Research Article

Phytochemical, *in vitro* antioxidant and antibacterial activity of flower extracts of *Tiliacora acuminata* (Lam.) Hook. f. & Thoms (Menispermaceae)

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Abstract

The present study was carried out to investigate the phytochemical, *in vitro* antioxidant and antibacterial activity of *Tiliacora acuminata* (Lam) Hook. f. &Thoms flower an important medicinal plant. Qualitative phytochemical analysis of the methanol and ethanol extracts prepared from *T. acuminata* flower revealed the presence of alkaloids, anthraquinones, catechins, coumarins, flavonoids, phenols, quinones, saponins, steroids, sugar, glycosides, tannins and xanthoproteins. The FT-IR spectrum confirmed the presence of hydroxyl group, alkyl group, alcohols, ethers, esters, carboxylic acid and anhydrides. Antioxidant activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of the flower of *T. acuminata* have been tested using various antioxidant model systems viz, DPPH, hydroxyl, superoxide, ABTS and reducing ability. This study indicates significant free radical scavenging potential of *T. acuminata* flower which can be exploited for the treatment of various free radical mediated ailments. The petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of flower of *T. acuminata* were tested against *Bacillus thuringiensis*, *Bacillus subtilis, Streptococcus faecalis, Streptococcus pyogens, Staphylococcus aureus, Staphylococcus aureus* (Methicillin sensitive), *Enterococcus faecalis, Salmonella paratyphi* A and B, *Salmonella paratyphi, Proteus mirabilis, Proteus vulgaris, Escherichia coli, Escherichia coli* (ESBL), *Klebsiella pneumoniae, Pseudomonas aeruginosa, Pseudomonas aeruginosa* (ESBL) and *Mycobacterium smegmatis*, by the agar disc diffusion method.

Keywords: *Tiliacora acuminata*, Phytochemical screening, FT-IR, antioxidant, antibacterial, *P. mirabilis* and *S. pyogenes*

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Introduction

The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raise serious concern of health delivery and accessibility due to untreatable bacterial infections. Therefore, there is an urgent need in search of new antimicrobial drug. Plants are important source of potentially useful structures for the development of new chemotherapeutic agents [1].

In many developing as well as developed countries, chronic diseases, such as cardiovascular diseases and cancer have become leading causes of death. Epidemiological evidence shows that intake of diets which includes mainly fruits and vegetables are associated with reduced risk of these diseases. Fruits and vegetables rich in phenolic and flavonoids components are known to have potent antioxidant properties that decrease oxidative stress in consumers

and therefore, are associated with various health benefits [2]. Herbs have been used for medical treatment since ancient times [3]. Recent investigations have shown that the positive treatment effects and antioxidant properties of medicinal plants could be correlated with bioactive components such as alkaloids, flavonoids, bioflavonoids, glycosides, coumarins, terpenoids, saponins, tannins and phenols [4,5].The use of these compounds as natural antioxidants play an important role in protecting and prevention of DNA damage, cancer, atherosclerosis and aging process [6,7]. Many of the pharmaceutical companies worlds over are utilizing plant based formulations in treatment of various diseases and disorders with marked pharmacological activities.

Tiliacora acuminata is a large woody climber; branches cinereous, striate. Leaves are long, ovate, acuminate, cordate, truncate or rounded at the base, flowers yellow in elongate, and axillary, racemose panicles belonging to the family Menispermaceae. This plant has been used as an ingredient in many of the ayurvedic preparations and regarded as an antidote for snake bite [8,9]. To the best of our knowledge, there is no record of work on the phytochemical screening, antioxidant and antibacterial activity of the flower of *Tiliacora acuminata*. Therefore, the present study was carried out to evaluate the phytochemical screening, *in vitro* antioxidant and antibacterial activity of the flower of *Tiliacora acuminata*.

Experimental

Materials and Methods

The flower of *T. acuminata* (Lam) Hook. f. &Thoms was collected from Ulakaruvi, Kanyakumari District, Tamil Nadu. The plant was identified with help of local flora and authenticated in Botanical Survey of India, Southern circle, Coimbatore, Tamil Nadu.

Preparation of extracts

Freshly collected flower sample of *T. acuminata* were dried in shade, and then coarsely powdered separately in a willy mill. The coarse powder (100g) was extracted successively with petroleum ether, benzene, ethyl acetate, methanol and ethanol, each 250 ml in a Soxhlet apparatus for 24 hrs. All the extracts were filtered though Whatman No.41 filter paper. All the extracts (petroleum ether, benzene, ethyl acetate, methanol and ethanol) were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedures [10,11,12] . All the extracts were concentrated in a rotary evaporator. The concentrated extracts were used for *in vitro* antioxidant activity. The methanol extract was used for the estimation of total phenolics and flavonoids.

FT-IR analysis

A little powder of plant specimen was mixed with KBr salt, using a mortar and pestle, and compressed into a thin pellet. Infrared spectra were recorded as KBr pellets on a Thermoscientific Nicot iS5 iD1 transmission, between 4000 − 400 cm^{-1} [13].

Estimation of Total Phenolics

Total phenolic content was estimated using Folin-Ciocalteau reagent based assay as previously described [14] with little modification. To 1mL of each extract (100µg/mL) in methanol, 5mL of Folin-Ciocalteau reagent (diluted ten-fold) and 4mL (75g/L) of Na_2CO_3 were added. The mixture was allowed to stand at 20ºC for 30min and the absorbance of the developed colour was recorded at 765nm using UV-VIS spectrophotometer. 1mL aliquots of 20, 40, 60, 80, 100 µg/mL methanolic gallic acid solutions were used as standard for calibration curve. The absorbance of solution was compared with gallic acid calibration curve. The total phenolic content was expressed as gallic acid equivalents (GAE g/100g dry weight of extract).

Estimation of Flavonoids

The total flavonoid content was determined according to Eom *et al* [15] . An aliquot of 0.5 mL of sample (1mg/mL) was mixed with 0.1mL of 10% aluminium chloride and 0.1mL of potassium acetate (1M). In this mixture, 4.3mL of 80% methanol was added to make 5mL volume. The mixture was vortexted and the absorbance was measured spectrophotometrically at 415nm. The value of optical density was used to calculate the total flavonoid content present in the sample.

DPPH radical scavenging activity

The DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant component. This method is based on the reduction of DPPH in methanol solution in the presence of a hydrogen donating antioxidant due to the formation of the non radical form DPPH-H [16].

The free radical scavenging activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to the previously reported method [16]. Briefly, an 0.1mM solution of DPPH in methanol was prepared, and 1mL of this solution was added to 3 ml of the solution of all extracts in methanol at different concentration (50,100,200,400 & 800µg/mL).The mixtures were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance was measured at 517 nm using a UV-VIS spectrophotometer (Genesys 10S UV: Thermo electron corporation). Ascorbic acid was used as the reference. Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability of scavenging the DPPH radical was calculated by using the following formula.

$$\% \text{ inhibition} = \{(A_0 - A_1)/A_0)*100\}$$

Where, A_0 is the absorbance of the control reaction, and A_1 is the absorbance in presence of all of the extract samples and reference. All the tests were performed in triplicates and the results were averaged

Hydroxyl radical scavenging activity

The scavenging capacity for hydroxyl radical was measured according to the modified method of Halliwell [17]. Stock solutions of EDTA (1mM), $FeCl_3$ (10mM), Ascorbic Acid (1mM), H_2O_2 (10mM) and Deoxyribose (10 mM), were prepared in distilled deionized water.

The assay was performed by adding 0.1mL EDTA , 0.01mL of $FeCl_3$**,** 0.1mL H_2O_2, 0.36mL of deoxyribose, 1.0mL of the extract of different concentration (50,100,200,400 &800µg/mL)dissolved in distilled water,0.33mL of phosphate buffer (50mM , pH 7.9), 0.1mL of ascorbic acid in sequence . The mixture was then incubated at 37^0C for 1 hour. 1.0mL portion of the incubated mixture was mixed with 1.0mL of 10%TCA and 1.0mL of 0.5% TBA (in 0.025M NaOH containing 0.025% BHA) to develop the pink chromogen measured at 532nm. The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

Superoxide radical scavenging activity

The superoxide anion scavenging activity was measured as described by Srinivasan *et al* [18]. The superoxide anion radicals were generated in 3.0 ml of Tris – HCL buffer (16mM, pH 8.0), containing 0.5mL of NBT (0.3mM), 0.5 ml NADH (0.936mM) solution, 1.0mL extract of different concentration (50, 100, 200, 400 & 800µg/mL), and 0.5mL Tris – HCl buffer (16mM, p^H 8.0). The reaction was started by adding 0.5mL PMS solution (0.12mM) to the mixture, incubated at 25°C for 5 min and the absorbance was measured at 560nm against a blank sample, ascorbic acid. The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula

Antioxidant activity by radical cation (ABTS. +)

ABTS assay was based on the slightly modified method of Huang *et al* [19]. ABTS radical cation (ABTS+) was produced by reacting 7mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The ABTS + Solution were diluted with ethanol to an absorbance of 0.70+0.02 at 734 nm. After addition of 100µL of sample or trolox standard to 3.9 mL of diluted ABTS+ solution, absorbance was measured at 734 nm by Genesys 10S UV-VIS (Thermo scientific) exactly after 6 minutes. Results were expressed as trolox equivalent antioxidant capacity (TEAC). The percentage inhibition was calculated by comparing the results of the test with those of the control using the above formula.

Reducing power

The reducing power of the extract was determined by the method of Kumar and Hemalatha [20]. 1.0 mL of solution containing 50,100,200,400 &800µg/mL of extract was mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH6.6) and potassium ferricyanide (5.0mL, 1.0%): The mixture was incubated at 50°C for 20 minutes. Then 5mL of 10% trichloroacetic acid was added and centrifuged at 980 g (10 minutes at 5°C) in a refrigerator centrifuge. The upper layer of the solution (5.0 mL) was diluted with 5.0 mL of distilled water and ferric chloride and absorbance read at 700 nm. The experiment was performed thrice and results were averaged.

Statistical analysis

Antioxidant activities like DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical activity, ABTS radical cation scavenging activity and reducing powers were estimated in triplicate determinations. Data were analyzed using the statistical analysis system SPSS (SPSS software for windows release 17.5; SPSS Inc., Chicago IL, USA) Estimates of mean, standard error for aforesaid parameters were calculated.

Microorganisms
Bacterial strains of *Bacillus thuringiensis*(+), *Bacillus subtilis*(+), *Streptococcus faecalis*(+), *Staphylococcus aureus*(+), *Staphylococcus aureus* (Methicillin sensitive) (+), *Staphylococcus pyogenes*(+), *Enterococcus faecalis*(+), *Salmonella paratyphi-A & B*(-), *Salmonella paratyphi* (-), *Proteus mirabilis*(-), *Escherichia coli*(-), *Escherichia coli* (ESBL) (-), *Proteus vulgaris*(-), *Klebsiella pneumoniae*(-), *Serratia marcescens*(-), *Pseudomonas aeruginosa* (-),*Pseudomonas aeruginosa* (ESBL) (-) and *Mycobacterium smegmatis* (+) bacterial strains were obtained from Department of Microbiology, Bharathidasan University, Trichy, Tamil Nadu, India. The bacteria were incubated on a nutrient agar-slant (Stationary cultures) for 48h at 37ºC, followed by inoculation in Muller Hinton Agar (MHA) medium.

Antibacterial assay
Antimicrobial study was carried out by disc diffusion method [21] against the pathogens. A loopful of bacteria was taken from the stock culture and dissolved in 0.1ml of saline. All the tests were done by placing the disc (6mm diameter) impregnated with (20mcg) respective different extracts on the Muller Hinton Agar surface previously inoculated with 10ml of MHA liquid medium with Gram Positive and Gram Negative bacteria. Respective solvents without plant extract served as negative control. Standard antibiotic of tetracycline (30mcg/disc) was used as reference or positive control. Plates were incubated at 37ºC for 24 hours. After the incubation period, the diameter of the inhibition zone around the plant extracts saturated discs were measured and also compared with the diameter of inhibition zone of commercial standard antibiotic discs. The inhibition zone and antibacterial activity against the pathogenic bacteria were recorded. The experiments were repeated in triplicate and the results were documented.

Results
Phytochemical screening

Table 1 Preliminary phytochemical screening of flower extract of *Tiliacora acuminata*

Bioactive components	Nature of extract				
	Petroleum ether	Benzene	Ethyl acetate	Methanol	Ethanol
Alkaloids	+	+	+	+	+
Anthroquinones	_	_	_	_	_
Catechin	-	-	+	+	+
Coumarin	_	_	+	+	+
Flavonoids	_	_	_	+	+
Phenols	+	+	+	+	+
Quinones	_	_	+	+	+
Saponins	+	+	_	+	+
Steroids	+	+	+	+	+
Tannins	+	+	_	+	+
Terpenoids	_	_	_	+	+
Sugar	+	+	+	+	+
Glycosides	+	+	+	+	+
Xanthoprotein	+	+	+	+	+
Fixed oil	+	+	+	–	–

The results of the phytochemical screening revealed the presence of alkaloid, catechin, coumarin, flavonoid, phenol, quinine, saponin, steroid, tannin, terpenoid, sugar, glycosides and xanthoprotein in the methanol and ethanol extracts of *T. acuminata* flower (Table 1). Maximum degree of chemical diversity is seen in *T. acuminata* flower methanol and ethanol extracts in which thirteen out of fifteen different chemical tests gave positive results. Ethyl acetate extract of *T. acuminata* flower gave positive results for ten different chemical tests. Petroleum ether and benzene extracts showed minimum degree of chemical diversity with the positive results for only nine different chemical tests i.e. for

alkaloid, phenol, saponin, steroid, tannin, sugar, glycoside, xanthoprotein and fixed oil. Alkaloid, phenol, steroid, sugar, glycoside and xanthoprotein present in flower of *T. acuminata* of all the five solvent studied.

FT-IR Spectroscopy studies

The FT-IR spectroscopy studies of ethanol and methanol extracts of *T. acuminata* flower gave the following characteristic absorption peaks as shown in table 2. It is also given in fig.1 and 2.

Table 2 FT-IR Spectroscopic data of ethanol and methanol extracts of *T. acuminata* flower

S.No	Group	Stretching Frequency (cm^{-1})	
		Ethanol	Methanol
1	O-H	3316.16	3320.06
2	C-H Stretching (alkyl)	2972.50	2944.35
3	C=O	-	1653.48
4	C-H Stretching (carbonyl compounds)	2879.89	2832.88
5	C-O	1378.02	1406.34
6	C-F Stretching	1044.49	1018.55

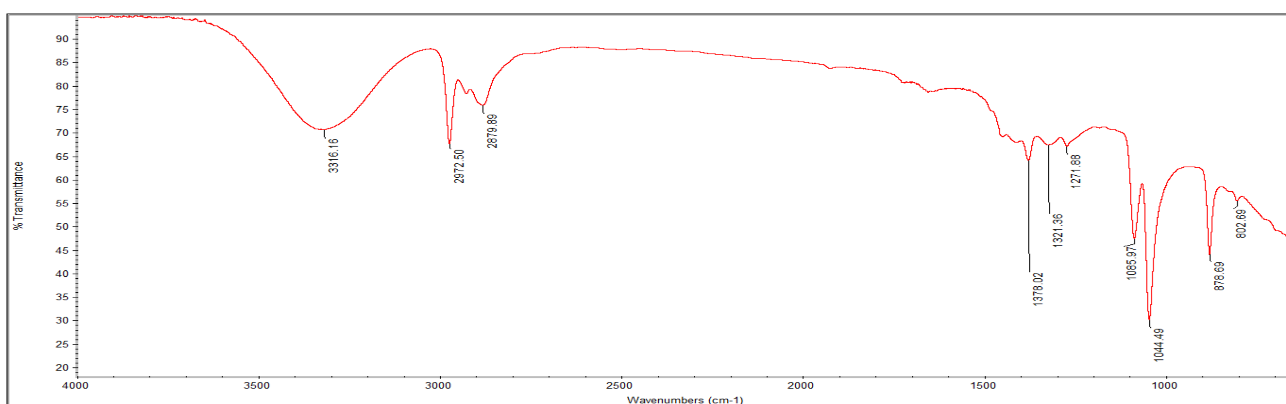

Figure 1 FT-IR spectrum of ethanol extract of flower of *Tiliacora acuminata*

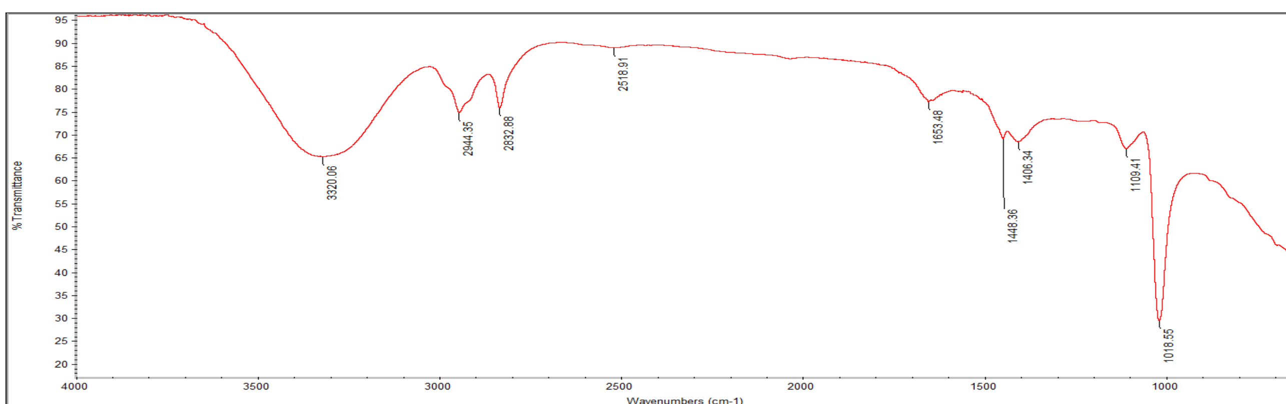

Figure 2 FT-IR spectrum of methanol extract of flower of *Tiliacora acuminata*

Antioxidant activity

Total phenolics and total flavonoid content

The total phenolic content and total flavonoid content of the methanol extract of *T. acuminata* flower were found to be 0.81g100g^{-1} and 1.02g100g^{-1}respectively.

DPPH radical scavenging activity

DPPH radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *T. acuminata* flower was shown in Fig 3. The scavenging effect increases with the concentration of standard and samples. Among the solvent tested, petroleum ether extract of *T. acuminata* flower exhibited highest DPPH radical scavenging activity. At 800µg/ml concentration, petroleum ether extract of *T. acuminata* possessed 90.20% scavenging activity on DPPH which is lower than the standard ascorbic acid whose scavenging activity is 95.28%.

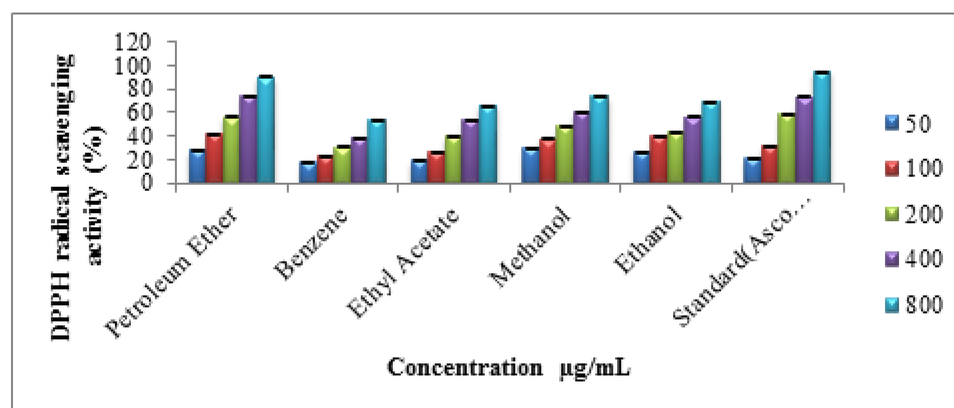

Figure 3 DPPH radical scavenging activity of different extracts of *Tiliacora acuminata*

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *T. acuminata* flower was shown in Fig 4. Methanol extract showed very potent activity. At 800µg/ml concentration, methanol extract of *T. acuminata* possessed 72.16% scavenging activity on hydroxyl radical.

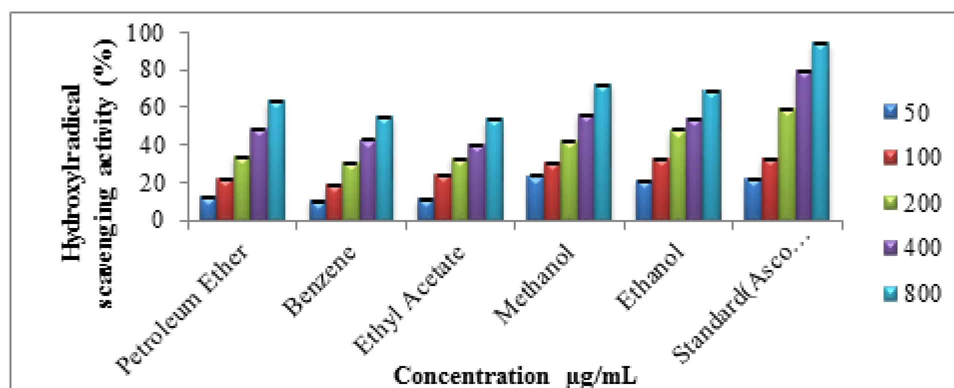

Figure 4 Hydroxyl radical scavenging activity of different extracts of *Tiliacora acuminata*

Superoxide radical scavenging activity

The *T. acuminata* flower extracts were subjected to the superoxide radical scavenging assay and the results were shown in Fig 5. It indicates that ethanol extract of *T. acuminata* flower (800µg/ml) showed the maximum superoxide scavenging activity of 106.93% which is higher than the standard ascorbic acid whose scavenging activity is 93.27%.

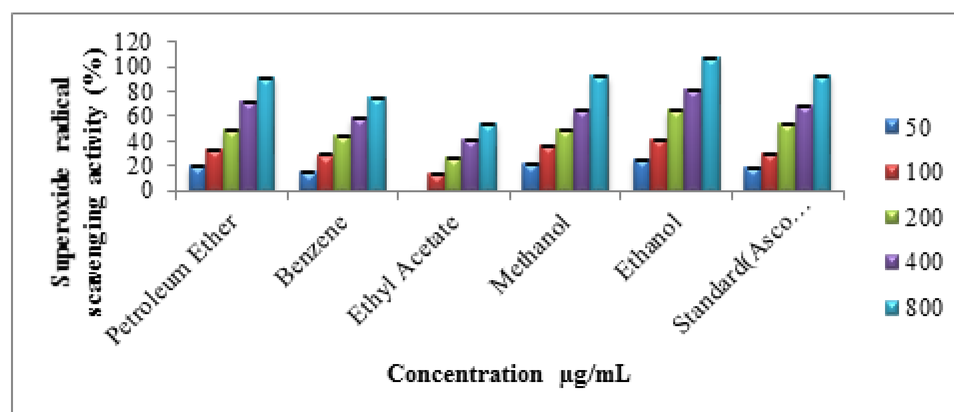

Figure 5 Superoxide radical scavenging activity of different extracts of *Tiliacora acuminata*

ABTS radical cation scavenging activity

The *T. acuminata* flower extracts were subjected to the ABTS radical cation scavenging activity and the results were shown in Fig 6. The petroleum ether extract exhibited potent ABTS radical cation scavenging activity in concentration dependent manner. At 800µg/ml concentration, *T. acuminata* flower possessed 89.05% scavenging activity on ABTS which is lower than the standard ascorbic acid whose scavenging activity is 90.88%.

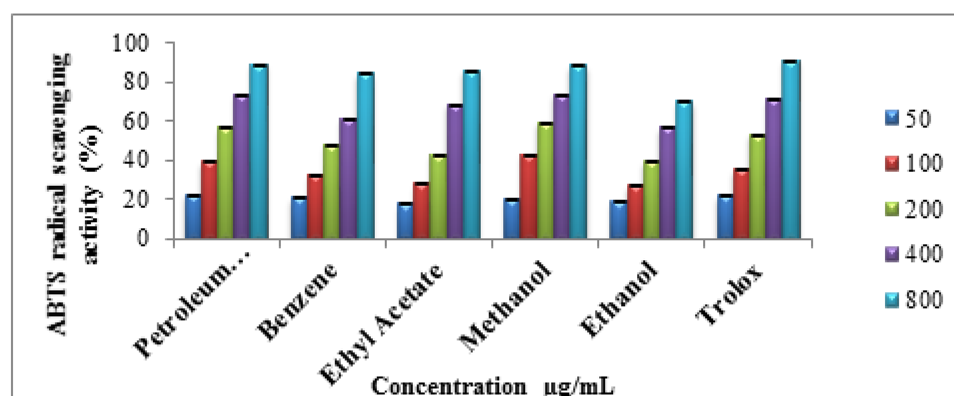

Figure 6 ABTS radical scavenging activity of different extracts of *Tiliacora acuminata*

Reducing Power

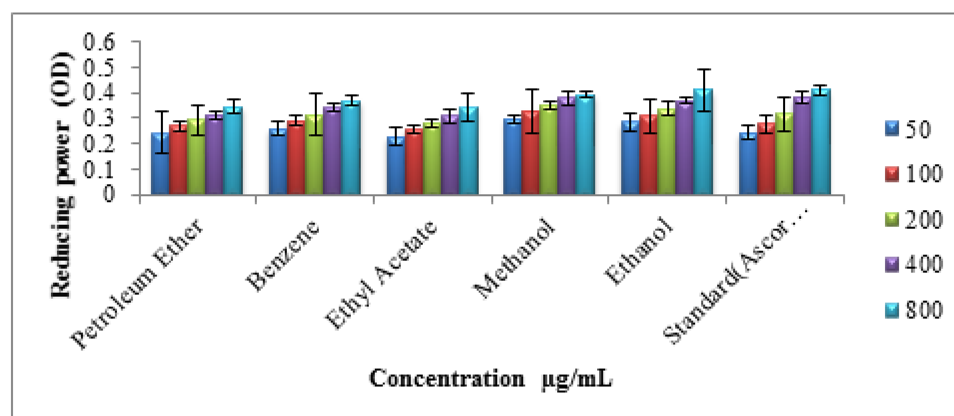

Figure 7 Reducing power ability of different extracts of *Tiliacora acuminata*

Figure 7 showed the reducing power ability of different solvent extracts of *T. acuminata* flower compared to ascorbic acid. Absorbance of the extract was increased when the concentration increased. A higher absorbance indicates a higher reducing power. Among the solvent tested, ethanol extract exhibited higher reducing activity.

IC_{50} values

IC_{50} values of petroleum ether extract of *T. acuminata* flower and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging and trolox for ABTS radical cation scavenging were found to be 19.36µg/mL and 20.12µg/mL; 17.46µg/mL and 21.83µg/mL; 19.58 µg/mL and 21.18µg/mL and 20.89µg/mL and 21.16µg/mL respectively. IC_{50} values of benzene extract of *T. acuminata* flower and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging and trolox for ABTS radical cation scavenging were found to be 15.05µg/mL and 20.12µg/mL; 16.84µg/mL and 21.83µg/mL; 17.47µg/mL and 21.189µg/mL and 20.11µg/mL and 21.16µg/mL respectively. IC_{50} values of ethyl acetate extract of *T. acuminata* flower and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging and trolox for ABTS radical cation scavenging were found to be 16.12µg/mL and 20.12µg/mL; 16.08µg/mL and 21.83µg/mL; 15.23µg/mL and 21.18µg/mL and 20.63µg/mL and 21.16µg/mL respectively. IC_{50} values of methanol extract of *T. acuminata* flower and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging and trolox for ABTS radical cation scavenging were found to be 29.65µg/mL and 19.11µg/mL; 18.77µg/mL and 20.84µg/mL; 28.05µg/mL and 23.79µg/mL and 20.93µg/mL and 21.18µg/mL respectively. IC_{50} values of ethanol extract of *T. acuminata* flower and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging and trolox for ABTS radical cation scavenging were found to be 28.13µg/mL and 19.11µg/mL; 16.13µg/mL and 20.84µg/mL; 26.16µg/mL and 23.79 µg/mL and 19.27µg/mL and 21.18µg/mL respectively (Table 3).

Table 3 IC_{50} values of different solvent extracts of flower of *Tiliacora acuminata*[a]

Solvent	IC_{50} (µg/mL)			
	DPPH	**Hydroxyl radical**	**Superoxide**	**ABTS**
Petroleum ether	19.36	17.46	19.58	20.89
Benzene	15.05	16.84	17.47	20.11
Ethyl acetate	16.12	16.08	15.23	20.63
Methanol	17.73	19.28	20.78	20.45
Ethanol	16.36	18.16	22.96	17.84
Ascorbic acid	20.12	21.83	21.18	-
Trolox	-	-	-	21.16

All the values are mean by triplicate determines*

Antibacterial activity

The petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *T. acuminata* flower were examined for their antibacterial activity against the selected pathogens. The antibacterial activity has been observed in the petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *T. acuminata* flower against all the tested bacteria with varied activity.

The petroleum ether extract of flower of *T. acuminata* illustrated the highest zone of inhibition against five pathogens viz; *Bacillus subtilis* (15mm), *B. thuringiensis*, *Streptococcus faecalis, Salmonella paratyphi B* and *Proteus mirabilis* (12mm each). The benzene extract of *T. acuminata* flower showed the highest zone of inhibition against *Enterococcus faecalis* (16mm), *Streptococcus faecalis*, *Staphylococcus aureus, S. pyogenes*, *Proteus mirabilis* and *Klebsiella pneumoniae* (13mm each). The ethyl acetate extract of *T. acuminata* flower showed the highest zone of inhibition against six pathogens viz; *Staphylococcus pyogenes*(20mm), *Pseudomonas aeruginosa, Mycobacterium smegmatis* (15mm each), *Streptococcus faecalis*(14mm), *Bacillus subtilis, Staphylococcus aureus* and *Escherrichia coli* (12mm each). The methanol extract of *T. acuminata* flower illustrated the highest zone of inhibition against four pathogen viz; *Enterococcus faecalis, Proteus mirabilis* (15mm each), *Staphylococcus aureus*(13mm) and *Streptococcus faecalis*(12mm). While the ethanol extract of *T. acuminata* flower demonstrated maximum zone of inhibition against the pathogen *Proteus mirabilis* (23mm), *Bacillus subtilis, Pseudomonas aeruginosa* (15mm each) and *Enterococcus faecalis* (12mm) (Table 4).

Table 4 Antibacterial activity of different extracts of flower of *Tiliacora acuminata*

Microorganisms	Name of the extract/ Zone of Inhibition (mm)					
	Petroleum ether	Benzene	Ethyl acetate	Methanol	Ethanol	AB
1.*Bacillus thuringiensis*	12	06	10	07	10	29
2.*Bacillus subtilis*	15	07	12	10	15	25
3.*Streptococcus faecalis*	12	13	14	12	6	26
4.*Staphylococcus aureus*	10	13	12	13	10	25
5.*Staphylococcus pyogenes*	10	13	20	10	10	23
6.*Enterococcus faecalis*	8	16	10	15	12	25
7. *Salmonella paratyphi A*	10	7	6	10	9	25
8.*Salmonella paratyphi-B*	12	10	8	9	8	27
9. *Salmonella paratyphi*	10	8	5	9	9	25
10.*Proteus mirabilis*	12	13	10	15	23	25
11. *Escherrichia coli*	10	8	12	10	8	25
12. *Serratia marcescens*	9	-	10	10	5	23
13.*Klebsiella pneumoniae*	-	13	10	8	6	17
14. *Proteus vulgaris*	-	8	8	-	10	15
15.*Pseudomonas aeruginosa*	-	10	15	10	15	22
16.*Mycobacterium smegmatis*	10	7	15	10	6	13
17.*Staphylococcus aureus* (Methicillin sensitive)	-	8	9	10	-	20
18.*Pseudomonas aeruginosa* (ESBL)	8	-	5	10	9	15
19. *Escherrichia coli* (ESBL)	5	-	11	8	-	15

Discussion

The medicinal plants are rich in secondary metabolites which include alkaloids, glycosides, flavonoids, steroids, saponins, phenols, tannins and terpenoids, which are of great medicinal value and have been extensively used in the drug and pharmaceutical industry. Recently a number of studies have been reported on the phytochemicals of medicinal plants, particularly on the plant parts like flowers [22-27]. In the present investigation, flower of *T. acuminata* have been screened for the presence of various groups of bioactive compounds along with *in vitro* screening for antioxidant and antibacterial activity.

Qualitative phytochemical investigation revealed that the extracts contained some phytoconstituents. Saponins, tannins, alkaloids and flavonoids are present in the five different extracts. These bioactive components including thiocynate, nitrate, chloride and sulphates beside other water soluble components which are naturally occurring in most plant materials, are known to be bactericidal, pesticidal or fungicidal in nature thus conforming the antimicrobial property to plants [28-30].

Flavonoids, the major group of phenolic compounds are reported for their antimicrobial, antiviral and spasmolytic activity. Flavonoids are able to scavenge hydroxyl radicals, superoxide anion radicals and lipid peroxy radicals, which high lights many of the flavonoids health promoting functions in organisms. They are important for prevention of diseases associated with oxidative damage of membrane, proteins and DNA. Flavonoids on the other hand, are potent water soluble antioxidants and few radical scavengers, which prevent oxidative cell damage and have strong anticancer activity [31]. Flavonoids have been referred to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergen, virus and carcinogens. They show anti-allergic, antiinflammatory and anticancer activity medicines [27, 31]. Phenolics have antioxdative, antidiabetic, anticarcinogenic, antimicrobial, antiallergic, antimutagenic and antiinflammatory activities [32, 33]. Plant

derived natural products such as flavonoids, terpenoids and steroids etc have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity [34,35]. Many tannin containing drugs are used in medicine as astringent. They are used in the treatment of burns as they precipitate the proteins of exposed tissues to form a protective covering. They are also medically used as healing agents in inflammation, leucorrhoea, gonorrhoea, burns, and piles and as antidote. Tannins have been found to have antiviral, antibacterial, antiparasitic effects, antiinflammatory, antiulcer and antioxidant property for possible therapeutic applications. Tannins are known to possess general antimicrobial and antioxidant activities. Recent reports show that tannins may have potential value as cytotoxic antineoplastic agents [36]. It was also reported that certain tannins were able to inhibit HIV replication selectively and was also used as diuretic [37]. Coumarin has been used as anticoagulant drugs and to treat lymphedema [38]. Plant steroids are known to be important for their cardio tonic activities, possess insecticidal and antimicrobial properties [22]. These observations cited on phytochemical compounds support the present findings on the usefulness of flower of *T. acuminata* in various medicaments. It suggests that the *T. acuminata* plant can be used as antimicrobial, antioxidant, antiallergic, antiinflammatory, antidiabetic, anticarcinogenic and anticancer agents in the future.

The FT-IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. By using the macroscopic fingerprint characters of FT-IR spectrum, we can judge the origin of different extracts accurately and effectively, trace the constituents in the extracts, identify the medicinal materials true or false and even evaluate the qualities of medicinal materials. So, FT-IR spectrum reflecting objectively the panorama of chemical constituents in complex system is a most credible method to validate and identify the mix-substance systems such as traditional medicine and herbal medicine [39].

The FT-IR analysis of a ethanol and methanol extracts of *T. acuminata* flower results revealed that suggest the presence of different functional groups ranging from O-H stretching, hydroxyl (3316.16 & 3320.06 cm^{-1}), C-H stretching, alkyl (2972.50 & 2944.35 cm^{-1}), C=O stretching, carboxylic, carbonyl (1653.48cm^{-1}), C-H stretching, carbonyl (2879.89 & 2832.88 cm^{-1}), C-O stretching, alcohols, ethers, esters, carboxylic acid and anhydrides (1378.02 & 1406.34 cm^{-1}) and C-F stretching, fluorinated compounds (1044.49 & 1018.55 cm^{-1}).

The FT-IR analysis on *T. acuminata* flower displayed new phytochemical markers as useful analytical tool to check not only the quality of the plant but also to identify the medicinally important plant.

In vitro antioxidant activity of the petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of flower of *T. acuminata* were investigated in the present study by DPPH, hydroxyl, superoxide and ABTS radical cation scavenging activities. These methods have proven the effectiveness of the extracts in comparison to that of the reference standard antioxidants, ascorbic acid and trolox.

DPPH is one of the stable free radicals which are commercially available and widely been used for evaluating scavenging activity of antioxidant standards and herbs/ plant extracts [40]. DPPH radical which is of violet color, accepts an electron or hydrogen atom from the antioxidant compounds and is converted into a colourless or somewhat yellow diamagnetic DPPH molecule [41]. Among the solvent tested, petroleum ether extract of flower of *T. acuminata* exhibited more DPPH radical scavenging activity.

The hydroxyl radical is one of representative reactive oxygen species generated in the body. These radicals are produced through various biological reactions; one of the common reactions is the Iron (II)-based Fenton reaction [42]. Though the DPPH sacavenging model is a useful indicator, it has some limitations. Therefore the extracts ability to scavenge hydroxyl radical was assessed. Among the solvent tested, methanol extract of flower of *T. acuminata* possessed more hydroxyl radical scavenging activity when compared with standard ascorbic acid.

In cellular oxidation reactions, superoxide radical is normally formed first and its effect can be magnified because it produces other kinds of cell-damaging free radicals and oxidizing agents. Superoxide anion radical actively participate in the initiation of lipid oxidation. Oxidation of unsaturated fatty acids in biological membranes leads to formation and propagation of lipid radicals, uptake of oxygen, and rearrangement of the double bonds in unsaturated lipids and eventual destruction of membrane lipids, which produce breakdown products [43]. The present study showed potent superoxide radical scavenging activity of *T. acuminata* flower extracts. The ethanol extracts showed potent superoxide radical scavenging activity with IC_{50} values 22.96 µg/mL compared to ascorbic acid 21.18 µg/mL respectively.

The basic principle underlying the ABTS decolorization assay is that ABTS on reaction with $K_2S_2O_8$ forms a greenish blue radical cation. Standard and sample antioxidants that are able to transfer an electron to ABTS radical scavenge the color of the solution proportionate to their amount. The extent of scavenging depends both upon the

concentration of antioxidant and time duration for the reaction. In the present study, petroleum ether extract of *T. acuminata* were fast and effective scavengers of ABTS radical and this activity was higher in trolox standard. Proton radical scavenging is an important attribute of antioxidants. ABTS a protonated radical has characteristic absorbance maxima at 734nm which decreases with the scavenging of the proton radicals [44].

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the antioxidant activity of antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging antioxidant activity [45]. In the present study, increase in absorbance of the reaction mixture indicates the reductive capabilities of flower extracts of *T. acuminata* in concentration dependent manner when compared to the standard ascorbic acid.

In the present investigation, *in vitro* antibacterial efficacy of the petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *T. acuminata* flower was quantitatively assessed on the basis of zone of inhibition. All the studied flower extracts in the present investigation exhibited varying degree of inhibitory effect against the selected bacterial human pathogens. Eloff [46] reported that methanol was the most effective solvent for plant extraction than hexane and water. In the present study, petroleum ether, benzene, ethyl acetate, methanol and ethanol were used for extraction.

The present study indicates antibacterial property of flower of *T. acuminata* against the selected strains of human pathogenic bacteria varies depends upon the solvent medium used for extraction. Ethyl acetate extract shows highest activity (19/19 pathogens) against the bacterial pathogens followed by methanol extract (18/19 pathogens) and ethanol extract (17/19 pathogens). Based on the previous literature, there is no report on antimicrobial activity of flower extracts of *T. acuminata*. Thus, the present study shows the presence of antibacterial activity in *T. acuminata* flower for the first time. The maximum antibacterial activity was shown by ethanol flower extract of *T. acuminata* against *Proteus mirabilis* and followed by ethyl acetate against *Staphylococcus pyogenes*. In general the above two bacteria are known to cause gastroenteritis, food borne illness, urinary tract infections, neonatal meningitis, nosocomial infections, wound and septicemia [47]. The ethanol and ethyl acetate extracts of *T. acuminata* flower demonstrated the antibacterial activity against *P. mirabilis* and *S. pyogenes* respectively, it inferred that the selected plant can be used to treat above said diseases caused by these pathogens. Based on the present study, it is concluded that the flower of *T. acuminata* contains various bioactive compounds with high degree of antibacterial activity against various pathogens.

In conclusion, the present study provides the evidence that the methanol extract of *T. acuminata* flower, contains phenolic and flavonoids. These *in vitro* assays demonstrate that this plant extract is an important source of natural antioxidant which may be preventive against oxidative stresses. This is the first report on the antioxidant and antibacterial property of this plant. Therefore, further studies should be carried out to isolate active principles having antioxidant and antibacterial properties.

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