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Phytodrug on an assortment of solvent extract accelerates virulence to combat threatening antimicrobial resistance, phenolic constituents and antioxidant capacity of *Plumeria obtusa*

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Abstract
Medicinal plants have been identified and used traditionally throughout the world from the beginning of human civilization. In recent years, antibacterial resistance has become a major public health concern globally. One of the effective approaches could be the discovery and development of new antibacterial agents that have clinical significant importance from natural occurring phytochemicals and discover new antibacterial agents in the pharmaceutical in order to replace currently available antibacterial. The result of inhibitory action of four different extracts suggests that The result of antibacterial activity of four different extracts suggests that n-butanol extract against *E. coli* was the most resistant strain to plant extracts followed by *Staphylococcus* while aqeuous extracts were susceptible against *Salmonella* and *E. coli*. n-butanol extract against *Fusarium* and *Aspergillus* possess highest inhibitory activity, whereas aqueous against *Fusarium* and acetone against *Aspergillus* were sensitive to the extract. The result showed that n-butanol extract exhibited higher antioxidant capacity compared to aqueous, acetone and methanol.

Keywords: phytomedicine, inhibitory spectrum, resistance, antioxidant potential

Introduction
The use of medicinal plants has a long history in the treatment of a variety of diseases, and these years plant species have been tested for their medicinal properties. Plant-derived substances are tolerated and accepted by patients and seem a reliable source of antimicrobial compounds [1]. Microbicidal potential of these traditional medicinal plants are based on their secondary metabolites of plants [2]. Preliminary result imply most endemic medicinal plants contain bioactive compounds. The existence of these bioactive ingredients can inhibit the growth of not only reference strains but also multidrug resistant pathogens. Various studies established that medicinal plants contain phytoconstituents which possess inhibitory action on human pathogens [3, 4, 5]. This bactericidal action acts by perturbating the cell stability, cell membranes permeability and hinder the film bound ATPase activity, and as a consequence, dropping the development rate of pathogens and disrupting substance influx and even cell fatality [6, 7].
P. obtusa falls within the family Apocynaceae, generally known as white frangipani (Australia), temple tree and Singapore grave- yard flower. The plant is prevalent in Hawaii and is indigenous to North-Central America, the Greater Antilles and Belize, Florida Keys [8, 9]. In conventional medication, *P. obtusa* is used to take care of burning, bronchitis, cough, headache, purgative and as blood disorders [10, 11]. In investigational representation, the plant has shown bactericidal, fungicidal, antioxidant and anti-inflammatory action [12-16]. Terpenoids are the main phytoconstituents found in the leaves and flowers of this plant [17-23].

Materials and Methods
Sample collection
Fresh leaves of *Plumeria obtusa* were collected in local Virudhunagar.

Crude extract preparation
Leaves of *P. obtusa* were cut into the small section as the process of drying might be faster. Afterward the leaves are dehydrated below the shadow for two weeks. Next the desiccated leaves resources are pulverized into a fine particle by the electrical mill. The plant particles are accumulated in a desiccator that is utilized for the extraction procedure. For further study, the rough plant particles are fractionated into four diverse solvents.

Fractionation of crude extract
Preparation of crude extract by adding 5g of each sample plant material soaked in 25 ml of different solvent and kept in a shaker at room temperature for 24 hours. Subsequently all the plant extract is filtered throughout filter paper to get the filtrate of extract. After that it is permitted to disperse for few hours. The plant extract is accumulated in a sealed container under a refrigerator or in cooling condition.

Preliminary phytochemical analysis
The occurrence of bioactive constituents in plant was carried out for flavonoids, saponins, terpenoids, carbohydrate and steroids [24].

Test for flavonoids
20% sodium hydroxide is added to 2 ml of plant extract, yellow colour is developed. 70% hydrochloric acid is added to this and the yellow colour was vanished. The vanishing of yellow colour signify the presence of flavonoids.

Test for saponins
6ml of distilled water was mixed with 2 ml of extract and shaken vigorously, formation of bubble or persistent foam indicates the presence of saponins.

Test for terpenoids
0.5ml of chloroform was added with 1ml of plant extract and minute drops of concentrated sulphuric acid was added, reddish-brown precipitate was developed which indicates the presence of terpenoids.

Test for carbohydrate
1ml of plant extract was added with few drops of Benedict's or Fehling's solution and add 1ml of concentrated sulphuric acid at the side of the examination tube. The combination was subsequently permitted to rest for 2 to 3 minutes. The development of red colour indicates the presence of carbohydrates.

Test for steroids
1ml of plant extract was dissolved in 10ml of chloroform. To this combination the same amount of intense sulphuric acid was added at the side of the examination tube. The superior layer becomes red as the inferior layer sulphuric acid turns yellow with green fluorescence indicates the presence of steroids.

Antimicrobial analysis
The plant extract of diverse solvents is utilised for testing microorganisms by antibacterial activity and antifungal activity [25].

Bactericidal activity
Plumeria obtuse leaves are subjected to bactericidal investigation. The microorganism is preserved in slant. To the disinfected nutrient broth one loop of culture from the slant was relocated. For overnight then it is incubated at the appropriate condition. Examine the turbidity of culture as a result it is ready for testing antibacterial activity.

Preparation of inoculum
Nutrient agar and broth medium were prepared in distilled water and it is sterile under autoclave at 121°C for 45 minutes. Subsequently nutrient agar is dispensed into Petri dishes and permitted to harden. Bacteria is inoculated in nutrient broth.

Agar well diffusion method
Antibacterial activity of aqueous and other solvent extract was determined by Agar well diffusion technique. On nutrient agar plates the inoculum containing each bacterial culture with a sterile swab was moistened with the bacteria. The agar medium was punched with wells of 6 mm diameter and filled with 100 µl of plant extract. For 24 hours the plates were then incubated in the upright position at 37°C. Subsequent to incubation, the thickness of the growth inhibition zones was calculated in mm. For each of the test organism two replicates were carried out.

Fungicidal activity
P. obtusa leaves are studied for testing fungicidal assessment. The fungi are preserved in slants. A loopful of culture was transmitted into a sterile nutrient broth. Afterward it was developed under appropriate situation.

Preparation of inoculum
Potato dextrose broth and agar are prepared and autoclaved at 121°C for 45 minutes. Potato dextrose agar was decanted into petridishes and permitted to coagulate. The fungi was inoculated in potato dextrose agar and potato dextrose broth.

Microorganism test
Inoculate the fungal culture in potato dextrose agar and broth under the sterile condition in a laminar air flow chamber. Fungal cultures are incubated in appropriate conditions for 2 to 3 days.

Agar well diffusion method
Agar well diffusion method was used to screen the antifungal property of solvent extract. Potato Dextrose Agar for fungi was next poured into the Petri dishes. Fresh fungal culture was spread upon solidification. Wells are made by means of sterile tips into agar plates including inoculums. Subsequently, 100µlof each one extract was supplemented to the relevant wells. Afterward the plates were incubated at 37°C for 48 hours. Antifungal activity was identified by quantifying the zone of inhibition including the well diameter that appeared after the incubation period. Two replicates were conceded out for every extract against each of the examining organisms. Wells enclosing the similar quantity of solvents serve as a negative control.

Antioxidant
Total Phenolic Content
The total phenolic content of each plant solvent such as aqueous, acetone, n-butanol and methanol extract is filtered by using filter paper. 4.3 mg of solvents was suspended with 10 ml of methanol. The combination was mixed for 5 minutes to obtain the homogenized solution. To 1 ml methanol, 3.16 ml distilled water and 200 µl Folin-Ciocalteu reagent were added to 300µlof this solution taken in an examination tube. 600µl of sodium carbonate solution was added after 8 minutes of incubation at room temperature, and the test tube was covered with aluminium foil and incubated in a hot water bath at 40°C. A blank was arranged by means of the similar process but replacing the plant extract with an equal amount of methanol. By means of a colorimeter the absorbance of the test was determined [26].

Formula
The absorbance was calculated to decide the entire phenolic content by using the formula

$$C=c/V\times m$$

Where C=Entire phenolic content
c=Concentration of Gallic acid in mg/ml
V= Volume of extract in ml
m= Weight of the plant extract in g

Phosphomolybdate examination for total antioxidant assessment
To determine total antioxidant capacity of *P. obtusa* extract as per phosphomolybdate assessment. For sample preparation, 250µg plant extract was dissolved in 1 ml methanol and sonicated for 5 minutes to get a homogeneous mixture. In an examination tube, 300 µl plant extract was mixed with 3 ml phosphomolybdate reagent. The experiment tube was enclosed with aluminium foil and

incubated at 95°C. The combination was then permitted to attain room temperature. By means of a colorimeter the absorbance of the sample was measured. Blank was run by means of the similar procedure but containing an equal volume of methanol in place of the plant sample [27].

Results and discussion

The leaves of *P. obtusa* were subjected to extraction for two weeks to acquire the solvent extracts. The extracts were utilised for the investigation of microbicidal and antioxidant activity.

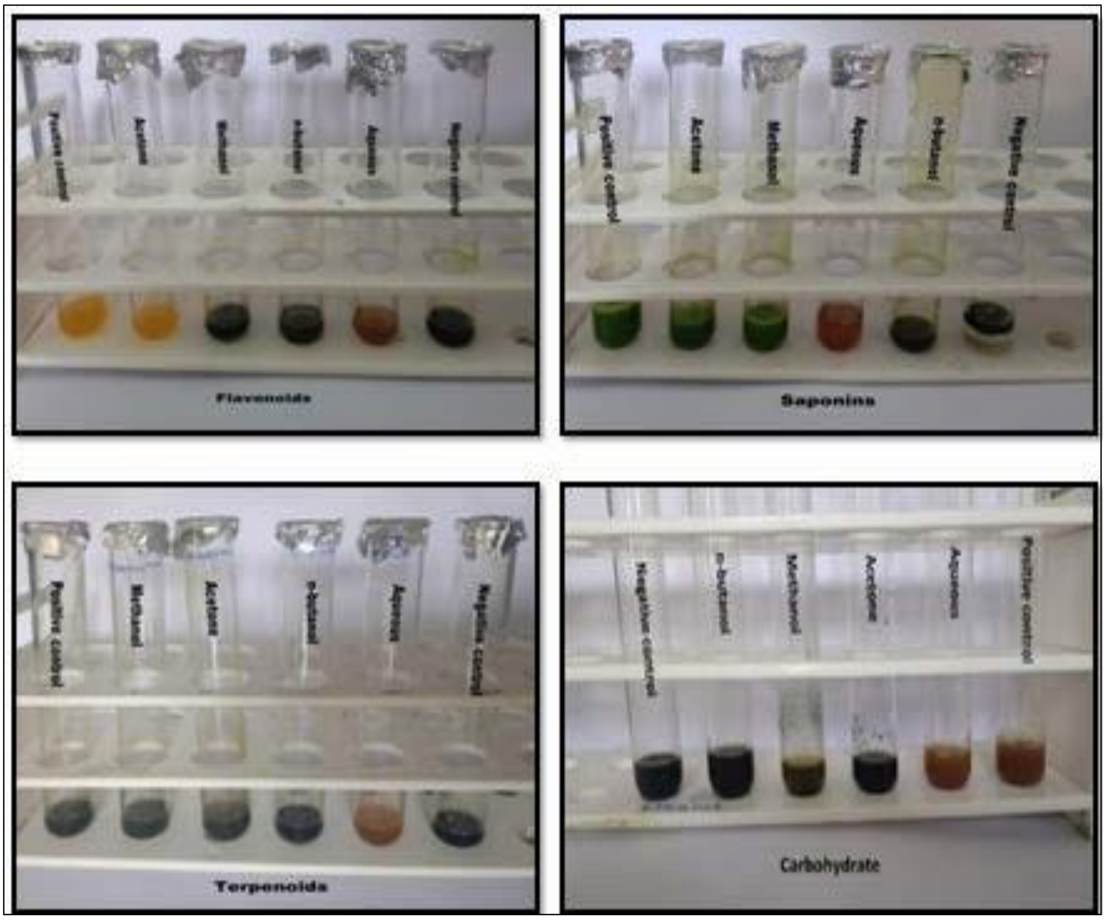

Fig 1: Phytoconstituents in *Plumeria obtusa leaf extract* with different solvents

Table 1: Preliminary bioactive screening of *Plumeria obtusa*

Phytochemical test	Aqueous	Acetone	n-butanol	Methanol
Flavonoids	-	+	-	-
Saponins	-	+	-	+
Terpenoids	-	+	-	+
Carbohydrates	+	-	-	-
Steroids	+	-	-	+

Phytochemical analysis conducted on the plant extract revealed the presence of constituents that are known to exhibit medicinal as well as physiological activity Figure: 1& Table 1. From the table, it might be seen that saponins, terpenoids, flavonoids, carbohydrates and steroids were present. Various investigation have revealed that each medicinal plant has various phytochemical compounds namely flavonoids, phenolic, coumarins, terpenoids, alkaloids, tannins, lectin, and polyacetylenes [28–30]. Flavonoids were not present in n-butanol, aqueous, methanol and plant extract as saponins were absent in aqueous and n-butanol. In aqueous and n-butanol extract terpenoids were absent. The occurrence of these pytoconstituents can show bactericidal or bacteriostatic effects on multidrug resistance pathogenic bacteria and also as precursor for developing antibiotics for treating contagious agents, chiefly from urinary tract illness causing microbes. Carbohydrates were deficient in acetone, methanol and n-butanol extract. In acetone and n-butanol extract steroids were deficient.

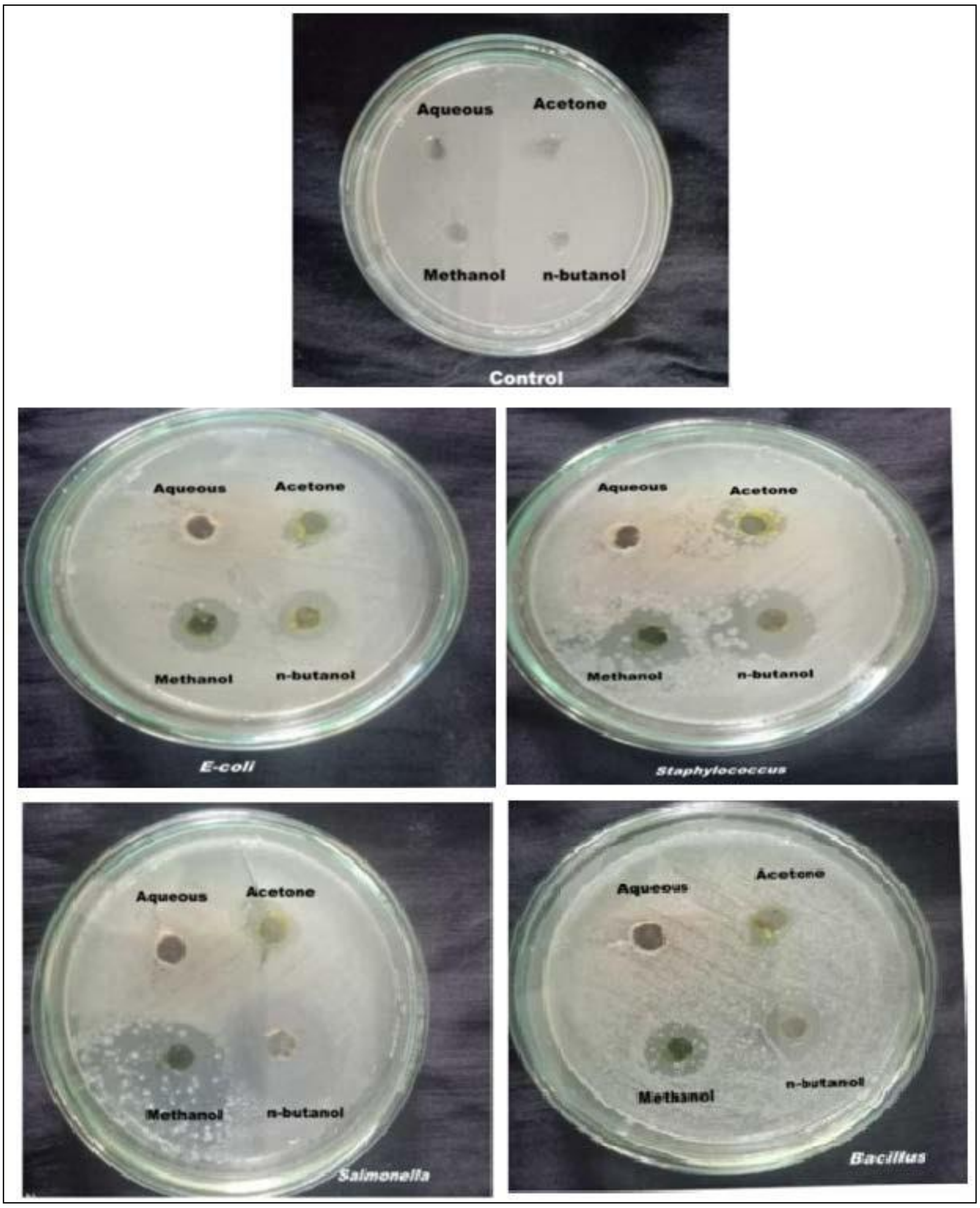

Fig 2: *In vitro* bactericidal activity of *Plumeria obtusa*

Table 2: *In vitro* bactericidal potential of *Plumeria obtusa*

Microorganism	Solvent Used			
	Acetone	Aqueous	n-butanol	Methanol
Bacillus	10 mm	7 mm	10 mm	15 mm
E.coli	10 mm	1 mm	22 mm	15 mm
Salmonella	7 mm	4 mm	8 mm	9 mm
Staphylococcus	8 mm	6 mm	16 mm	16 mm

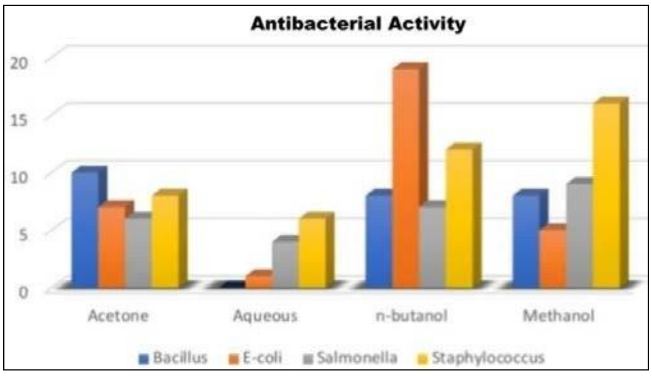

Fig 3: *In vitro* bactericidal screening of *Plumeria obtusa*

Bactericidal potential of *P. obtusa* by agar well diffusion assay

The crude plant extract was evaluated for antibacterial potential against *E-coli*, *Bacillus*, *Staphylococcus* and *Salmonella* were depicted in Figure: 2, Table: 2 and Figure: 3. Methanol and n-butanol plant extract is proven to be a better solvent compared to other plant solvents as aqueous and acetone for inhibitory potential. An intermediate ability zone in bactericidal activity of the extract towards pathogen was observed. The extracts had inhibitory spectrum against both reference and clinical isolates of *E. faecalis S. aureus* and *P. aeruginosa* [31]. The effect of activity of four different extracts suggests that n-butanol extract (22 mm) against *E. coli* was the most opposing strain to extracts followed by *Staphylococcus* (16 mm) while aqeuous extracts were susceptible against *Salmonella* (4 mm). The potential extracts of *A. corrorima*, *A. angustifolium* (Sonn.) and *V. amygdalina* were owing to the existence of phenol, saponin, terpenoids and flavonoids [32]. Therefore the research was performed to verify the spectrum against the most susceptible bacteria. The effect of this existing study is in agreement with other findings supporting that most compounds in medicinal plants are more extracted in n- butanol.

Table 3: *In vitro* fungicidal activity of *Plumeria obtusa*

Microorganism	Solvent Used			
	Acetone	Aqueous	n-butanol	Methanol
Aspergillus	5mm	18 mm	24mm	13mm
Fusarium	8mm	6mm	27mm	13 mm

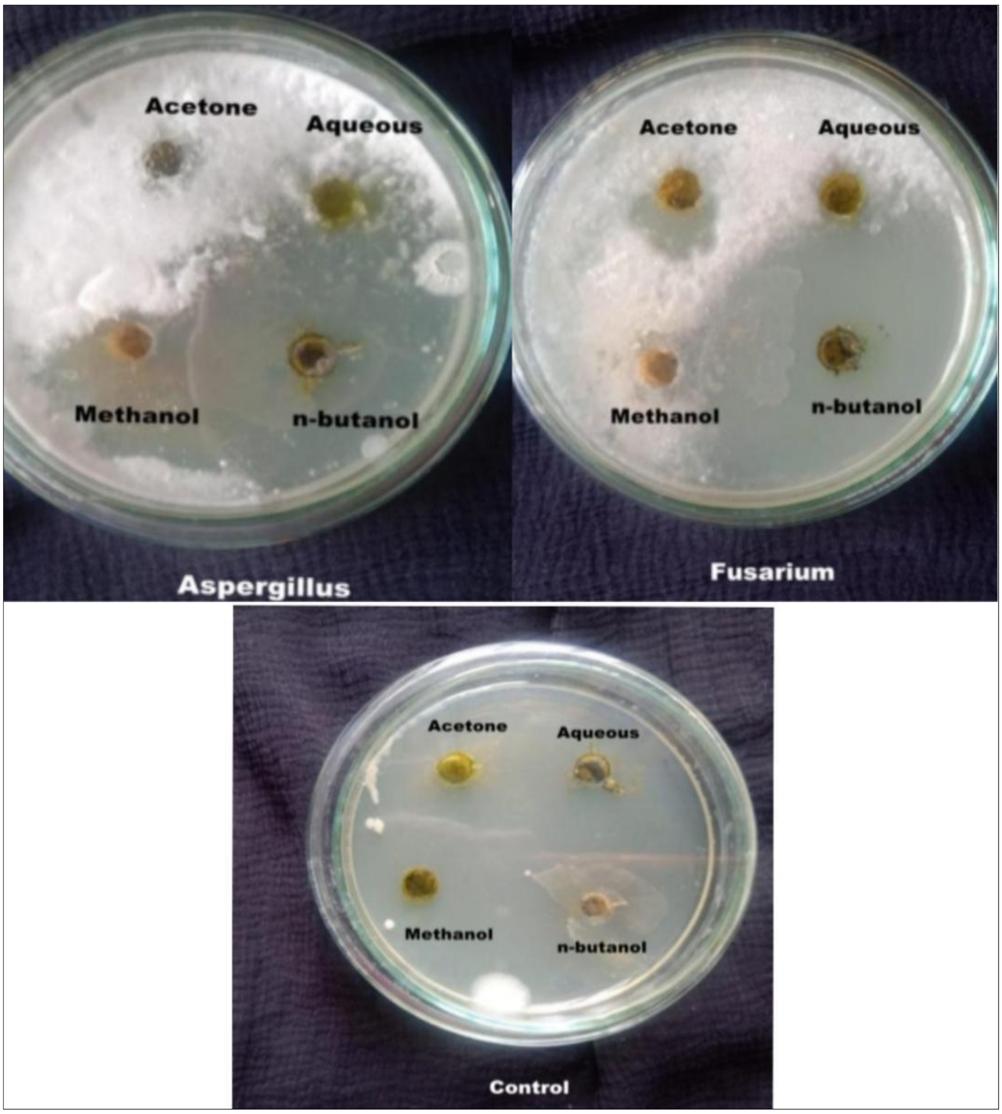

Fig 4: *In vitro* fungicidal potential of *Plumeria obtusa*

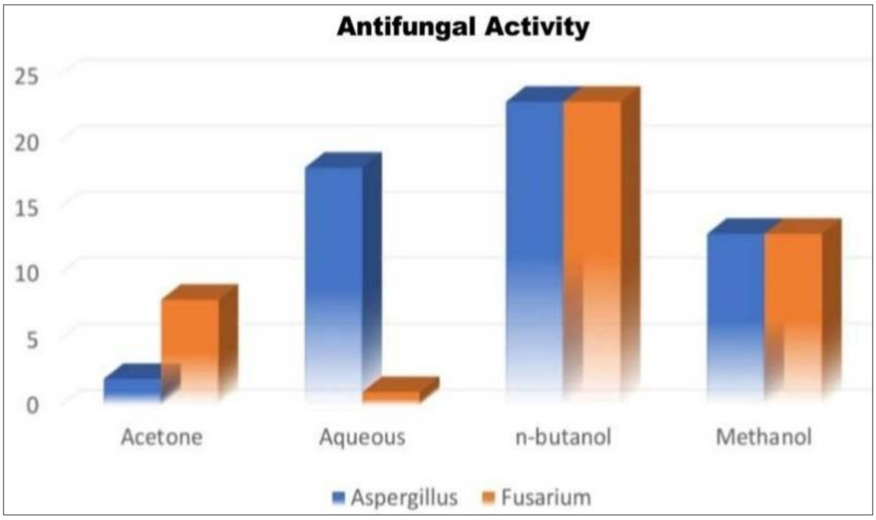

Fig 5: Fungicidal screening of *Plumeria obtuse*

Antifungal activity of *Plumeria obtusa* by agar well diffusion method

Crude plant extract is evaluated for antifungal activities against *Aspergillus* and *Fusarium* were revealed in Figure: 4, Figure: 5 & Table: 3. Extracts of n-butanol is proven to be a better solvent compared to other solvents for fungicidal activity. The microbicidal activities of these traditional medicinal plants are based on their secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins and glycosides [33]. Extracts of n-butanol in opposition to *Fusarium* (27 mm) and *Aspergillus* (24 mm) presented the highest action, while aqueous (6 mm) against *Fusarium* and acetone (5 mm) against *Aspergillus* were sensitive. This concurs with preceding findings on the antifungal activity of medicinal plants containing different secondary metabolites that inhibited microbes [34, 35, 36]. The consequence of this existing research is in concurrence with other findings supporting that most compounds in medicinal plants are most extract in n-butanol.

Antioxidant Activity

Phytochemical screening of the four different plant extract was accomplished and the extract demonstrated the occurrence of phenolics. The entire phenols was revealed using the Folin-Ciocalteu method.

Table 4: Total Phenolic Capacity

Concentration of extract(mg/ul)	Aqueous	Acetone	n-butanol	Methanol
100	1.25854	12.5854	-0.3659	20.7317
200	1.99024	17.4634	6.95122	25.6098
300	2.96585	19.9024	16.7073	40.2439
400	1.67317	27.2195	26.4634	50
500	5.89268	46.7317	36.2195	79.2683

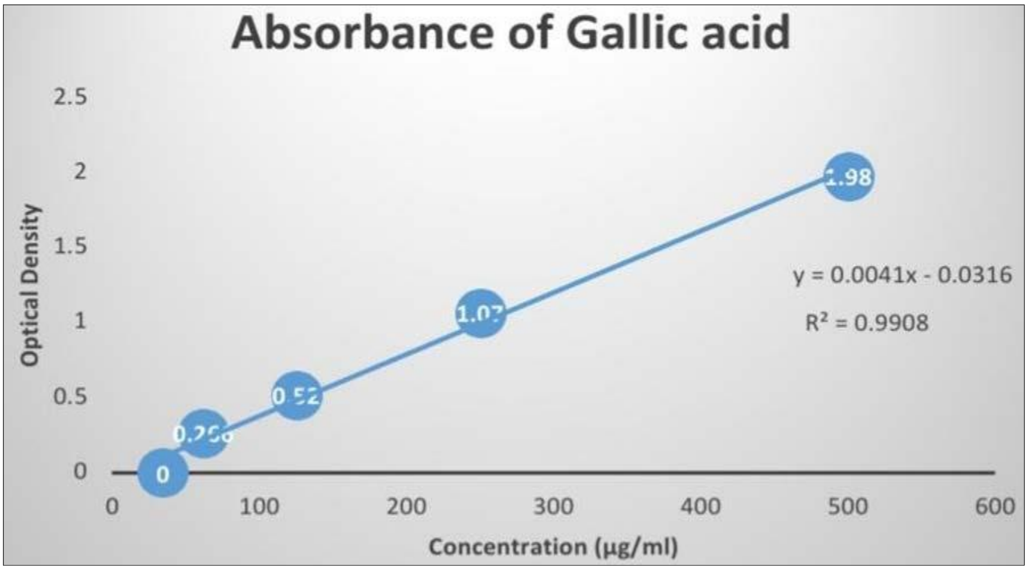

Fig 6: Absorbance of Gallic Acid

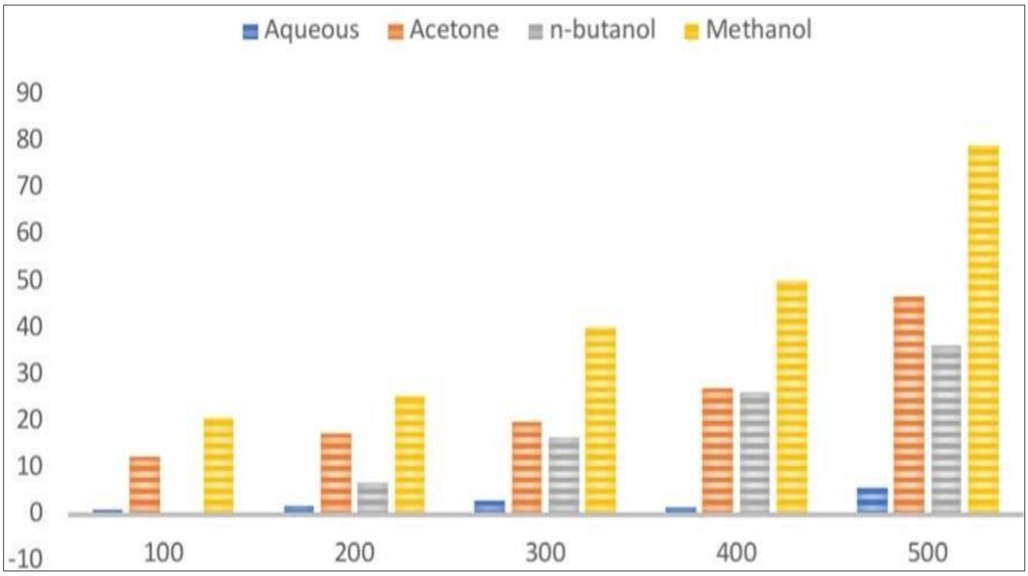

Fig 7: Total Phenolic Potential

The total phenolic solvent of different plant extract of *Plumeria obtusa* were represented in Figure: 6 & 7, Table: 4. Phenolic constituents are plant secondary metabolites that offer medical and nutritional properties in habitual diets [37]. The consequence depicted that methanol extract exhibited higher TPC as compared to aqueous, acetone and n-butanol which are just about 43.17mg GAE/g consequently followed by acetone as 24.77 mg GAE/g, n-butanol as 17.19 mg GAE/g and aqueous as 2.7 mg GAE/g. These variation in the quantity of TPC may be due to varied efficiency of the extracting solvents to dissolve diverse constituents. Numerous studies depicted that TPC differed with polarity of solvent used in process [38]. For extraction of phenolics, methanol was used as an enhanced solvent. The consequence revealed that the extract of methanol had the highest amount of yield, compared to that of other samples. The alcoholic solvents have the ability to extract phenolic compounds such as flavonoids which may contribute to the antifungal activity [39]. Lower phenolic content was observed in n-butanol, acetone and aqueous extracts.

Table 5: Phosphomolybdate assessment For Total Antioxidant Capacity

Phosphomolybdate Assay For Total Antioxidant Capacity				
Concentration of extract (mg/ul)	**Aqueous**	**Acetone**	**n-butanol**	**Methanol**
100	49.0196	58.8235	82.3529	33.3333
200	56.8627	31.3725	76.4705	9.8039
300	66.6666	29.4117	72.5490	68.6274
400	70.5882	64.7058	60.7843	70.5882
500	70.5882	70.5882	41.1764	70.5882

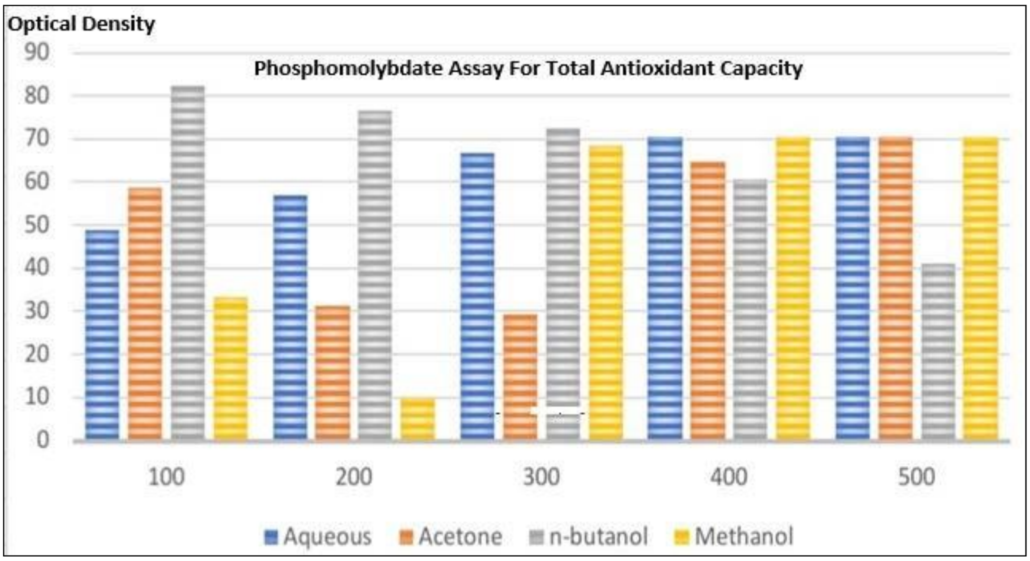

Fig 8: Phosphomolybdate assay for Total Antioxidant Capacity

Total antioxidant capacity of different extract of *P. obtusa*, according to assay were revealed in Table: 5 & Figure: 8. The result depicted that n-butanol exhibited higher antioxidant capacity compared to aqueous, acetone and methanol. n-butanol has approximately (66.86) followed by aqueous (62.74), methanol (50.58) and acetone (37). The phosphomolybdenum technique is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of green phosphate/Mo (V) complex with absorption at 695 nm [40]. Aqueous and n-butanol extract revealed much higher activity than the other two extracts. Least active solvent was methanol and acetone. From this examination, the entire antioxidant capacity of *Plumeria obtusa* could be as a result of the presence of phenolics and flavonoids in the plant aqueous and methanol extracts higher than acetone and n-butanol extract.

Conclusion

The phytochemical screening of *Plumeria obtusa* has revealed the presence of phytoactive components. The antimicrobial potential have been confirmed throughout the *invitro* qualitative by agar well diffusion process. Extracts of n-butanol exhibited higher antioxidant capacity compared to aqueous, acetone and methanol. Therefore there is a scope for future investigation to be focused on the clinical studies by using compounds isolated from *P. obtusa*. Such investigation will profit future pharmaceutical application of plants.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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