Antibacterial Activity and Phytochemical Analysis of Solvent Extracts of *Psidium guajava* and *Albizia amara* Leaves Against Selected Bacterial Strains

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Abstract: *Microbial infections exert a health problem throughout the world, and plants are a possible source of antimicrobial agents. According to World Health Organization (WHO), plants are a best source to obtain a variety of drugs and compounds derived from medicinal plants. The present study was conducted to investigate the antimicrobial activity of Psidium guajava and Albizia amara against selected bacterial strains Escherichia coli and Staphylococcus aureus by agar well diffusion method and phytochemical analysis were carried out to find out the secondary metabolites by standard protocols. The solvents used for extraction of plant powders were ethanol, chloroform and acetone. Among the three extracts, acetone extract of A. amara exhibited maximum antibacterial activity against E. coli and S. aureus and ethanol extract of P. guajava exhibited maximum antibacterial activity against E. coli and S. aureus. Our results showed that the P. guajava and A. amara is abundant with anti bacterial compounds and it may be useful in industrial extraction and isolation of antibacterial compounds which may found place in pharmaceutical industry as constituents of antibiotics.*

Keywords: *Antibacterial activity; Plants, Solvents, Extracts, Agar well diffusion, Zone of inhibition, Phytochemicals*

1. INTRODUCTION:

The usage of herbs and medicinal plants is a universal phenomenon. All the cultures on earth has relied on the huge variety of natural chemistry found in healing plants for their therapeutic properties. As per World Health Organization (WHO), about 80% of world population use medicinal plants to treat human disease [1]. The medicinal plants are the plants whose parts (leaves, seeds, stems, roots, fruits etc), extracts, infusions, decoctions, powders are used in the treatment of different diseases of humans, plants and animals [2].

The antimicrobial activity have been screened because of their great medicinal relevance with the recent years, infections have increased to a great extent and resistant against antibiotics, becomes an ever increasing therapeutic problem [3]. Natural products of botanicals may give a new source of antimicrobial agents. Many researchers 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].

The presence of phytochemicals makes the plant useful against different diseases and has a strong of providing useful drugs of human use. Phytocompounds are regarded as secondary metabolites produced at very little amount as the plant have little need for them. They are produced naturally in whole parts of the plant body; like bark, leaves, stem, root, flower, etc. [5]. The quantity and quality of phytochemicals present in plant parts may differ from one part to another [6].

Microbes have become resistance to many antibiotics due to increased use of drugs, which is decreasing efficiency of conventional medicines. So, it has become necessary to find out new antimicrobial agents [7]. Now a days, antibiotic resistance against medically important bacteria is the major problem faced by the world. The indiscriminate usage of synthetic antimicrobial drugs has resulted in multiple drug resistance. Antibiotics may also cause adverse effects on the host including allergies, hypersensitivity and immune-suppression. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases [8].

2. MATERIALS AND METHODS:

2.1 Collections of test materials

Leaves of *P. guajava* and *A. amara* were collected from the College Campus 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.

2.2 Preparation of leaf powder and extracts

Fresh leaves of *P. guajava* and *A. amara* were air dried under shade. Dried leaves were powdered using an electric pulverizer. Fine powder was obtained by sieving. The powder was subjected to extraction [9,10]. 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 obtained was stored in tightly closed glass vials in the refrigerator for further use. Antibacterial activity was investigated**.**

2.3 Test microorganism

The bacterial strains used were the clinical isolates obtained from a Hospital inCoimbatore. The bacterial strains used were *E. coli* and *S. aureus*.

2.4 Antibacterial assay

The activity of various solvent extracts of leaves of *P. guajava* and *A. amara* on selected bacterial strains was assayed by agar well diffusion method. For agar well diffusion, method of Murray *et al* [131later modified by Olurinola [12] was used. Antibacterial susceptibility was tested on solid media in petriplates. For bacteria Nutrient agar was used for developing surface colony growth.

Reagents: Nutrient Agar

Nutrient agar 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 bacterial strains. Wells 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. About 50µl of different solvent extracts of the leaves of *P. guajava* and *A. amara* was added using sterile syringe into the wells and allowed to diffuse at room temperature for 2 h. Amoxillin was used as positive antibacterial control.

The plates were incubated at 37°C for 18-24 h for bacterial pathogens. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around well [13]. 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.

2.5 Statistical Analysis

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

2.6 Phytochemical screening

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

Test for Alkaloids

- **Mayer's test [14]:** 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 [15]:** 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 [16]:** 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 [17]:** 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 [18]:** 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 [19,20]:** 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 [17]:** 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 [19,20]:** 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 [21]:** 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 [22]:** 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'stest [23]:** 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 [24]:** 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 [24]:** 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 [20]:** To 1 ml of extract, 1 ml of Conc. H_2SO_4 was added and observed for the formation of red colour.
- **HCl test [25,26]:** 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:

3.1 Antifungal activity of *Albizia amara* against *Escherichia coli*

Antibacterial activity of the three solvent extracts was tested and zone of inhibition was recorded in millimeters. In this study, against the bacteria *E. coli* maximum antibacterial activity was exhibited by acetone extract in which zone of inhibition was recorded as 32.4±1.4 mm at a concentration of 40 µl and 30.5±1.6 mm at a concentration of 30µl respectively. Positive control Amoxicillin showed an inhibition zone of 33.2±1.2 mm (Fig 1 & 2).

Followed by acetone, ethanol extract showed a sensitivity of 29.7 mm at 40µl concentration; hence it showed a moderate antibacterial efficacy. Antibacterial activity was seen to be dose dependant. In the present investigation it was noted that the zone of inhibition determined by agar well diffusion method varied with the different extracts, of the same plant. It may be due to the solvent used for extraction, and the organism tested. The findings parallel to present study has been reported by (Dahiya and Purkayastha [27] in which they have assessed an *in vitro* antibacterial activity of various solvents and water extracts of *Aloe vera*, *neem*, bryophyllum, lemongrass, tulsi, oregano, rosemary and thyme on 10 multidrug resistant clinical isolates. Results showed that inhibition varied with the various extracts, of the plant.

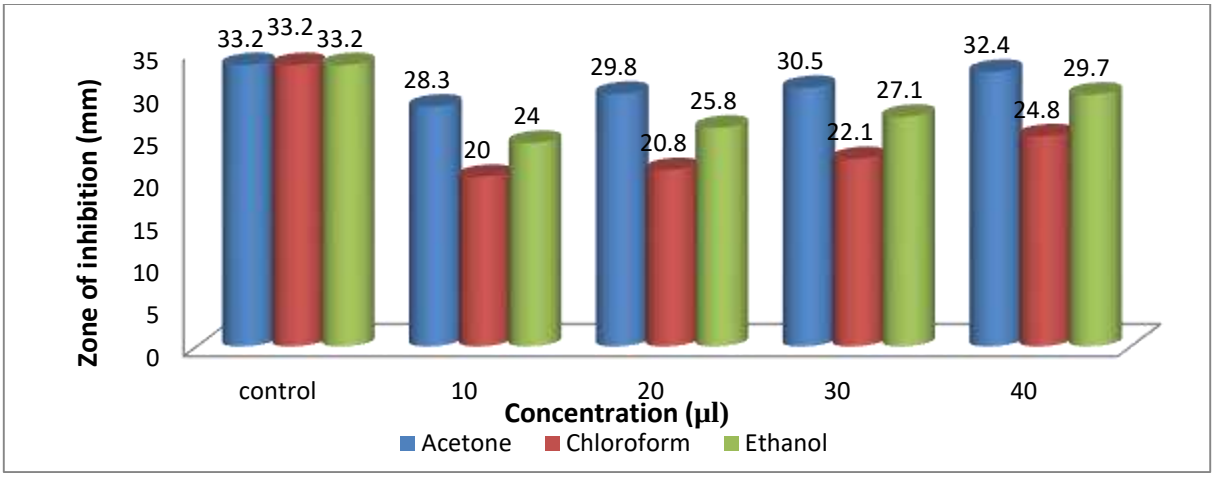

Fig 1: Graph showing antibacterial activity of *Albizia amara* leaf extracts against *E. coli*

Fig 2: Plates showing antibacterial activity of *A. amara* against *E. coli*

3.2 Antibacterial activity of *A. amara* against *S. aureus*

Against *S. aureus*, among the three extracts, maximum antibacterial potency was shown by acetone extract of *A. amara* leaf giving a inhibitory zone of 30.2±0.7 mm at a concentration of 40 µl and 29.5±1.4 mm at a concentration of 30 µl respectively. And the positive control showed 31.8 mm inhibition zone. Followed by the activity of acetone was that of ethanol extract which provided a zone of inhibition of 29.2±0.6 mm at a concentration of 40µl (Fig 3 & 4). Antibacterial activity was found to be directly proportion to the concentration of the extract. Dahiya and Purkayastha [27] has stated that ethanol and methanol extracts were found to be more potent being capable of exerting significant inhibitory activities against majority of the bacteria investigated. In present study also antibacterial efficacy exerted by ethanol extract of *A. amara*, was good providing an inhibitory zone of 29.2 mm against *S. aureus*.

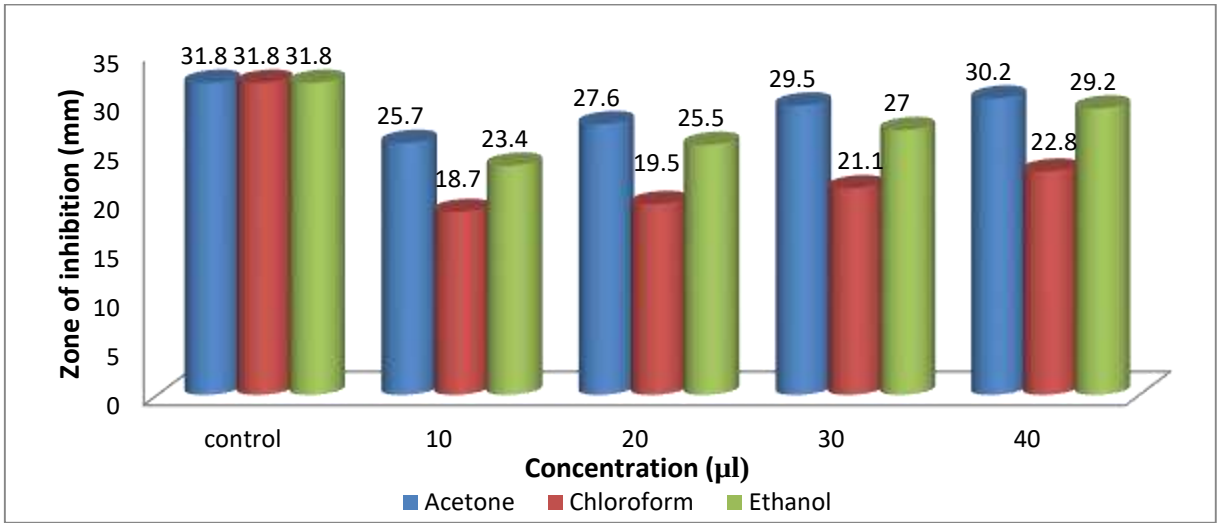

Fig 3: Graph showing antibacterial activity of *A. amara* leaf extracts against *S. aureus*

Fig 4: Plate showing antibacterial activity of *A. amara* against *S. aureus*

Among the two test organisms, *E. coli* species showed more resistance against the leaf extract of *A. amara* than *S. aureus.* In this study maximum antibacterial activity was exhibited by acetone extract of *A. amara* against *E. coli* species (32.4±1.4 mm), which was followed by ethanol extract against *S. aureus* which showed a zone of inhibition of 30.2±0.7 mm.

3.3 Antibacterial activity of *Psidium guajava* against *E. coli*

In this study, against the *E. coli* maximum antibacterial activity was exhibited by ethanol extract in which zone of inhibition was recorded as 33.7±0.5 mm at a concentration of 40µl and 32.7±0.6 mm at a concentration of 30µl respectively. Positive control Amoxycillin showed an inhibition zone of 33.2±1.2 mm (Fig 5 & 6). Gowri and Vasantha [28] reported that the methanolic extract of *P. guajava* showed the highest zone of inhibition against *E. coli in* 40 µl concentration. He also stated that the aqueous extract of *P. guajava* showed moderate antibacterial activity against *S. aureus.* In the present study, the antibacterial activity was showed to be dose dependent. Likewise Ishtiaq and Hayat [29] reported that antibacterial activity of organic and aqueous extracts of *N. sativa* seeds in comparison with standard drugs Ceftriaxone, Amoxycillin, Gentamycin was determined and found to proceed in dose dependent manner against major clinical isolates.

Followed by ethanol, acetone extract showed a sensitivity of 30±0.7mm at 40 µl concentration, hence it exhibited moderate antibacterial efficacy. At 30 µl concentration the zone diameter was found to be 28.4 mm. Many researchers have carried out work similar to our present study. Similar study was conducted by Jasna and Dhivya [30] in that antibacterial activity of solvent extracts of *Nigella sativa* seeds were tested against gram positive bacteria *Staphylococcus aureus* and gram-negative bacteria *Salmonella typhimurium*. According to the results, against the bacteria *S. aureus* maximum inhibitory zone was exhibited by acetone extract.

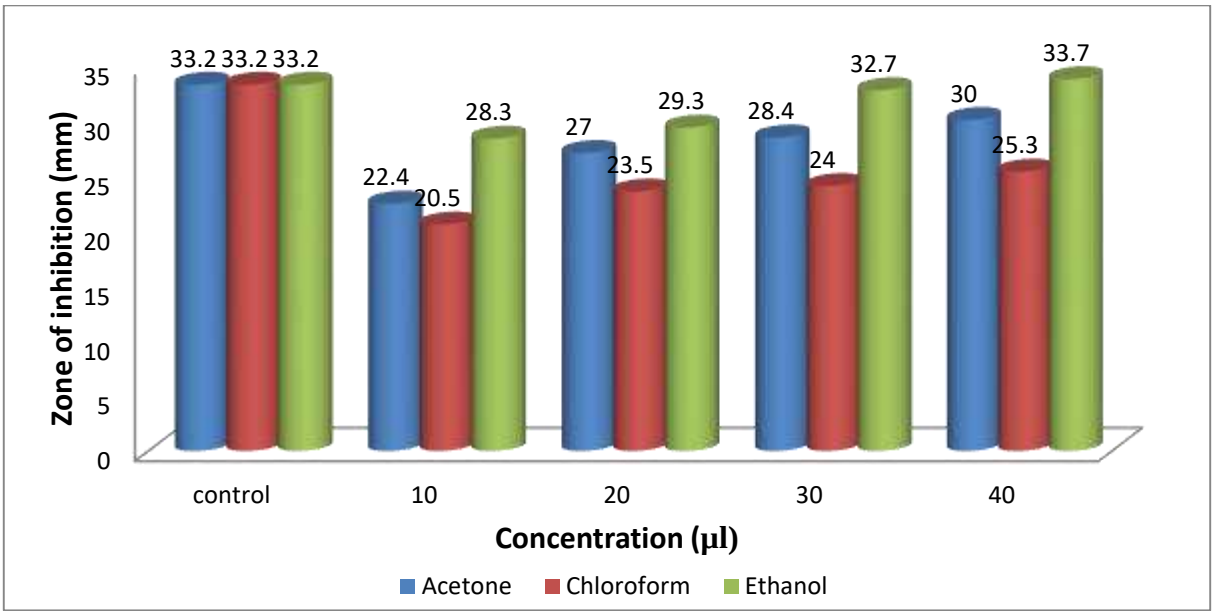

Fig 5: Graph showing antifungal activity of *P. guajava* leaf extracts against *E. coli.*

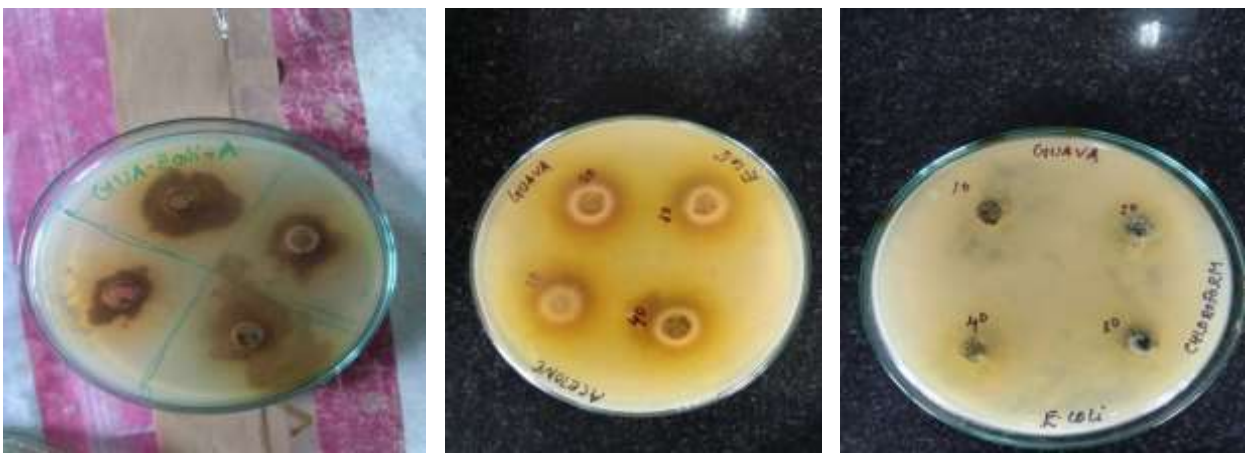

Fig 6: Plate showing antibacterial activity of *P. guajava* against *E. coli*

3.4 Antibacterial activity of *P. guajava* against *S. aureus*

The *P. guajava* leaf extract showed a remarkable antibacterial activity against the selected bacterial strain *S. aureus*. Against *S. aureus* among the three extracts, maximum antibacterial potency was shown by ethanol extract of *P. guajava* leaf giving a inhibitory zone of 32.04±1.6 mm at a concentration of 40µl and 30.0±0.3 mm at a concentration of 30µl respectively (Fig 7 & 8). And the positive control showed 31.8±1.8 mm inhibition zone. Aqil *et al* [31] reported significant inhibitory effect of ethanol extracts of various Indian medicinal plants on both clinical isolates of β-lactamase producing MRSA.

Followed by the activity of ethanol was that of acetone extract which provided a zone of inhibition of 30.2±0.2mm at a concentration of 40µl. Positive control showed an inhibition zone of 31.8 mm. Gnan and Damello [32] reported a complete inhibition of growth of *Staphyllococcus aureus*; aqeous guava leaf extract Vieira *et al* [33] reported the microbicidal effect of guava sprout extract (acetone). Abdelrahim *et al* [34] also reported a complete inhibition of *Bacillus subtilis*, *Staphyllococcus aureus* with extract of the guava leaf.

Among the two test organisms, *E. coli* showed more resistance against the leaf extract of *P. guajava* than *S. aureus.* In this study maximum antibacterial activity was exhibited by ethanol extract of *P. guajava* against *E. coli* species (33.7±0.5), which was followed by ethanol extract of *S. aureus* which showed a zone of inhibition of 32.04.±1.6 mm. Similar results in accordance with present study were reported by Zhao *et al* [35] in which it is stated that Gram negative bacteria are surrounded by the cell wall which disrupts diffusion of hydrophobic compounds through its lipopolysaccharide covering. The absence of barrier in Gram positive bacteria allows the direct contact of the essential oil's hydrophobic constituents with the phospholipids bilayer of the cell membrane. This causes an increase of ion permeability and leakage of vital intracellular constituents, or impairment of the bacterial enzyme systems. Venkataswamy *et al* [36] has reported that two groups of bacteria differ in their structure of cell wall.

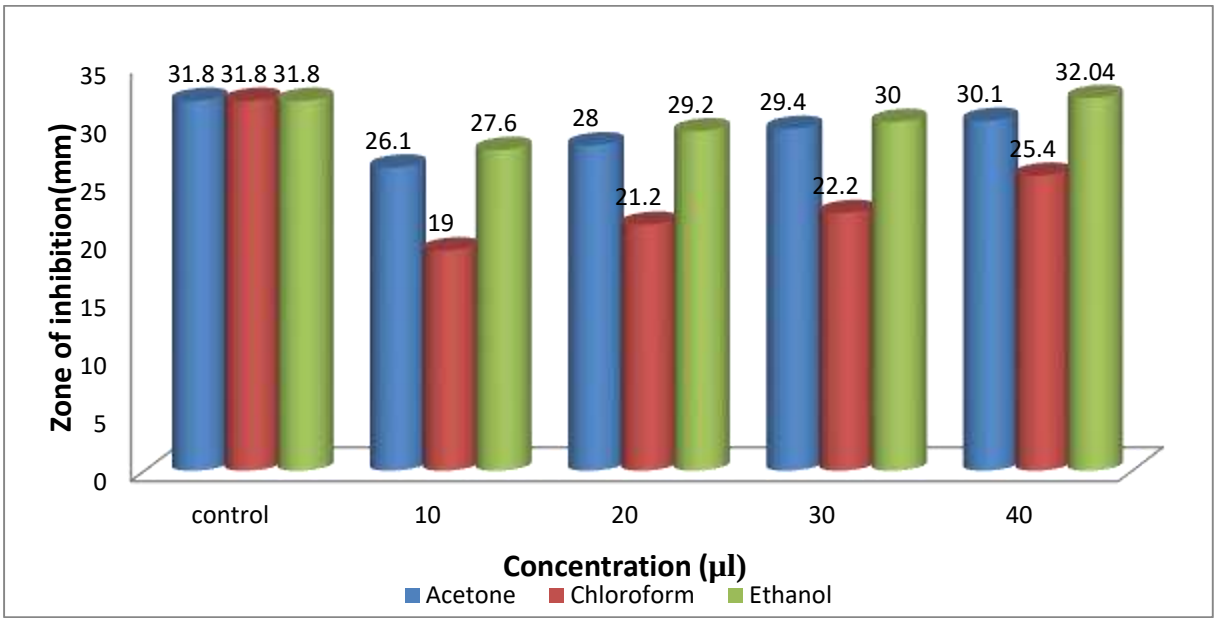

Fig 7: Graph showing antibacterial activity of *P. guajava* leaf extracts against *S. aureus*

Fig 8: Plate showing antibacterial activity of *P. guajava* against *S. aureus*

Phytochemical analysis is carried out in ethanol and acetone extracts of *A. amara* and *P. guajava* leaf extracts as they displayed excellent antibacterial activities to find out the number of secondary metabolites present in them. The results are presented in Table 1 & 2.

3.5 Phytochemical analysis of *Albizia amara* leaf

A. amara leaf extract tested for its antimicrobial activity against *S. aureus*, showed that acetone extract has high antimicrobial potential and moderate antibacterial activity against *E. coli*. Therefore the extract was tested for its phytochemical constituents and it showed the presence of saponins, tannins, terpenoids, sterols and proteins. Leaf extract of *A. amara* that showed highest inhibitory activity against the fungus *A. flavus* and *Fusarium sp.* has secondary metabolites like alkaloids, flavonoids, terpenoids, anthroquinones, phenols, saponins, tannins, proteins and quinones. Our phytochemical analyses are in agreement with the reports of other workers. Jyoti *et al* [37] has isolated ten phytochemicals like carbohydrate, proteins, starch, aminoacids, steroids, glycosides, flavonoids, alkaloids, tannins and saponins from the methanol extracts of rhizomes of *A. amara.*

Moderate antibacterial potential against the bacteria *S. aureus* and maximum activity against *E. coli* was exhibited by ethanol extract and its phytochemical analysis showed the presence of compounds such as flavonoids, saponins, tannins, terpenoids, phenols, sterols, quinones and proteins. alkaloids, flavonoids, sterols, phenols, saponins, tannins, proteins and quinones. The occurrence of alkaloids, phenols, phytosterols, saponins, sterols, tannins, flavonoids, terpenoids in the aqueous root extracts of *A. amara* was also reported earlier by Ali and Blunden [38]. Results parallel to present study was given by Srivastava *et al* [39] who has reported the presence of flavonoid, tannin, steroid and triterpine, saponin, alkaloid, cardiac glycoside and reducing compounds. The antibiotic resistance problem demands that a renewed effort be made to screen various medicinal plants for their potential antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant.

Table 1: Phytochemicals present in *Albizia amara* leaf extracts

Sl. No.	Constituents	*Albizia amara* 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

The presence of these chemical constituents in the leaf extracts of *A. amara* supports its antimicrobial activity. These phytochemicals are known to show medicinal as well as physiological activity as reported by Sofowora [22].

3.6 Phytochemical analysis of *Psidium guajava* Leaf

High antimicrobial activity against the bacteria strain *E. coli* and moderate antibacterial activity against *S. aureus* was exhibited by acetone extract of *P. guajava.* Therefore acetone extract was tested for phytochemical which showed the presence of secondary metabolites like alkaloids, flavonoids, sterols, anthroquinones, saponins and proteins. Swadhini *et al* [40] determined six phytochemicals viz., alkaloids, flavonoids, tannins, saponins, cardiac glycosides and phenol from aqueous extract of turmeric.

Ethanol extract of *P. guajava* exerted moderate antibacterial activity against *E. coli* and minimum activity against *S. aureus*. The phytochemical analysis of ethanol extract of *P. guajava* showed the presence of phytochemical compounds such as alkaloids, flavonoids, sterols, terpenoids, tannins and proteins. In concordance to the present study Rajeshwari *et al* [41] analysed phytochemical constituents in various analysis were carried out in *Aegle marmalos, Ruta graveolens, Opuntia dilleni, Euphornia royaleanna* and *Euphoria antiquorum* that were extracted using five different solvents. The solvents like acetone and ethanol exhibited phytochemical activity and revealed the presence of proteins, saponins and flavanoids.

Table 2: Phytochemicals present in *Psidium guajava* leaf extracts

Sl. No.	Constituents	*Psidium 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

The antimicrobial activity of the selected leaf and seed 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 [42] and Padayana *et al* [43] in which they have reported that antibacterial activity of leaf extracts can be attributed due to the presence of these phytochemicals. The results of the present study suggested that the use of this plant is beneficial to treat human diseases as it is a potential source of bioactive substances. Awoyinka *et al* [44] studied eight bioactive compounds from dry leaf of *Cnidoscolus aconitifolius* for its phytocompounds. In the solvents, methanol and acetone solvents showed the presence of phenols, tannins. The ethanol and chloroform solvents revealed the presence of glycosides, carbohydrates and tannins.

4. CONCLUSION:

The present study observed the sensitivity pattern of the selected pathogens towards the extracts of *A. amara* and *P. guajava*, as well as standard antibiotics. The result suggested that both the plants could be used as a curative agent for different ailments. In addition, phytochemicals evaluation of leaf extracts of *P. guajava* of *A. amara* provided information about a number of medicinally important secondary metabolites, which impart antimicrobial characteristics. It is suggested that using natural products as therapeutic agents will probably not cause any health issues and the present study supports this statement. In conclusion, the results of this study showed that leaf extracts of *P. guajava* of *A. amara* have antibacterial activity against the most common bacterial strains *S. aureus* and *E. coli* involved in human infectious diseases.

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