28,29]**:** To 1 ml of the extract, 5 ml of HCl was added and observed for the presence of yellow colour precipitate.

3. RESULTS AND DISCUSSION:

Antifungal activity of *Albizia amara* against *Aspergillus flavus*

In this study, against the *A. flavus* maximum antifungal activity was exhibited by acetone extract in which zone of inhibition was recorded as 33.1±1.8mm at a concentration of 40 µl and 29.7±1.2mm at a concentration of 30µl respectively (Fig 1 & 2). As the concentration increased, antifungal activity was found to increase. Followed by acetone, ethanol extract showed a sensitivity of 29.6 mm at 40µl concentration, hence presented a moderate antifungal efficacy. And the positive control exhibited an inhibition zone of 29.4±1.3 mm.

The antifungal activity was found to be dose dependent. Varying levels of sensitivity of test organisms of fungi may be due to the intrinsic tolerance of microorganisms and the nature and combinations of phytocompounds presents in the crude extracts [30]. Study similar to our present study has been carried out by Bazie *et al* [31] in which, the methanolic extract of *A. albida* showed the best antifungal activity (19 mm) against *Colletotrichum musae*, followed by *D. abyssinica* (11.7 mm) and *A. mexicana* (11.0 mm).

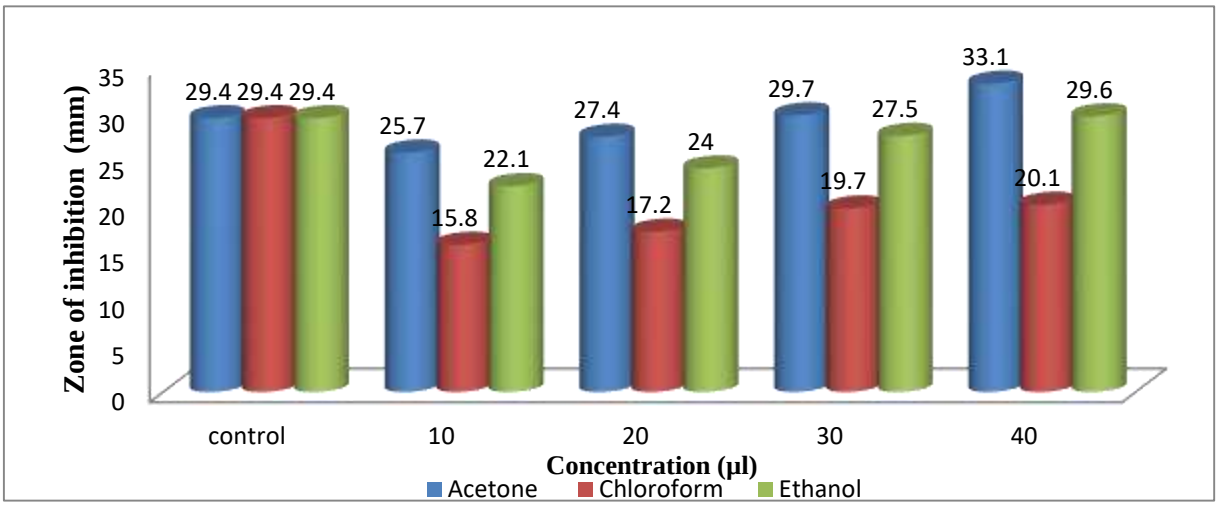

Fig 1: Graph showing Antifungal activity of *Albizia amara* leaf extract against *A. flavus.*

Fig 2: Plate showing Antifungal activity of *A. amara* against *A. flavus*

Antifungal activity of *Albizia amara* against *Fusarium sp*:

Against *Fusarium sp.* among the three extracts, maximum antifungal potency was shown by acetone extract of *A. amara* leaf giving a inhibitory zone of 31.5±1.6 mm at a concentration of 40µl and 29.7±2.2 mm at a concentration of 30µl respectively. Followed by the activity of acetone was that of ethanol extract which provided a zone of inhibition of 26.7±1.7 mm at a concentration of 40µl (Fig 3 & 4). Reports in harmony with these finding were given by Sales *et al* [32] in which the *in vitro* trials has showed that the selected species, *A. sativum*, *A. vera*, *G. glabra*, *M. balsamum*, *R. mangle* and *P. heptaphyllum*, displayed consistent antifungal activity against *F. guttiforme* and *C. paradoxa*.

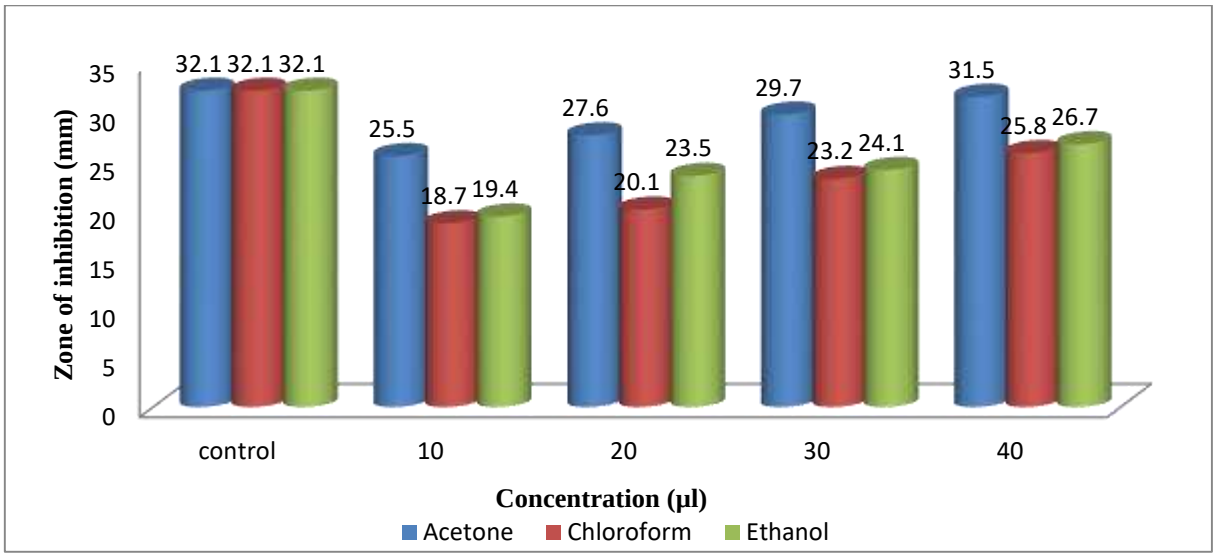

Fig 3: Graph showing Antifungal activity of *Albizia amara* leaf extracts against *Fusarium sp.*

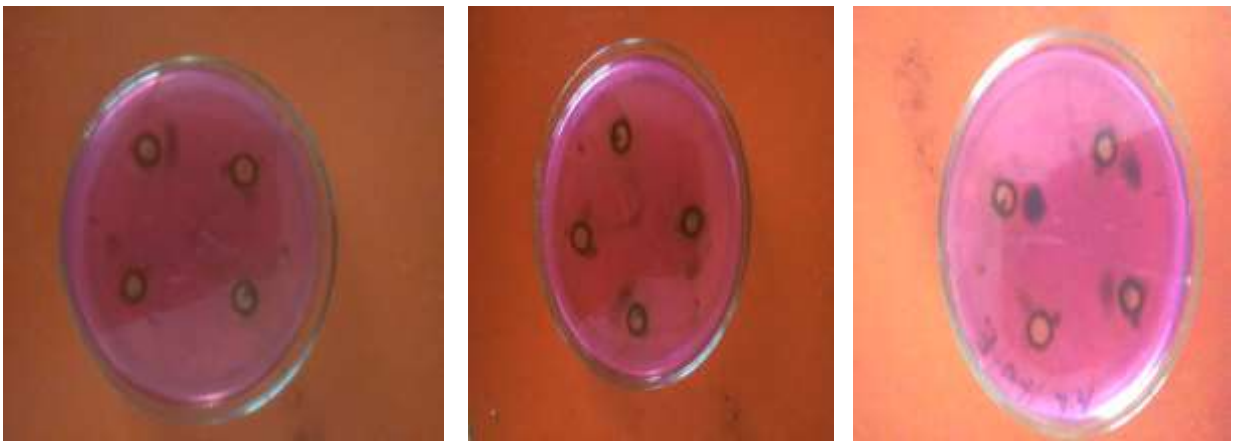

Fig 4: Plate showing Antifungal activity of *A. amara* against *Fusarium species*

Antifungal activity of *Psidium guajava* against *Aspergillus flavus*:

In this study, against the *A. flavus* maximum antifungal activity was exhibited by ethanol extract in which zone of inhibition was recorded as 27.3±1.8 mm at a concentration of 40µl and 25.2±1.5 mm at a concentration of 30µl respectively. Followed by ethanol, acetone extract showed a sensitivity of 24.1 mm at 40µl concentration (Fig 5 & 6); hence it showed a moderate antifungal efficacy. The results showed that different extracts varied in their effectiveness in inhibiting fungi growth. The fungi-toxic effect of aqueous extracts of *P. juliflora* against *Aspergillus* species as reported by Satish *et al* [33] and *Fusarium* species as reported by Satish *et al* [34] is in line with present result.

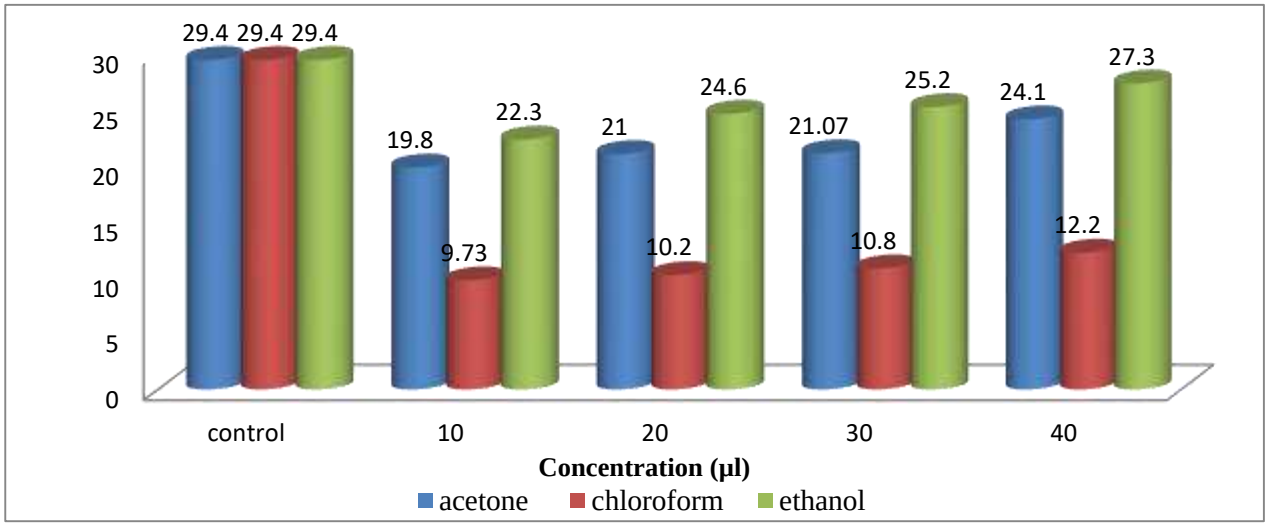

Fig 5: Graph showing Antifungal activity of *Psidium guajava* leaf extract against *A. flavus*

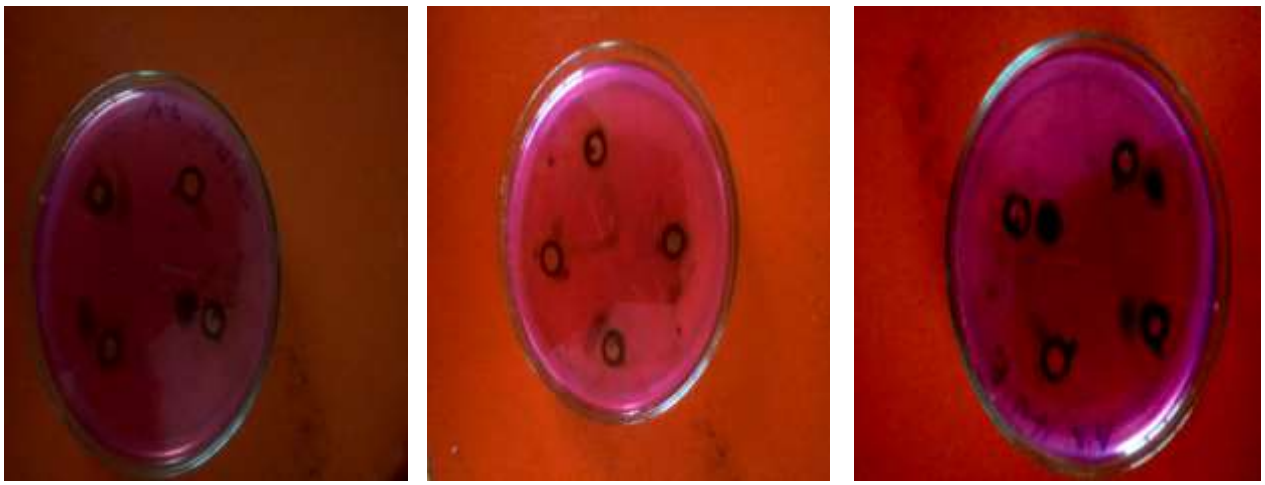

Fig 6: Plate showing Antifungal activity of *P. guajava* against *A. flavus*

Antifungal activity of *Psidium guajava* against *Fusarium sp*:

Against *Fusarium sp.*, among the three extracts, maximum antifungal potency was shown by ethanol extract of *P. guajava* leaf giving an inhibitory zone of 31.1±1.7 mm at a concentration of 40µl and 29.8±1.3 mm at a concentration of 30µl respectively. Followed by the activity of ethanol was that of acetone extract which provided a zone of inhibition of 25.6±1.2mm at a concentration of 40 µl (Fig 7 & 8). Positive control showed an inhibition zone of 32.1±1.2 mm. In parallel to the present study Carole [35] has reported that high rate of inhibition of *Pythium myriotylum* was observed with 3 % of *P. guajava* in aqueous and ethanol media.

The results obtained from the present investigation revealed that among the solvent extracts of *A. amara* highest antifungal activity was exhibited by the acetone extract against both tested fungal strains. Likewise among the three solvent extracts of *P. guajava*, ethanol extract displayed maximum resistance toward both fungal species tested. Therefore, phytochemical analysis of both these extracts was carried out using standard protocols.

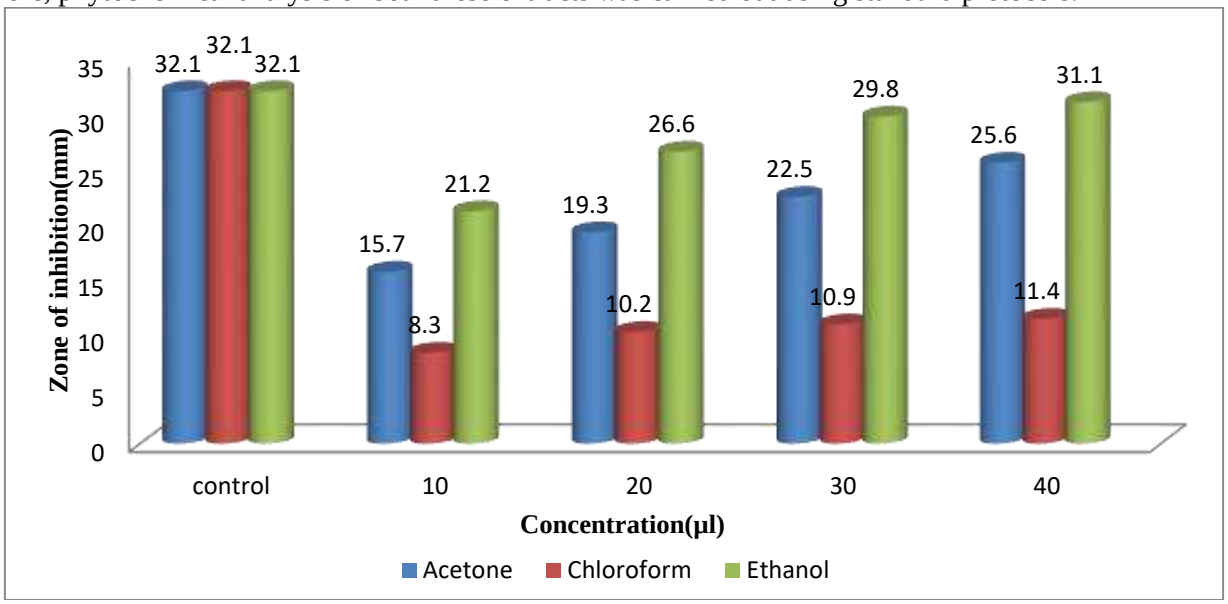

Fig 7: Graph showing Antifungal activity of *Psidium guajava* leaf extracts against *Fusarium sp.*

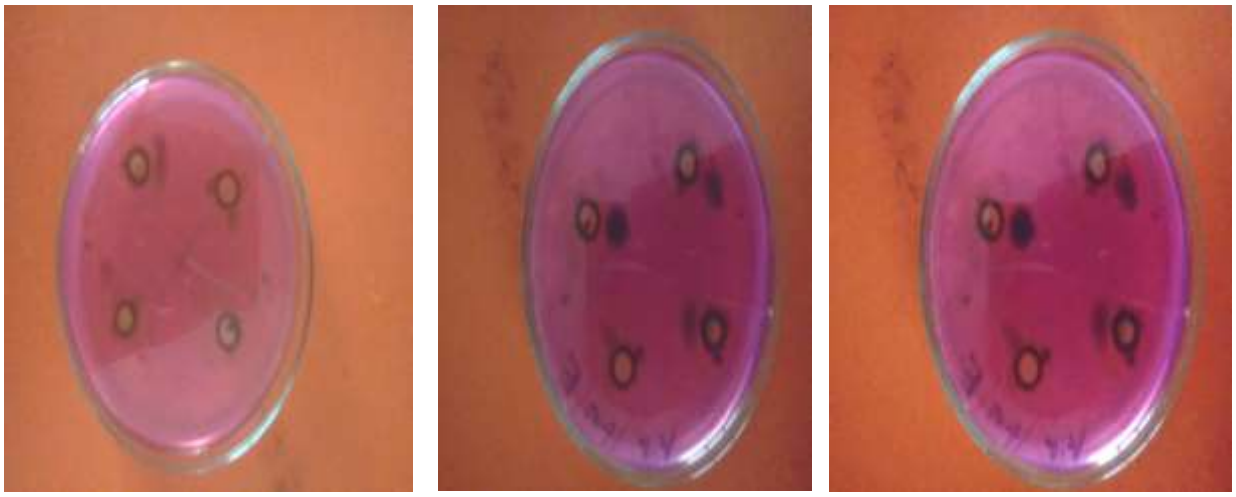

Fig 8: Plate showing Antifungal activity of *P. guajava against Fusarium species*

Acetone extract *A. amara* leaf that had highest antifungal potential, when tested for the phytochemicals showed the presence of saponins, tannins, terpenoids, sterols and proteins. The antifungal activity of the selected leaf extracts of *P. guajava* and *A. amara* respectively may be due to the presence of various phytochemicals seen in them. Similar observations were recorded by Cowan[10] and Padayana *et al* [36] in which they have reported that antibacterial activity of leaf extracts can be attributed due to the presence of these phytochemicals.

The presence of variety of flavonoids and phenolic compounds in the leaf extract of *A. amara*, may have the capacity to rupture the cytoplasmic membrane of the fungal cells and damage the intracellular compounds or they may interact with lipid bilayer or inhibit the protein and nucleic acid synthesis of the fungal cell. Janaranjani and Rajalakshmi[37] has revealed the presence of alkaloids, flavonoids, terpenoids, glycosides, carbohydrates, phenols, quinines, betacyanins, saponins, tannins, oils and lipids using organic solvents such aqueous, ethanol, chloroform and benzene extracts of *A. amara* leaves.

Table 1: Phytochemicals present in *Albizia amara* and *Psidium guajava* leaf extracts

Sl. No.	Constituents	*A. amara* leaf	*P. guajava* leaf
		Acetone extract	Ethanol extract
1	Alkaloids	-	+
2	Flavonoids	+	+
3	Sterols	+	+
4	Terpenoids	+	+
5	Anthroquinones	-	-
6	Phenols	+	-
7	Saponins	+	-
8	Tannins	+	+
9	Proteins	+	+
10	Quinones	+	-

"+" Presence "-" Absence

Among the three extracts, maximum antifungal potency was shown by ethanol extract of *P. guajava* leaf against both the rest fungal strains. The phytochemical analysis of ethanol extract of *P. guajava* showed the presence of phytochemical compounds such as alkaloids, flavonoids, sterols, terpenoids, tannins and proteins. The potency of ethanol extract may be due to the presence of active secondary metabolites. Wilkins and Board[38] has suggested that this may be because of the fact that some essential oils contain metabolic function of microbial cells. Antimicrobial activity of oils may be due to impairment of variety of enzyme systems that are involved in the production of energy or synthesis of structural component in the microbial cells.

P. guajava and *A. amara* are good resource of bioactive compounds due to its content of various phytochemicals. However, most of the literature shows that the compounds of *P. guajava* and *A. amara* are antifungal in nature and the present study supports this statement. The present study also observed the sensitivity pattern of the selected pathogens towards the extracts of *P. guajava,* and *A. amara* as well as standard fungicide fluconosole.

4. CONCLUSION:

The use of botanicals in the treatment of pathogenic diseases associated with the infection of these pathogens is validated, and scientifically supported by the results obtained in this work. The leaf extracts of *P. guajava* and *A. amara* appears to be a rich source of different phytoconstituents, with antifungal compounds and also supported the statement of applicability of plant in traditional system of treatment. The result suggested that both the plants could be used as a curative agent for different diseases. The present findings reveal the antifungal potential of selected medicinal plants. The study can be extended for purification and evaluation of bioactive compounds in novel drug discovery.

REFERENCES:

1. Olajide, O.A., Awe, S.O., Makinde, J.M, (1999): Pharmacological studies on the leaf of *Psidium guajava*. *Filotherapia*, 70, 25.
2. Meckes, M., Calzada, F., Tortoriellobn, J., Gonzalez, J.L., Martinezm M, (1966): Terpenoids isolated from *Psidium guajava* hexane extract with depressant activity on central nervous system. *Phytotherapy Research,* 10, 600-603.
3. Lozoya, X., Meckes, M., Abouaaid, M., Tortoriello, J., Nozzolillo, C., Arnasonm J.T, (1994): Querectin glycosides in *Pisdium guajava* L. leaves and determination of a spasmocytic principle. *Arch Med Res*, 25, 11.
4. Begum, S., Hassan, S. I., Ali, N.S., Siddiqui, B.S, (2004): Chemical constituents from the leaves of guajava. Natural Product Res. *Journal of Pharmaceutical Research,* 18, 135-140.
5. Preethi, R., Devanathan, V.V., Loganathan, M, (2010): Antimicrobial and antioxidant efficacy of some medicinal plants against food borne pathogens. *Advanced Biomedical Research,* 4(2), 122-125.
6. Abu-shanab, B., Adwan, G., Abu-safiya, D., Jarrar, N., Adwan, K, (2004): Antibacterial activities of some plant extracts utilized in popular medicine in Palestine. *Turkish Journal of Biology*, 28(1), 99-102.

7. Onyeagba, R.A., Ugbogu, O.C., Okeke, C.U., Iroakasi, O, (2004): Studies on the antimicrobial effects of garlic (*Allium sativum* Linn.), ginger (*Zingeber officinale* Roscoe) and lime (*Citrus aurantifolia* Linn.). *African Journal of Biotechnology*, 3(10), 552-554.
8. Anjana, S., Rani, V., Padmini, R,(2009): Antibacterial activity of some medicinal plants used by Tribals against Uti causing pathogens. *World Applied Science Journal*, 7(3), 332-339.
9. Sandhu, D.S., and Heinrich, M, (2005): The use of health foods, spices and other botanicals in the skin community in London. *Phytotherapy Research*, 19, 633-642.
10. Cowan, M.M, (1999): Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, 12(4), 564-582.
11. Mojab, F., Kamalinejad, M., Naysanch, G., Hamid, (2003): Phytochemical screening of some species of Iranian plants. *Iranian Journal of Pharmaceutical Research*, 2(2), 77-82.
12. Harbourne, J.B. (1973): Phytochemical Methods. Chapman and Hall, Ltd, London, p. 49-188.
13. Vogel, A.I. (1978): Text book of practical organic chemistry. The English language book society and Longman, London, p. 1368.
14. Murray, P.R., Baron, E.J., Pfaller, M.A., Tenover, F.C., Yolken, H.R. (1995): Manual of Clinical Microbiology, 6th Ed. ASM Press, Washington DC, p. 15-18.
15. Olurinola, P.F. (1996): A laboratory manual of pharmaceutical microbiology, Idu, Abuja, Nigeria, p. 69-105.
16. NCCLS. (1993): Performance standards for antimicrobial disc susceptibility tests approved standards, NCCLS publication M2-A5, Villanova, PA, USA.
17. Evans, W.C. (1997): Trease and Evans Pharmacology. 14th Edn. Harcourt Brace and company. Asia. Pvt. Ltd. Singapore.
18. Wagner, H. (1993): Pharmazeutische Biology 5th edn. AUFI. 15 BN 3-437-20 498-X. Gustav fisher Vwelag. Stuttgart. Germany.
19. Wagner HXS, Bladt Z, Gain EM. Plant drug analysis. Springer Veralag. Berlin. Germany; 1996.
20. Trease GE and Evans WC. Pharmacognosy. 15th Ed. London: Saunders Publishers; 2002. p. 42–44. 221–229, 246–249, 304–306, 331–332, 391–393.
21. Mace, M.E. (1963): Histochemical localization of phenols in healthy and diseased tomato roots. *Phytopathology*, 16, 915-925.
22. Brain, K.R., and Turner, T.D. (1975): Wright Scien Technica. 1st Edn. Bristol: Practical evaluation of phyto pharmaceuticals, p. 144.
23. Evans, W.C. (1996): Pharmacognosy. 14th Edn. WB Saunders Co. Ltd. Singapore, 9, 713-734.
24. Finar, I.L. (1986): Stereo Chemistry and the chemistry of Natural products. Longman. Singapore. Vol.2.
25. Sofowora, A. (1993): Medicinal plants and traditional medicinal in Africa. Screening Plants for Bioactive Agents, 2nd ed. Sunshine House, Spectrum Books Ltd. Nigeria: Ibadan, p. 134–156.
26. Yasuma, A., and Ichikawa. (1953): Ninhydrin-Schiff and 5-Schiff staining. A new histochemical staining method for proteins. *J Lab Clin Med*, 41, 296-299.
27. Gahan, P.B. (1984): Plant Histochemistry and cytochemistry: An introduction. Academic press, Florida; U.S.A.
28. Kokate, C.K. (2000): Practical Pharmacognosy, Vallabh Prakashan, Delhi, p. 107-111.
29. Harbone, J.B. (1999): Phytochemical Methods, Chapman & Hall, London, p. 60-66.
30. Jamuna, S., Paulsamy, S., Karthika, K, (2017): *In-vitro* antibacterial activity of leaf and root extracts of *Hypochaeris radicata* L. (Asteraceae) – a medicinal plant species inhabiting the high hills of Nilgiris, the Western Ghats. *International Journal of Pharmacy and Pharmaceutical Sciences*, 5(1), 175-178.
31. Bazie, S., Ayalew, A., Woldetsadik, K, (2014): Antifungal activity of some plant extracts against (*Colletotrichum musae*) the cause of postharvest banana anthracnose. *J Plant Pathol Microb*, 5(2), 1000226.
32. Sales, M.E., Atega, A., Afifa, O., Fouzy, A, (2016): Qualitative and quantitative analysis of phytochemicals of various extract for *Ephedra altissima* from Libya. *Journal of Medicinal Plant Studies*, 4(3), 119-121.
33. Satish, S., Mohana, D.C., Ranhavendra, M.P., Raveesha, K.A, (2007): Antifungal activity of some plant extracts against important seedborne pathogens of *Aspergillus sp*. Journal of Agricultural Technology 2007; 3: 109-119.
34. Satish, S., Raghavendra, M.P., Raveesha, K.A, (2009): Antifungal potentiality of some plant extracts against *Fusarium sp*. *Archives of Phytopathology and Plant Protection*, 42, 618-625.
35. Carole, D.S, (2014): Evaluation of antifungal activities of *Psidium guajava* L. (Myrtaceae) leaves extract on *Pythium myriotylum* Drechsl. *Int J Med Plants*, 107, 549-557.
36. Padayana, S., Ashalatha, M., Prajna, P.S., Yende, A., Bhatt, R, (2011): Evaluation of antibacterial and antioxidant properties of *Uvaria narum* (Dunal) Wall. *Int. Research J. Pharm*, 2(5), 142-144.
37. Janaranjani, D., and Rajalakshmi, A.G, (2018): Phytochemical screening of *Albizia amara* leaf extracts. *International Journal of Scientific Research in Multidisciplinary Studies*, 4(9), 22-25.
38. Wilkins, K.M., and Board, R.G, (1989): Natural antimicrobial system. In: Gould GW, editor. Mechanism of Action of Food Preservation Procedures. New York: Elsevier, p. 285-362.