Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 10 [5] April 2021 : 15-27

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Evaluation of Antibacterial and Antioxidant Potential of *Melia. azedarach* Linn. and *Psidium guajava* Linn. Leaf Extracts

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ABSTRACT
The present study is intended to evaluate the total phenol, flavonoid contents, analyze in-vitro antibacterial and antioxidative activities of leaf extracts of Melia azedarach Linn (M. azedarach L.) and Psidium guajava Linn (P. guajavaL.). In-vitro antibacterial activity was measured using agar well diffusion assay against Pseudomonas aeruginosa and Staphylococcus aureus bacterial strains. In-vitro antioxidant activity was estimated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. Among both plants high minimum inhibitory concentration values are observed with P. guajava L. ethanol extract at 47.6 µg/ml for S. aureus and 43.7 µg/ml for P. aeruginosa, respectively. The highest concentrations of Total phenolic and flavonoid content values of 469, 496 µg/mg respectively were seen with ethanolic, followed by methanol extracts for P. guajava L. The results revealed that 20 µg/ml ethanol extracts of P. guajavaL. exhibited the highest radical % scavenging activity (76.24%) succeeded by methanol extracts (72.78%), respectively. We demonstrated that for both bacterial strains, ethanol followed by methanol extracts showed significant antibacterial activity in P. guajava L. against the bacterial strains in dose dependent manner indicating the presence of high flavonoids, tannins, and steroids. The results revealed that the ethanol extract fraction of P. guajava L. exhibited the highest radical scavenging activity followed by methanol extracts indicating they are effective solvents to extract phenolic compounds. Our results revealed that both plants are vital reservoirs of phytochemicals with antibacterial and antioxidant capacities hence could be of use for the supportive therapy of dengue fever.
Keywords: *Melia azedarach L., Psidium guajava L., Antioxidant activity, Antibacterial activity, Dengue fever, Total phenol content, Total flavonoid content.*

Received 18.02.2021 Revised 03.04.2021 Accepted 30.04.2021

INTRODUCTION

Dengue feverzis a mosquito borne disease that shows resurgence in tropical and subtropical regions of the world. Regardless of its serotypes, it is transmitted from person to person by *Aedes aegypti* and *Aedes albopictus (A albopictus)* mosquitoes as primary vectors in the domestic environment [1].Most of the people infected with the dengue virus are asymptomatic, the symptoms might start 4-7 days after the infected aedes mosquito bite which include muscle pain, high fever (≥104°F), chills, nausea, eye pain, vomiting, or abdominal pain, skin rashes, feeling tired and sometimes joint pain. However, in few patients it may proceed to dengue haemorrhagic fever with persistent abdominal pain, severe bleeding, decreased blood platelet count or thrombocytopenia. Therefore, supportive therapy is imperative for relieving the symptoms at the earliest.

During dengue infection there is every possibility of co-infections when clinical course alters or when clinical features cannot be described by dengue virus and these facts are supported by some previously reported case reports and case series [2]. However, these individual co-infections can be bacterial infection, enteric fever, hepatitis A, chikungunya, or zika virus co-infection. Among these categorial co-infections bacterial infections are more common and leading cause for increased incidence of disease worldwide. A report by Prasan Kumar Panda et al., reveled case study regarding bacterial co-infection due to *P. aeruginosa* and *S. aureus* in sputum culture of dengue patient[3].

To our knowledge, there are limited studies explaining treatment strategies for bacterial co-infections and oxidative stress associated with dengue using antibacterial and antioxidants respectively [4]. However, no study is proposed to treat these conditions using medicinal plants. Medicinal plants constitute the major constituents of most native medicines and many of them contain one or more

components of plant origin for curing the life threatening infections caused by pathogenic microorganisms.

An imbalance between antioxidants and reactive oxygen species results in oxidative stress induced cellular damage. Oxidative stress occurs due to intracellular calcium ions (Ca^{2+}) and thiol (E.g.:GSH) imbalance. It is linked to most of the diseases such as cancer, diabetes, ageing, atherosclerosis, ischemia injury, rheumatoid arthritis, inflammation, neurodegenerative, and pulmonary disorders[5]. Recent findings revealed oxidative stress in response to pathogenesis of severe dengue disease prognosis and therapeutics [6].Epidemiological studies reinforce that the prevalence of oxidative stress-associated conditions is mitigated by the consumption of fruits and vegetables abundant in compounds retaining high antioxidant activity [7].

Taking this into consideration our present study is aimed to assess antibacterial and antioxidant activities of various solvent extracts (ethanol (E.E), methanol (M.E) , pet. ether (P.E.E), aqueous (A.E))extracts obtained from *Melia. azedarach* Linn (*M. azedarach* L.) and *Psidium guajava* Linn(*P. guajava* L.) leaves, as well as evaluating their total phenol and flavonoid contents.

MATERIAL AND METHODS

Collection of the plant materials:

Leaves of *M. azedarach* and *P. guajava* were collected from various locations of Andhra Pradesh, India and authenticated by taxonomist P. Satyanarayana Raju at the Department of Botany &Microbiology, ANU, India.

Preparation of the plant extracts:

The dried powdered leaves are extracted continuously at 55 ^{o}C with 500 ml of 50% (v/v) of different solvents petroleum ether (Pet.ether), ethanol, methanol, and water by soxhlet extraction [8]. The extracts obtained were filtered, evaporated using rotary evaporator at various temperatures depending on the solvent. The concentrated solutions obtained were freeze-dried, lyophilized, and then stored in sterile glass desiccators until further analysis. Dimethyl sulfoxide (DMSO)is used to prepare dose extracts that can be orally administered.

Chemicals and reagents:

Pet.ether, Ethanol, Methanol, Chloroform, DMSO, Ampicillin were purchased from Anka chem, Telangana, India. 2, 2- Diphenyl-2-picryl hydrazyl (DPPH), rutin, gallic acid andAscorbic acid, Nutrient agar, luria broth for bacterial cultureare obtained from (Himedia Lab., India).

Test microorganisms:

The two Gram-positive bacteria, *Pseudomonas aeruginosa* (*P. aeruginosa)* and *Staphylococcus aureus (S. aureus)* bacterial samples were obtained from (MTCC1144, & 6388).

In-vitro antibacterial and antioxidant activities of *M. azedarach* and *P. guajava* leaves*:*

Antibacterial activity using Agar well diffusion method.

Agar well-diffusion method was followed to determine the antibacterial activity [9]. Solidified nutrient agar plates were cleansed using sterile cotton swabs with 8 hours old broth culture of respective bacteria. Wells (2 cm apart and 10 mm diameter) were made in each of these plates using sterile cork-borer. The control for experiment was made of DMSO solution. Stock solution of both extracts were prepared at a concentration of 1 mg/ml in different solvents viz. petroleum ether, ethanol, methanol, water. About 100 µl concentrations of *M. azedarach* L. and *P. guajava* L. solvent extracts were added individually using sterile syringe into the wells of agar medium containing petridish and done twice in a row and allowed to diffuse at room temperature for about 2 hours [Figure 1].

Pure sub-cultures of *P. aeruginosa* and *S. aureus* were used in the study and were obtained from college microbiology lab that were initially procured from the Microbial type of culture collection and gene bank (MTCC1144, 6388). Control experiments comprising only inoculums without plant extracts were set up. The plates were further incubated at 37°C for 18-24 h for growth of pathogenic bacteria. For the antibacterial activity, the inhibition zone diameter (IZ) (mm) was measured, and the activity index (AI) was also calculated. Triplicates were maintained, the average values were recorded [10].Also, the activity index is calculated for both plant extracts using the formula, AI- Activity index = IZ of test sample / IZ of standard.

Microdilution method:

The Minimal inhibitory concentration (MIC) and Minimum bacterial concentration (MBC) were performed by a serial dilution technique employing 96-well microtiter plates. The serial dilution of the extracts was made with Luria broth for bacterial culture with respective inoculum. The microplates were incubated for 72 hours at 28^{o}C, respectively. The MICs were determined by serial sub-cultivation of 2 µl into microtiter plates containing 100 µl of broth per well and further incubation for 72 hours. The optical density of each well was measured at a wavelength of 655 nm by Microplate reader (Bio Rad, iMark-

1.02.01)and compared with the standard ampicillin for bacteria as the positive control. All experiments were performed in triplicate [Figure 1].

Phytochemical Analysis:

The preliminary qualitative phytochemical screening of various extracts was done to determine the presence of bioactive components. The presence of phenolics (ferric chloride test), alkaloids (dragendroff test), flavonoids (ammonium test), tannins (lead acetate), triterpenes/ steroids (liebermann burchardt test), saponins (foam test) and glycosides was determined (legals test) [11, 12]. These are further confirmed using the quantitative tests.

Determination of total phenolics:

The total phenolics content inextracts were determined with slight modifications using Folin- Ciocalteu's phenol reagent method [13]. Stock solutions of extracts and gallic acid were prepared by solvating 1 mg of *M. azedarach* L. &*P. guajava* L. aliquots separately and gallic acid in 1 ml of extract. A total of 50µl of different concentrations of extracts ranging from 10µg to 100µg were taken into series of test tubes. To each tube 250µl of 50% Folin- Ciocalteu's reagent was added and properly mixed and allowed to stand for 10 minutes. Then add 500µl 20% sodium carbonate (Na_2CO_3) to the above mixture. The mixtures were then vortexed to which autoclaved distilled water was added to make up the final volume up to 5 ml [14].

A set of standard solutions of gallic acid (10, 20, 40, 60, 80 and 100 ug/ml) in distilled water were prepared. The absorbance of the blue colored complex was measured for test and standard solutions against the reagent blank at 750nm after 30 min incubation at room temperature. The results were expressed as gallic acid equivalent (GAE) (µg/mg of extracted compound) using gallic acid calibration curve [15].

Determination of flavonoids:

The total flavonoids content (TFC) in extracts were measured through Aluminum chloride colorimetric assay as described by Moneim [16]. 1mg/ml of stock solutions were prepared in distilled water for different dilutions of rutin standard and *M. azedarach* L &*P. guajava L* extracts (10-100µg/ml) concentrations separately in a series of test tubes. The total volume was made up to 5 ml using distilled water. Around 0.3ml of 5% $NaNO_2$ was added to the above mixture. Then after 5 minutes 0.3ml of 10% aluminum chloride ($AlCl_3$) was incorporated to the mixture resulting in the appearance of a yellow color [17]. The resultant mixture was incubated for 6 minutes at room temperature. After the incubation period 2 ml of 1M NaOH was incorporated into the mixture. The total volume of about 10 ml was made up with distilled water. The absorbance was measured against a reagent blank devoid of the extract at 510 nm wavelength in the spectrophotometer after the solution was mixed well. The results were expressed in terms of rutin equivalent (µg/mg of extracted compound) calibration curve.

***In-vitro* antioxidative activity using DPPH assay:**

The antioxidant activity of the extracts was determined by free radical scavenging ability in correspondence to stable 2, 2- diphenyl-2-picrylhydrazyl (DPPH) method[18]. The scavenging activity was in correspondence to hydrogen donating ability and was determined by the method described by Brand-Williams *et al.*[19] with minor modification. The DPPH radical discolorizes in presence of antioxidants (catechol moieties) indicating the scavenging potential towards the free DPPH* radical by *in-vitro* method. A linear correlation was founded between the total phenol content estimation (Folin-Ciocalteu assay) and the free radical scavenging potential [20].

The extracts were prepared i.e., 20µg/ml, 40µg/ml, 60µmg/ml, 80µmg/ml, and 100µg/ml. 5 ml of each prepared concentration was mixed with 0.5ml of 1mM DPPH solution in DMSO with ethanol and methanol, pet. ether and aqueous solutions, respectively. Experiment was done in triplicate. The test tubes were incubated for 30 min at room temperature with intermittent shaking and then, absorbance measured at 517nm using UV- Visible spectrophotometer, lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. Vitamin C (0.1 mg/ml) was used as a standard and the same concentrations were prepared as the test solutions [Figure 2]. The difference in initial absorbance between the test extracts and the control (DPPH in solvent) was calculated and expressed as % scavenging activity of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation. Scavenging effect (%) = (1-AS/AC) ×100, AS is the absorbance of the sample at t =0 min. AC is the absorbance of the control at t =30 min, which varied with different concentrations.

Statistical Analysis

All experimental results were expressed as mean ± standard deviation (SD)or standard error of mean (SEM), n = 3. Statistical analysis of the data was performed by one-way Analysis of Variance (ANOVA) and mean comparison using Student's t-test. Graphical representation is performed using software GraphPad prism software 9.0.0.121 version. P <*0.05,<**0.01 was considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening

The significance in the yields of *M. azedarach* L.& *P. guajava* L. leaf extracts ($p \leq 0.05$) were shown in Table 1. The % (w/w) yields of pet. ether, ethanol, methanol, and aqueous extracts were 11.74gm (2.34%), 13.39gm (2.67%), 23.50gm (4.7%), and 25.37gm (5.07%), respectively while for *P. guajava* L.leaves the crude extracts weights were10.16gm (2.02%), 14.95gm (2.98%),23.86gm (4.77%) and27.43gm (5.48%)correspondingly. The variation in yields obtained is due to the presence of higher percent of polar content in the leaf extracts. Most of the polar contents are extracted when most polar aqueous solvent is used followed by methanol, while pet. ether and ethanol showed minimum yield.(Table 1).

The quantitative analysis of phytochemical constituents of crude extracts of both plants are shown in (Table 2). Both the plants data show the presence of flavonoids,phenols, tannins, glycosides in higher amount while other phytoconstituents saponins, steroids were present in trace amounts and anthraquinones are absent. *M. azedarach* L. showed presence of higher concentration of flavonoids while *P. guajava* L. indicated higher concentration of phenolics. The results obtained are in correspondence with existing data[21, 22].

Antibacterial activity

The antibacterial activity of M. azedarach L. (M. A. 1 & 2) & P. guajava L. P. G 1 & 2) leaf extracts was determined *in-vitro*using agar well diffusion method and micro dilution methods [23, 24]. Results were evaluated according to their zone of inhibition (IZ) against sample-1(*S. aureus)* and sample-2 (*P. aeruginosa)*pathogens (Figure3). The obtained IZ, AI values were compared with that of standard, viz., Ampicillin (1.0 mg/disc)as summarized in [25] (Table 3). For both the tested bacterial cultures, ethanol extract showed maximum IZ value in *P. guajava* L.(24.6mm for*S. aureus*) which is followed by *M. azedarach* L ethanol extract (23.4for *P. aeruginosa*).

The standard ampicillin IZ values for the two bacterial strains closely resemble the IZ values of ethanol extract of respective plants (23.25mm for *P. aeruginosa* and 20.13mm for *S. aureus*). The minimum IZ valuesof 8.9 and 11.7 mm are observed with aqueous extract of *M. azedarach* L.with respect to *S. aureus* and*P. aeruginosa* pathogens(Table 3 and Figure 4).More specifically, aqueous extract represented higher susceptibility to all bacterial strains . Overall, ethanol extract showed higher AI value with *P. guajava* L of 1.222 for *S. aureus* and for *M. azedarach* L. of 1.006 for *P. aeruginosa*. The minimum AI values are observed with *M. azedarach* L. aqueous (0.503 for *P. aeruginosa* and 0.442 for *S. aureus*) (Table 3, Figure 5).

Further, maximum MIC values are observed with *P. guajava* L. ethanol extract at 47.6 µg/ml for *S. aureus and* 43.7 µg/ml for *P. aeruginosa,* respectively. Aqueous extract of *P. guajava*L. showed least MIC values of 37.0 µg/ml & 35.9 µg/ml against *S. aureus* & *P. aeruginosa*, respectively. For *M. azedarach* L. maximum MIC values of 12.7 µg/ml for *S. aureus and* 17.8 µg/ml for *P. aeruginosa* are observed for ethanol extract, while minimum values are observed withaqueous extract at 7.0 µg/ml for *S. aureus and* 9.6 µg/ml for *P. aeruginosa,* individually (Table 4, Figure6).

In connection with MIC values the MBC values were estimated accordingly. Ethanol extract of *P. guajava* L. showed comparatively efficient MBC value of 94.4 µg/ml (*S. aureus)* and 96.4 µg/ml (*P. aeruginosa*) while aqueous extract has minimum value with 73.1 µg/ml (*S. aureus)* and 71.9 µg/ml (*P. aeruginosa*) (Table 4, Figure 6). Whilst ethanol extract of *M. azedarach* L. indicated maximum MBC of 24.8 µg/ml &34.6 µg/ml against *S. aureus* & *P. aeruginosa* correspondingly and aqueous extract showed minimum MBC of 14.8 µg/ml & 18.3 µg/ml against *S. aureus*& *P. aeruginosa* correspondingly. These results are in line with the findings of earlier reported activities of these plants and the high antibacterial activity of *P. guajava* L. extracts could be due to presence of high content of flavonols, isoflavones and flavones[25].

The results all together revealed that ethanol followed by methanol extract of*M. azedarach* L.and *P. guajava* L. plants have potent antibacterial activity against the two bacteria studied showing high degree of inhibition followed by pet. ether and aqueous extracts. The inhibition zone diameters differ for the plant extracts which might be due to diffusion capacity and amphiphilic character of substances used and their antibacterial action. The major components in studied extracts including quercetin among phenolics and low doses of gallic acid, oleic acid inhibit the growth of *S. aureus* and *P. aeruginosa* bacteria in accordance with study conducted by Dilika et al., [26].From the results of study, it was understandable that *P. guajava* L. shows better antibacterial action than *M. azedarach* L. extracts and could be used as a drug of choice in the treatment of bacterial co-infections against dengue.

Amongst types of dengue co-infections, majority of patients are affected with bacterial infection followed by viral, parasite, and then fungal. The quest for antibacterial from natural sources to treat for dengue co-infections has received much consideration in concern with morbidity and energies that have been put in to identifying compounds that can act as appropriate antibacterial agents and can replace synthetic ones.

Copious studies have been conducted with the extracts of various plants, screening antibacterial activity as well as for the discovery of new antimicrobial compounds [27,28]. Phytochemicals derived from plant products serve as a model to develop less toxic and more effective medicines in controlling the growth of microorganisms [29,30].

Though, the mechanism of the action of these plant constituents is not yet fully understood the efficiency of the extracts generally depends on the nature of solvent used. The organic extracts offered more powerful antibacterial activity as compared to aqueous extracts. This observation clearly indicates that the endurance of non-polar residues in the extracts leading to higher bactericidal and bacteriostatic capacities. Here, the MIC value of the active plant extracts obtained were lower than the MBC values suggesting that the plant extracts were bacteriostatic at lower concentration but bactericidal at higher concentration.

Antioxidant activity

The total phenolics (TPC) and flavonoids (TFC) content were determined usingfolin- ciocalteu's and aluminum chloride colorimetric methods, respectively.

Phenolic compounds in plants are responsible for their antioxidant activity due to presence of hydroxyl groups [31]. The results were reported as equivalents of GAE using gallic acid calibration curve (µg/mg) for TPC and equivalents of rutin (RE) (µg/mg) using rutin calibration curve for TFC (Table 5, Figure7)

The TPC values differed significantly between the various extracts as shown in Table 6 and Figure 8. The highest concentration of TPC 469 µg/mg was seen with ethanolic, followed by methanol (397 µg/mg), pet. ether (286 µg/mg) and least with aqueous (189µg/mg) extracts for *P. guajava* L. While the order for *M. azedarach* L. follows 431, 359, 248 and 148 µg/mg for ethanol, methanol, pet.ether and water extracts, respectively. However, the values of both extracts are comparatively lesser than the standard ascorbic acid. Totally, *P. guajava* L. extracts showed better TPC values indicating the presence of more phenolic compounds than *M. azedarach* L. extracts.

Flavonoids also serve as secondary antioxidant defense system in plants when exposed to variable stresses [32]. The TFC varied considerably between the M. *azedarach* L. &*P. guajava* L.as shown in Table 6 and Figure 9. The highest concentration of total TFC was seen in *P. guajava* L. (496 µg/mg -ethanol), followed by methanolic (481 µg/mg),pet. ether, and aqueous extract 465, 431 µg/mg, respectively.TFC values *P. guajava* L. are higher than the standard values. While in *M. azedarach* L. the values of 194, 184, 177and 120µg/mg were observed for ethanol, methanol, pet. ether and aqueous extracts, respectively. These results align with TPC values indicating the presence of more flavonoids along with phenolic compounds in *P. guajava* L. extracts.

Several assays are used to assess antioxidant activity but generally used methods are those that include production of free radical species which are formerly neutralized by antioxidant compounds [33]. Table7, Figure 11 shows the results of the free radical activity (DPPH) in terms of % scavenging action for *M. azedarach* L.&*P. guajava* L. *leaves* extract in comparison to standard vitamin C. The discoloration of the samplesin extract solvents was observed in accordance with addition of DPPH reagent (Figure10). The results showed that the decrease in scavenging activity with respect to increase in absorbance of the DPPH radical, was due to its reduction by various antioxidant concentrations of extracts relating to the standard. The results revealed that 20 µg/ml ethanol extract fraction of *P. guajava*L. exhibited the highest radical % scavenging activity (76.24%)succeeded by methanol,Pet. ether and aqueous extracts with 72.78%, 68.67%,and 65.78%, respectively. While lowest % scavenging activity of 74.79%, 70.46%, 67.15%, and 63.26%are demonstrated in *M. azedarach* L. 100 µg/ml ethanol, methanol, pet. ether, and aqueous extracts, respectively.

Finally, in the present study, both ethanolic extracts showed the highest % scavenging activity followed by methanolic extracts indicating they are effective solvents to extract phenolic compounds [35]. Ethanol is favored for the extraction of antioxidant compounds mainly because of its low toxicity [36]. Thus, it was apparent that DPPH free radical scavenging activity is related to the presence of bioactive compounds such as phenolic compounds in extracts [37]. Also, the antioxidant capability of these plants revealed that scavenging effect DPPH radical was proportional to phenolic content along with flavonoids contribution indicating that higher the phenolic content in plants then higher will be the radical scavenging action.

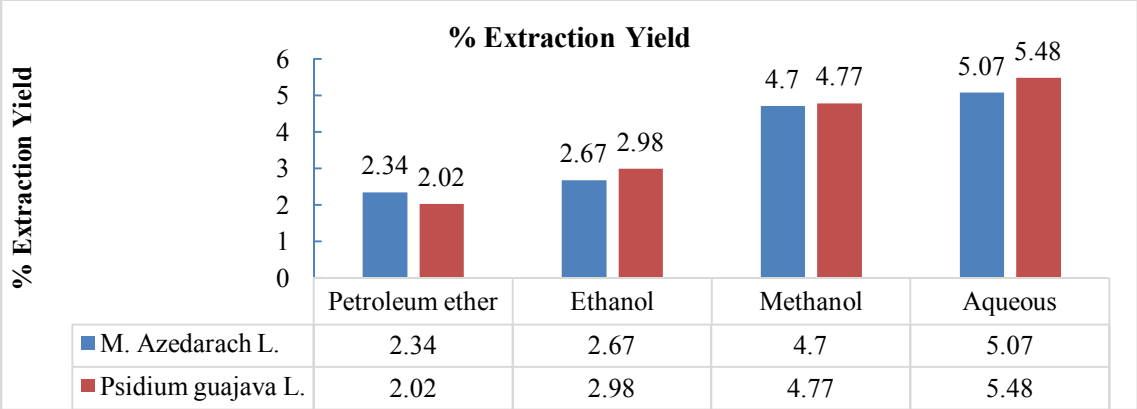


Table 1: Percent extract yield by solvents for *M. azedarach* L.& *P. guajava* L.leaves.

Table 2: Preliminary quantitative analysis of phytochemical constituents of different extracts of *M. azedarach*L. & *P. guajava* L. leaves.

Si.no	Constituents	*M. azedarach*L. extracts				*P. guajava* L. extracts			
		P.E	E.E	M.E	A.E	P.E	E.E	M.E	A.E
1.	Alkaloids	+	+	-	+++	-	++	+++	++
2.	Steroids	-	+++	+	+	-	+	-	-
3.	Tannins	+	++	++	++	-	+++	++	++
4.	Phenols	+++	++	++	-	+	+++	++	++
5.	Flavonoids	++	+	+++	+++	-	+++	++	++
6.	Glycosides	+++	+	+	+	-	+++	+	++
7.	Saponins	+	++	-	++	-	-	-	-
8.	Terpenes	+++	+++	++	++	+	-	+	+
9.	Reducing Sugar	-	-	+	+	+	++	+	++
10.	Anthraquinone	-	+	-	-	-	-	-	-

(+) indicates presence and **(-)** indicates absence of phytochemical constituents,(**++**) indicate moderate presence, (**+++**) indicates high presence.

Table 3: Antibacterial activity (zone of inhibition (IZ), mm, and activity index (AI)) of *M. azedarach* L. and *P. guajava* L. plant extracts.

Plant	Bacterial strain	Activity	E.E	M.E	P.E.E	A.E	Standard (Ampicillin)
M .azedarach L.	S. aureus	IZ	20.2±0.25	17.3±0.31*	15.0±0.35	8.9±0.15*	20.13
		AI	1.003	0.859	0.745	0.442	
M. azedarach L.	P. aeruginosa	IZ	23.4±0.14*	18.6±0.25*	14.0±0.13*	11.7±0.52	23.25
		AI	1.006	0.886	0.602	0.503	
P. guajava L.	S. aureus	IZ	24.6±0.15*	20.56±0.34	22.4±0.25*	16.3±0.13*	23.13
		AI	1.222	1.021	1.11	0.809	
P. guajava L.	P. aeruginosa	IZ	20.7±0.65	20.3±0.86	14.5±0.52	14.8±0.41	26.25
		AI	0.890	0.787	0.623	0.636	

Values are mean of triplicate readings (mean ± S.D), IZ- includes the diameter of disc (6 mm); Standard - Ampicillin (1.0 mg/disc). Some readings were found to be significant with P value <0.05.

Table 4: MIC (µg /ml), MBC performance of different extracts of *M. azedarach* L. and *P. guajava.* L. against pathogenic organisms

Bacterial strain	Plant Extract	Activity	E.E (µg/ml)	M.E (µg/ml)	P.E.E (µg/ml)	A.E (µg/ml)
S. aureus	*P. guajava* L.	MIC	47.6	45.6	40.7	37.0
		MBC	94.4	83.3	82.5	73.1
P. aeruginosa	*P. guajava* L.	MIC	43.7	41.7	39.8	35.9
		MBC	96.4	94.5	89.7	71.9
S. aureus	*M. azedarach* L.	MIC	22.7	21.27	19.7	17.0
		MBC	48.8	42.5	38.7	34.8
P. aeruginosa	*M. azedarach* L.	MIC	27.8	24.5	22.7	19.6
		MBC	54.6	48.3	44.5	38.3

Table-5: The calibration curve and R^2 value of gallic acid and rutin standards.

Standard	Regression equation	R^2 value
Gallic acid	y=0.0121x + 0.0642	0.9991
Rutin	$y=0.00669x + 2 \times 10^{-16}$	0.9998

Table 6: Total phenolic contents (TPC) and Total flavonoid contents (TFC) of *P. guajava* L.&*M. azedarach* L. extracts in comparison with standard.

Sl.no	Solvent extracts used	TPC (µg/mg) of Standard (gallic acid)	TPC (µg/mg) of *P. guajava*L. leaves	TPC (µg/mg) of *M. azedarach*L. leaves	TFC (µg/mg) of standard (rutin)	TFC (µg/mg) of *P. guajava* L. leaves	TFC (µg/mg) of *M. azedarach* L. leaves
1	Ethanol (E.E)	518± 0.67	469± 1.23	431± 1.42	340± 1.03	496± 0.79	194± 0.89
2	Methanol (M.E)	496± 1.21	397± 1.13	359± 0.98	321± 1.11	481± 0.84	184± 0.73
3	Pet. Ether (P.E.E)	374± 0.72	286± 0.18	248± 1.03	313± 0.67	465± 1.16	177± 1.19
4	Aqueous (A.E)	297± 0.67	189± 0.21	148± 1.35	299± 1.17	431± 1.17	120± 1.21

All data values were expressed as mean ± standard deviation (SD) (n=3).

Table 7: DPPH radical scavenging activity (%) of in various solvent extracts of *M. azedarach* L.,*P. guajava* L.leaves and standard.

Sl. no	Conc (µg/ml)	Scavenging effect (%) of Ascorbic acid (standard)				Scavenging effect (%) of *M. azedarach*L.				Scavenging effect (%) of *P. guajava*L.			
		E.E	M.E	P.E.E	A.E	E.E	M.E	P.E.E	A.E	E.E	M.E	P.E.E	A.E
1	20	84.76± 0.02	81.30± 0.05	77.19± 0.07	74.31± 0.06	74.79± 0.08	70.46± 0.04	67.15± 0.03	63.26± 0.07	76.24± 0.08	72.78± 0.04	68.67± 0.03	65.78± 0.04
2	40	82.13± 0.09	77.25± 0.10	74.75± 0.03	71.86± 0.07	72.66± 0.06	68.33± 0.06	65.02± 0.07	62.13± 0.05	74.11± 0.06	69.23± 0.04	66.73± 0.07	63.84± 0.09
3	60	80.57± 0.03	73.30± 0.07	71.95± 0.04	69.06± 0.04	71.11± 0.07	66.77± 0.08	63.46± 0.09	60.57± 0.03	73.83± 0.07	66.56± 0.08	65.21± 0.09	62.32± 0.06
4	80	77.31± 0.12	66.29± 0.08	68.13± 0.07	65.24± 0.03	67.84± 0.04	63.51± 0.07	60.20± 0.06	57.31± 0.09	71.56± 0.04	60.54± 0.07	62.38± 0.06	59.49± 0.07
5	100	71.04± 0.08	61.49± 0.04	60.28± 0.06	57.19± 0.07	61.57± 0.05	57.24± 0.10	53.93± 0.05	51.04± 0.03	69.89± 0.05	60.34± 0.10	59.13± 0.05	56.24± 0.03

Each value is expressed as the mean ± SD (n = 3). P value is significant <0.05.

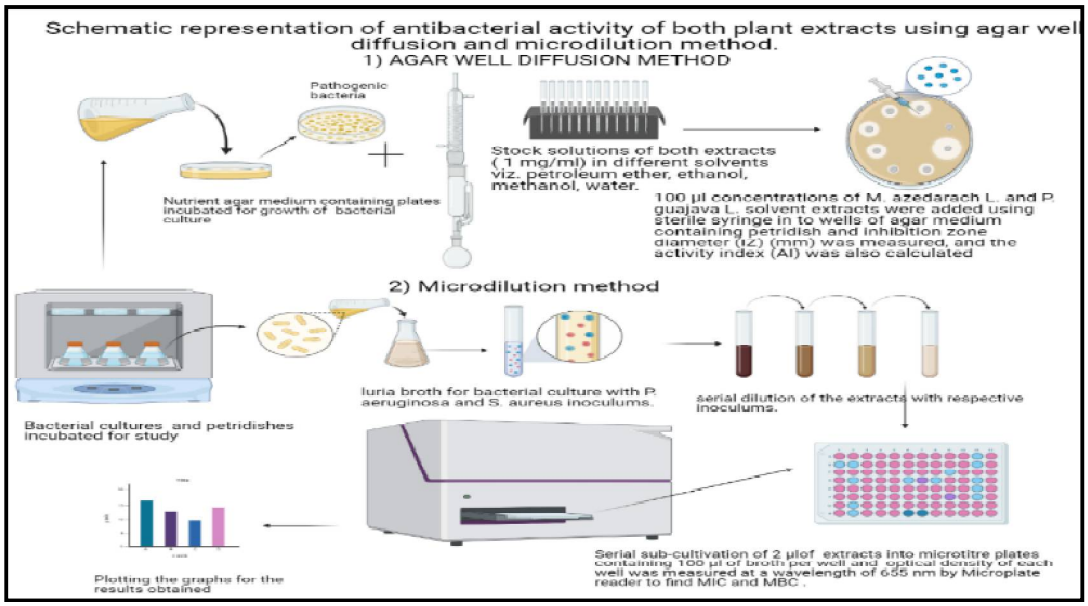


Fig 1 : Schematic representation of antibacterial activity of both plant extracts using agar well diffusion and microdilution method.

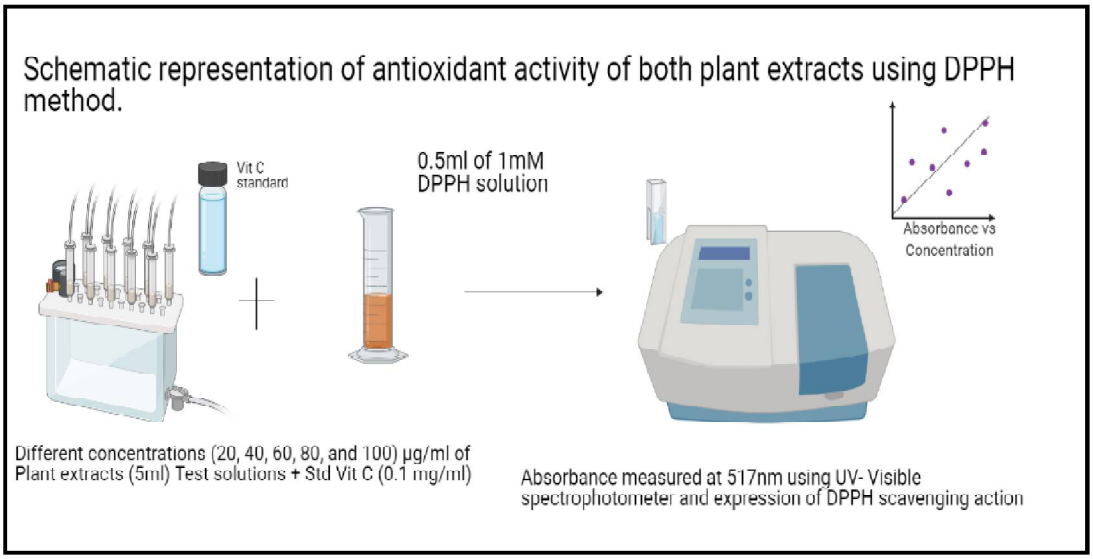


Fig 2 : Schematic representation of antioxidant activity of both plant extracts using DPPH method.

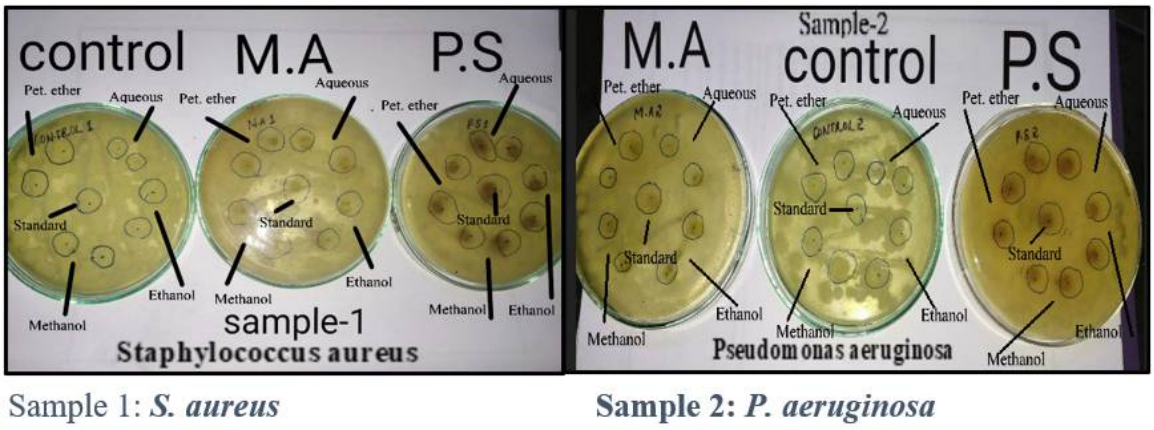


Fig 3: Antibacterial effect of various extracts of leaves of both plants against bacterial sample 1 & 2 strains by indicating zone of inhibition.

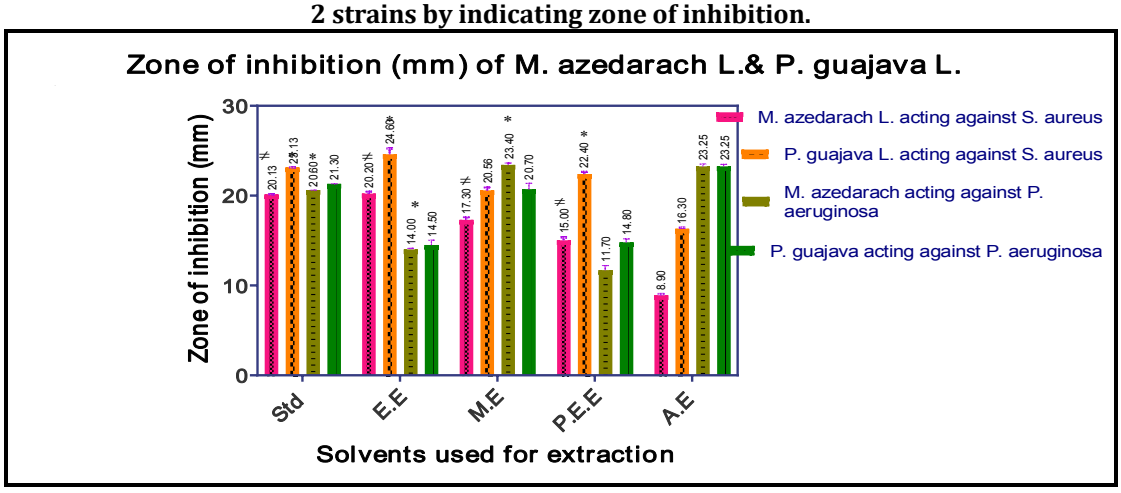


Fig 4: Zone of inhibition of E.E, M.E, P.E.E, A.E extracts of leaves of *M. azedarach* L. and *P. guajava* L. against bacterial strains observed against standard (S). P value is significant <0.05.

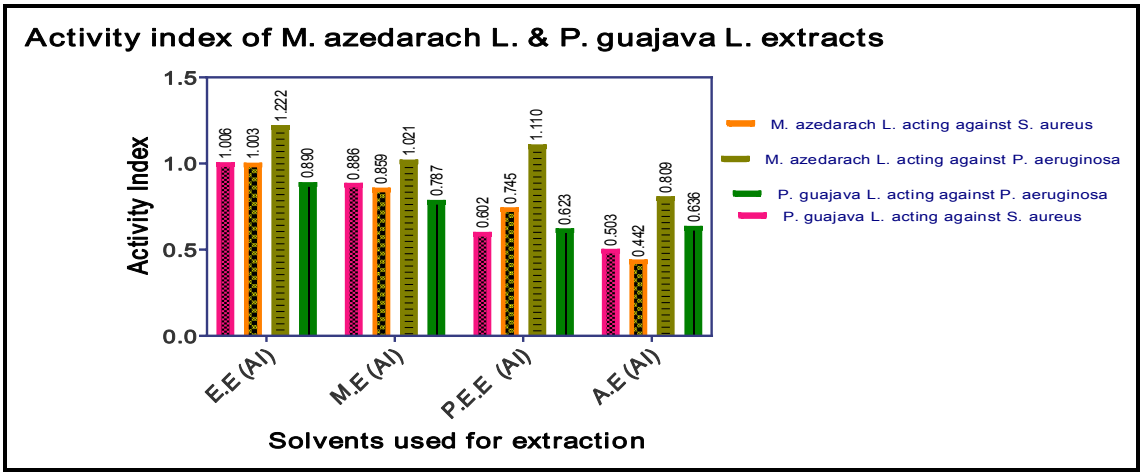


Fig 5: Activity index of E.E, M.E, P.E.E, A.E extracts of leaves of *M. azedarach* L. and *P. guajava* L. against bacterial strains.

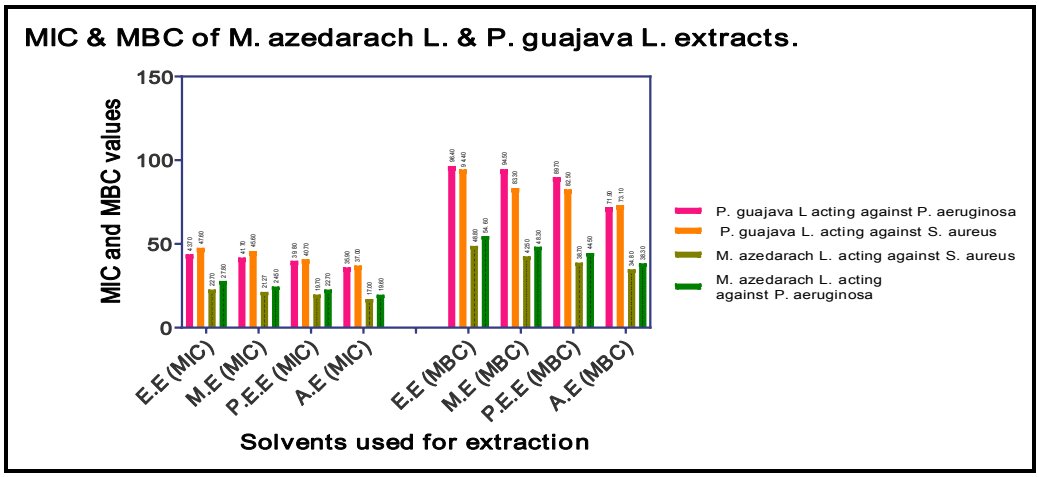
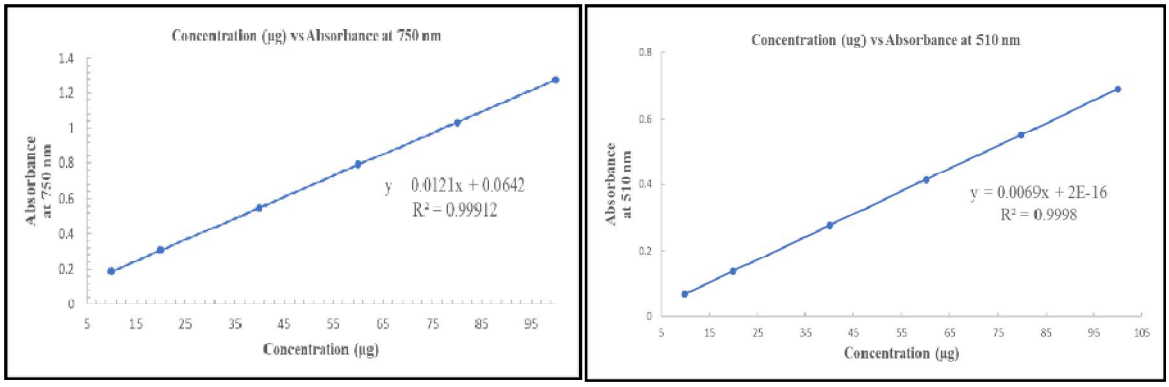


Fig 6: MIC & MBC of E.E, M.E, P.E.E, A.E extracts of leaves of *M. azedarach* L. and *P. guajava* L. against bacterial strains.



(A) Standard curve for gallic acid (B) Standard curve for rutin

Fig 7: Standard calibration curve for gallic acid (A) and rutin (B) standards.

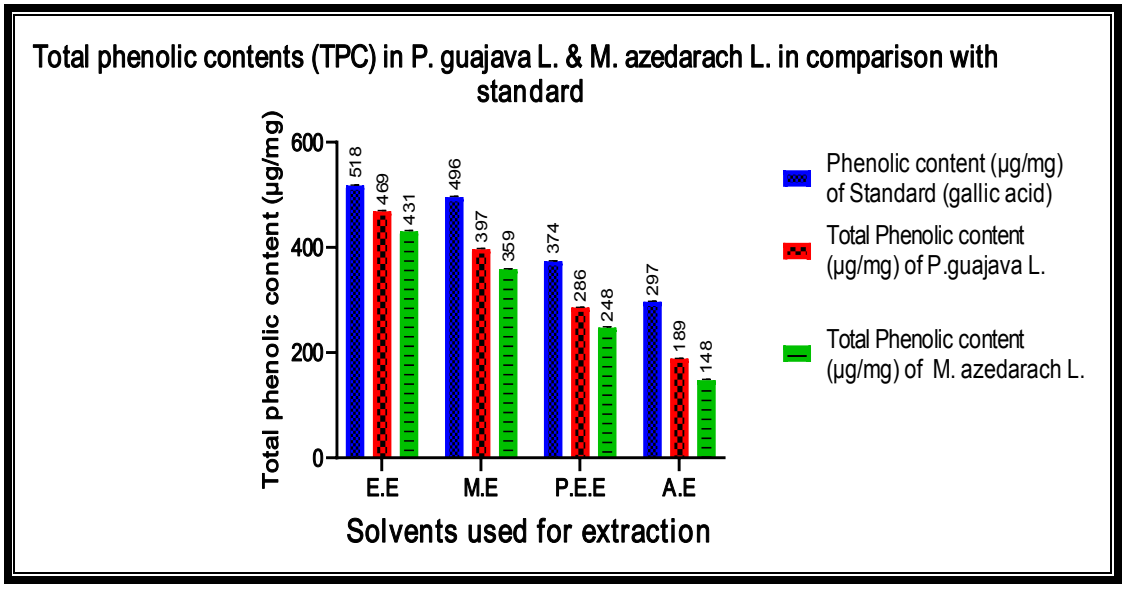


Fig 8: Total phenolic contents(µg/mg) of standard, *P. guajava* L.and *M. azedarach* L. with respect to E.E, M.E, P.E.E, A.E extracts. P value is significant <0.05.

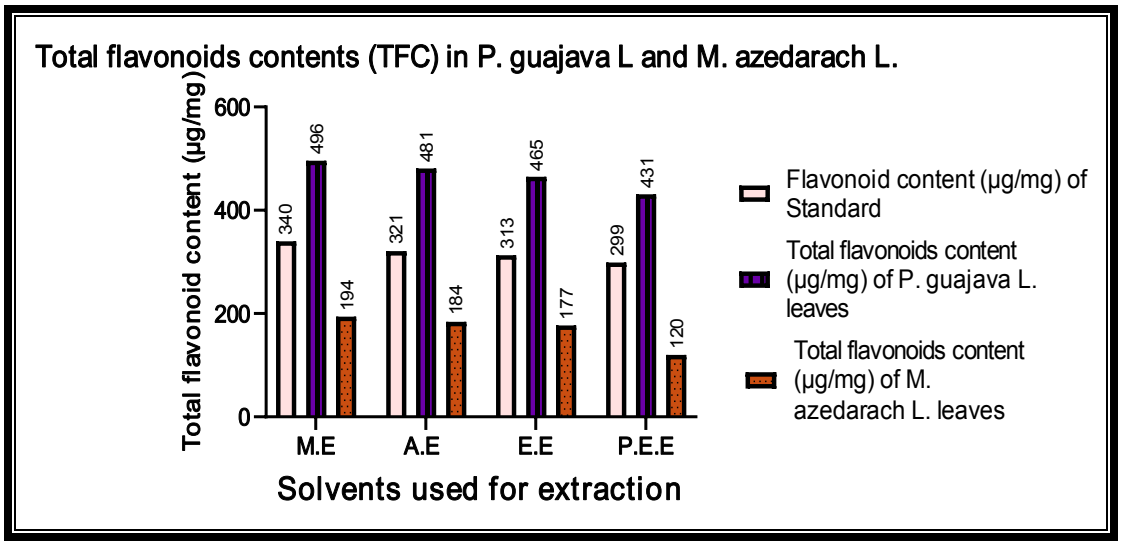
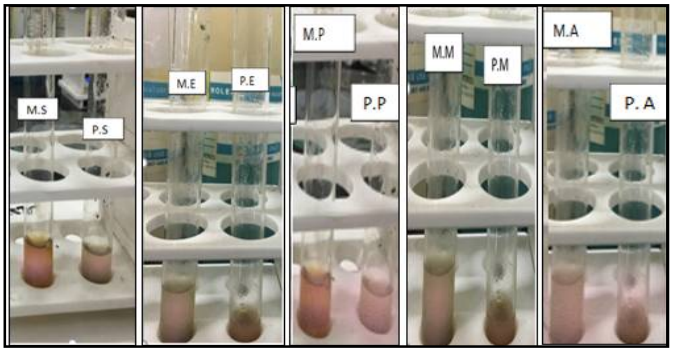


Fig 9:Total flavonoids contents (µg/mg) of standard, *P. guajava* L.and *M. azedarach* L. with respect to E.E, M.E, P.E.E, A.E extracts. P value is significant <0.05.



(A) *(B)* *(C)* *(D)* *(E)*

Fig 10: Discoloration of DPPH solution under the influence of *M. azedarach* L.(M), *P. guajava* L. (P) and standard (S) using ethanol (E), methanol (M), pet. ether (P) and aqueous (A) extracts. (A=M.S, P.S), (B=M.E,P.E), (C=M.P, P.P), (D=M.M, P.M), (E=M.A, P.A)

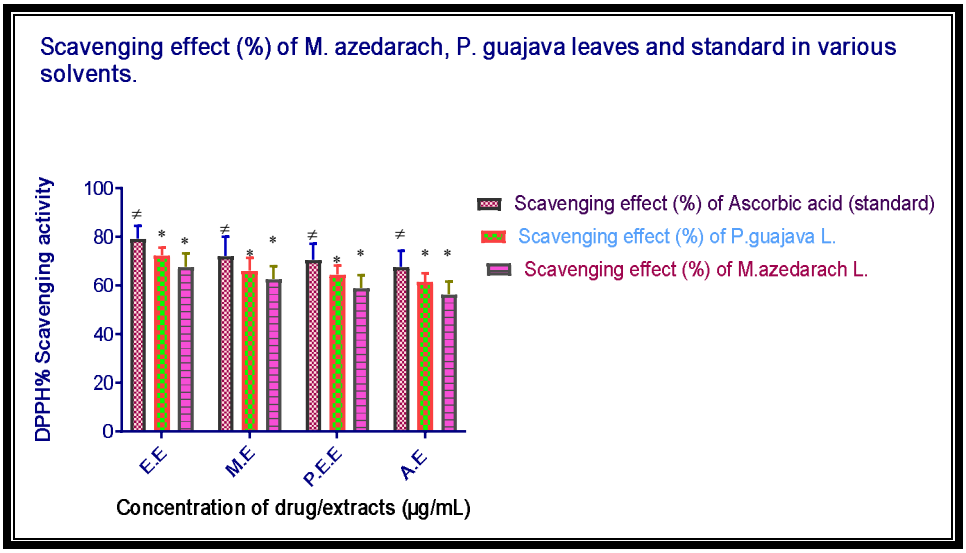


Fig 11: DPPH radical scavenging effect (%) of *M. azedarach* L.,*P. guajava* L. leaf E.E, M.E, P.E.E, A.E extractsat various concentrations.

Every value stands for the mean ± SD (n = 3) and in accordance with ascorbic acid.

CONCLUSION

The findings of this study suggest the ethanol and methanol solvent extracts of *M. azedarach* L. and *P. guajava* L. as antibacterial agents to treat various bacterial co-infections and act as a source of natural antioxidants by treating the oxidative stress associated with dengue fever [38]. The present investigation concludes *P. guajava L.* extracts contain potential antibacterial and antioxidant components over *M. azedarach* L. due to higher amounts of hydroxyl (-OH) groups in the phenolic compounds [39]. These phytochemical constituents may be of great use for the development of pharmaceutical drugs against dengue associated bacterial co-infections and to improve endogenous antioxidant system by inhibiting decomposition of hydroperoxides into free radicals[40]. Here we demonstrated the ethanolic, methanolic, petroleum ether, and aqueous extracts of these plants possess significant inhibitory effect against gram-positive *S.aureus* and *P.aeruginosa* pathogens and act as promising antioxidant agents. We further recommend prediction of active lead molecules and conduct experimentation to validate the claims of their use in dengue therapy management.

CONFLICT OF INTEREST

We, the authors declare that there is no conflict of interest associated with this work andpublication of this paper.

ACKNOWLEDGMENTS

We, the authors would like to thank the university college of pharmaceutical sciences, Acharya Nagarjuna University for providing support during this study. Authors would also like to thank Dr. Rajasekhar Reddy, Johns Hopkins University, USAfor his help in editing the final draft of the manuscript.

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CITATION OF THIS ARTICLE

G Kalyani, D. R S Reddy. Evaluation of Antibacterial and Antioxidant Potential of *Melia. azedarach* Linn. and *Psidium guajava* Linn. Leaf Extracts. Bull. Env.Pharmacol. Life Sci., Vol10[5] April 2021 :15-27