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PHYTOCHEMICAL, *IN VITRO* ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF SEED EXTRACTS OF *TILIACORA ACUMINATA* (LAN.) HOOK F AND THOMAS (MENISPERMACEAE)

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ABSTRACT

The present study was carried out to investigate the phytochemical, *in vitro* antioxidant and antibacterial activity of seed of *Tiliacora acuminata* (Lan) Hook.F&Thomas an important medicinal plant. Qualitative phytochemical analysis of the methanol and ethanol extracts prepared from *Tiliacora acuminata* seed revealed the presence of alkaloid, catechin, coumarin, flavonoid, phenol, quinone, saponin, steroid, tannin, terpenoid, sugar, glycoside, xanthoprotein and fixed oil. 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 seed of *Tiliacora acuminata* have been tested using various antioxidant model systems viz, DPPH, hydroxyl, superoxide, ABTS and reducing power. Methanol extracts of *Tiliacora acuminata* is found to possess higher DPPH, superoxide and ABTS radical cation scavenging activity. Ethanol extract of *Tiliacora acuminata* is found to possess higher hydroxyl radical scavenging activity. Methanol extract of seed of *Tiliacora acuminata* showed the highest reducing ability. This study indicates significant free radical scavenging potential of *Tiliacora acuminata* seed which can be exploited for the treatment of various free radical mediated ailments. The petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of seed of *Tiliacora 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 pneumonia, Pseudomonas aeruginosa, Pseudomonas aeruginosa* (ESBL) and *Mycobacterium smegmatis* by the agar disc diffusion method.

INTRODUCTION

Majority of the diseases/disorders are mainly linked to oxidative stress due to free radicals. Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and metabolism (**Tiwari, 2001**). The most common reactive oxygen species (ROS) include superoxide anion, hydrogen peroxide (H2O2), peroxyl (ROO-) radicals, and reactive hydroxyl (OH) radicals. The nitrogen derived free radicals are nitric oxide (NO) and peroxynitrite anion (ONOO). ROS have been implicated in over a hundreds of diseases states which range from arthritis and connective tissue disorders to carcinogenesis, aging, physical injury, infection and acquired immunodeficiency syndrome (**Joyce, 1987; Panda *et al.*, 2011**). Free radicals activities are controlled by a system of endogenous enzymatic and non-enzymatic antioxidants which eliminate pro-oxidants and scavenge free radicals. Current research is now directed towards finding naturally occurring antioxidants of plant origin. Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions. The antioxidative effect of plant extracts are mainly due to phenolic components, such as flavonoids, phenolic acids and phenolic diterpenes. Antioxidant agents of natural origin have attracted special interest because they can protect human body from free radicals (**Kiran *et al.*, 2012**). Plant and plant products are being used as a source of medicine since long.

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The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability (**Auudy *et al.*, 2003**). Over the past few decades, there has been much interests in natural materials as source of new antibacterial agents. Different extracts from traditional medicinal plants have been tested. Many reports show the effectiveness of traditional herbs against microorganisms as a result plants have become one of the bases of modern medicine (**Evans *et al.*, 2002**). Plants have been used for the treatment of disease all over the world before the advent of modern clinical drugs. Natural phytochemicals are known to contain substance that can be used for therapeutic purposes or as precursor for the synthesis of novel useful drugs.

Natural products of higher plants may give a new source of antibacterial agents with possibly a novel mechanism of action. The selection of crude plant extract for screening the crude antibacterial activity has the potential of being more successful in the initial steps than screening of pure compounds (**Chandrappa *et al.*, 2010**). Some organisms have developed resistance to the existing antibiotics, therefore the development of bacterial resistance to the currently available antibiotics has necessitated the research for new antibacterial agents (**Pranav *et al.*, 2010**). *Tiliacora acuminata* is a large woody climber belonging to the family Menispermaceae. This plant has been used as an ingredient in many of the ayurvedic preparations and regard as an antidote for snake bite (**Selvaraj *et al.*, 2008; Sri and Reddi, 2011**). To the best of our knowledge, there is no record of work on the phytochemical screening, antioxidant and antibacterial activity of the seed of *Tiliacora acuminata*. Therefore, the present study was carried out to evaluate the phytochemical screening, *in vitro* antioxidant and antibacterial activity of the seed of *Tiliacora acuminata*.

MATERIALS AND METHODS

The seed of *T. acuminata* 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 for phytochemical screening

Freshly collected seed 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 250mL in a Soxhlet apparatus for 24 hrs. All the extracts were filtered though Whatman No.41 filter paper.

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 (**Kareru *et al.*, 2008**).

Preparation of extract for antioxidant activity

The seed of *T. acuminata* was 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 were concentrated in a rotary evaporator. The concentrated extracts were subjected to qualitative tests for the identification of various phytochemical constituents as per standard procedures (**Saraf, 2010; Shajeela *et al.*, 2012; Murugan and Mohan, 2011**) and used for *in vitro* antioxidant activity. The methanol extract was used for the estimation of total phenolics and flavonoids.

Estimation of Total Phenolics

Total phenolic content was estimated using Folin-Ciocalteau reagent based assay as previously described (**McDonald *et al.*, 2001**) 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 Na2CO3 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* (2007**). 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 (**Shen *et al.*, 2010**). 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 (**Shen *et al.*, 2010**). 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&800 µg/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.

DPPH scavenging effect (% inhibition) = $\{(A_0 - A_1)/A_0)*100\}$

Where, A0 is the absorbance of the control reaction, and A1 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 (1987)**. Stock solutions of EDTA (1mM), $FeCl_3$ (10mM), Ascorbic Acid (1mM),H_2O_2 (10mM) and Deoxyribose (10mM) were prepared in distilled deionized water. The assay was performed by adding 0.1mL EDTA , 0.01mL of FeCl3**,** 0.1mL H2O2, 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 370C 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.* (2007)**. The superoxide anion radicals were generated in 3.0mL 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, pH 8.0). The reaction was started by adding 0.5mL PMS solution (0.12mM) to the mixture, incubated at 25^{o}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* (2011). ABTS radical cation (ABTS+) was produced by reacting 7mM ABTS solution with 2.45mM 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 734nm. After addition of 100µL of sample or trolox standard to 3.9mL 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 (2011).** 1.0mL 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^{o}C for 20 minutes. Then 5mL of 10% trichloroacetic acid was added and centrifuged at 980 g (10 minutes at 5^{o}C) in a refrigerator centrifuge. The upper layer of the solution (5.0mL) was diluted with 5.0mL of distilled water and ferric chloride and absorbance read at 700nm. The experiment was performed thrice and results were averaged.

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 (**Gokhale, 2009**) 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.

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.

RESULTS

Phytochemical screening

The results of the phytochemical screening revealed the presence of alkaloid, catechin, coumarin, flavonoid, phenol, quinone, saponin, steroid, tannin, terpenoid, sugar, glycoside,

xanthoprotein and fixed oil in the methanol and ethanol extracts of *T. acuminata* seed (Table 1). Maximum degree of chemical diversity is seen in *T. acuminata* seed methanol and ethanol extracts in which fourteen out of fifteen different chemical tests gave positive results. Ethyl acetate extract of *T. acuminata* seed gave positive results for thirteen different chemical tests. Petroleum ether and benzene extracts showed minimum degree of chemical diversity with the positive results for only eleven different chemical tests ie for alkaloid, catachin, coumarin, phenol, saponin, steroid, tannin, sugar, glycoside, xanthoprotein and fixed oil.

Alkaloid, catachin, coumarin, phenol, saponin, steroid, sugar, glycoside, xanthoprotein and fixed oil present in seed of *T. acuminata* of all the five solvent studied.

FT-IR Spectroscopy studies

The use of FT-IR fingerprinting for herbal extract tends focus on identification and assessment of the stability of the chemical constituents, functional groups as observed by FT-IR analysis. The results of FT-IR fingerprint for the ethanol and methanol extracts of seed of *T. acuminata* is shown in Fig 1 and 2. It is also given in Table 2.

Table 1. Preliminary phytochemical screening of seed extract of *T. acuminate*

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

Table 2. IR Spectroscopic data of ethanol and methanol extracts of *T. acuminata* seed

S.No	Group	Stretching Frequency (cm^{-1})	
		Ethanol	Methanol
1	O-H	3317.73	3310.19
2	C-H, methyl group	2972.51 2879.58	2942.59
3	N-H, amine	1653.84	1653.72
4	C=C stretching, aromatic	-	1560.35 1448.11
5	C=O	1378.30 1321.48	1407.54
6	C-O	1086.00	1109.00
7	C-F stretching	1044.41	1021.00

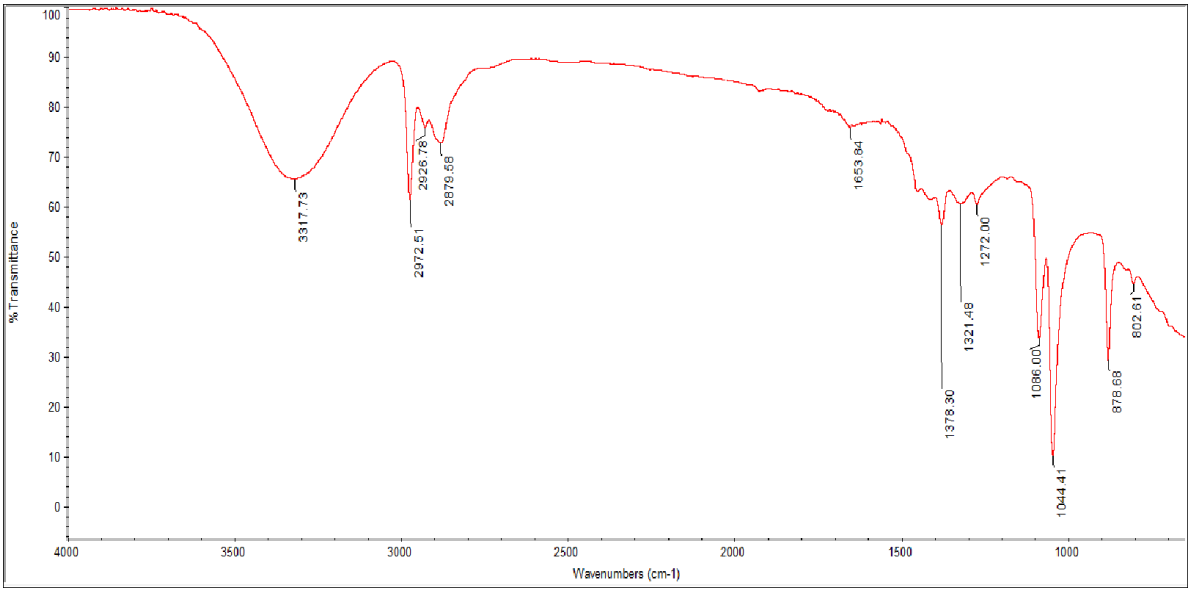

Fig. 1. FT-IR spectrum of ethanol extract of seed of *Tiliacora acuminata*

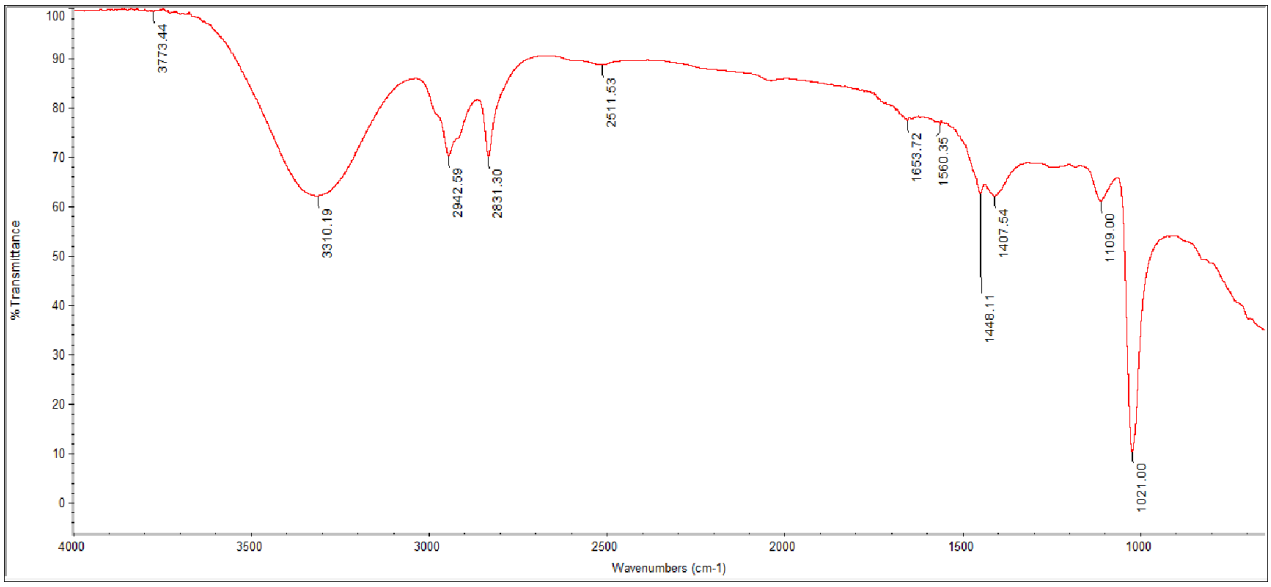

Fig. 2. FT-IR spectrum of methanol extract of seed of *Tiliacora acuminata*

FT-IR spectroscopic analysis showed the presence of phytoconstituents. The FT-IR gives broad peaks at 3317.73 and 3310.19cm^{-1} which indicated the presence of OH stretching of phenols and the strong absorption band observed at 2972.51, 2879.548 and 2942.59cm^{-1} may represent bonded C-H stretching of alkanes. Absorption bands at 1653.84 and 1653.72cm^{-1} are N-H stretching of amino compounds. The peak obtained at 1560.35 and 1448.11cm^{-1} indicated the presence of C=C stretching of aromatic compounds. The peak at 1378.30, 1321.48 and 1407.54cm^{-1} can be attributed as bonded C=O stretching of carbonyls. Peaks at 1086.00 and 1109.00cm^{-1} can be due to the presence of stretching of C-O of alcohols, ethers, carboxylic acids and ester groups of compounds. The peak obtained at 1044.41 and 1021.00cm^{-1} indicated the presence of C-F stretching of halogen compounds.

Antioxidant activity

Total phenolics and total flavonoid content

The total phenolic content and total flavonoid content of the methanol extract of *T. acuminata* seed were found to be 1.97g100g^{-1} and 3.49g100g^{-1} respectively.

DPPH radical scavenging activity

DPPH radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *T. acuminata* seed was shown in Fig 3. The scavenging effect increases with the concentration of standard and samples. Among the solvent tested, methanol extract of *T. acuminata* seed exhibited highest DPPH radical scavenging activity. At 800µg/mL concentration, methanol extract of *T. acuminata* possessed 109.16% scavenging activity on DPPH.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of *T. acuminata* seed was shown in Fig 4. Ethanol extract showed very potent activity.

At 800µg/mL concentration, ethanol extract of *T. acuminata* possessed 88.13% scavenging activity on hydroxyl radical.

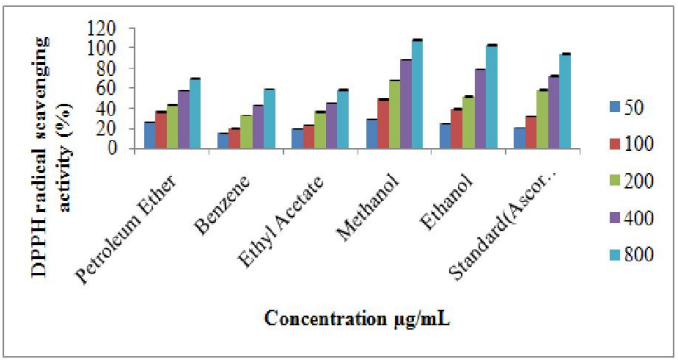

Fig. 3. DPPH radical scavenging activity of different extracts of *T. acuminata*

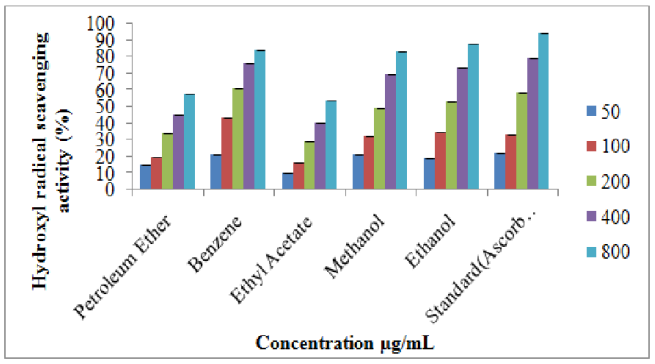

Fig. 4. Hydroxyl radical scavenging activity of different extracts of *T. acuminata*

Superoxide radical scavenging activity

The seed of *T.acuminata* extract was subjected to the superoxide radical scavenging assay and the result was shown in Fig 5. It indicates that methanol extract of *T. acuminata* seed (800µg/mL) exhibited the maximum superoxide radical scavenging activity.

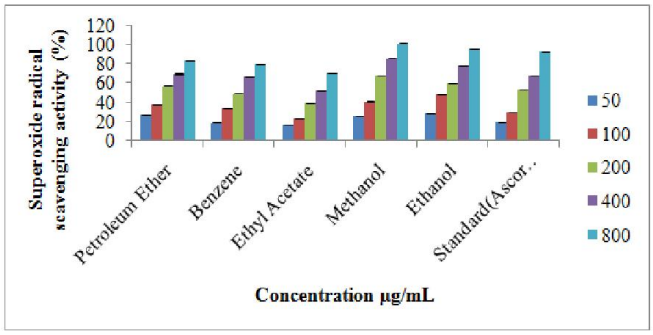

Fig. 5. Superoxide radical scavenging activity of different extracts of *T. acuminata*

ABTS radical cation scavenging activity

The seed of *T. acuminata* extract was subjected to the ABTS radical cation scavenging activity and the results was shown in Fig 6. The methanol extract exhibited potent ABTS radical cation scavenging activity in concentration dependent manner. At 800µg/mL concentration, methanol extract of *T. acuminata* seed possessed 96.24% scavenging activity on ABTS which is higher than the standard trolox whose scavenging activity is 90.88%.

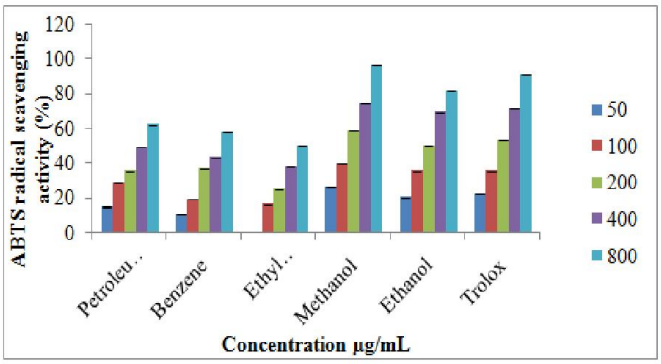

Fig. 6. ABTS radical scavenging activity of different extracts of *T. acuminata*

Reducing Power

Figure 7 showed the reducing power ability of different solvent extracts of *T.acuminata* seed 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, methanol extract exhibited higher reducing activity.

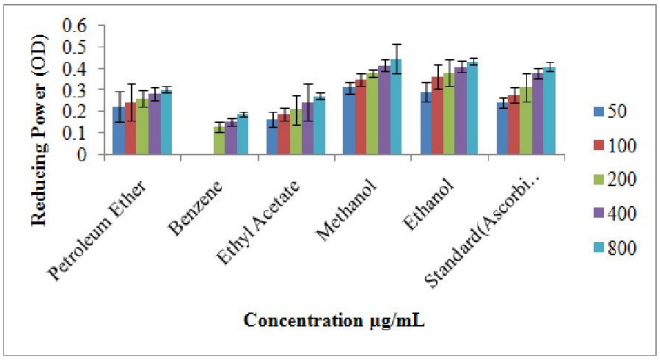

Fig. 7. Reducing power ability of different extracts of *T. acuminata*

IC_{50} values

IC_{50} values of Petroleum ether extract of *T. acuminata* seed and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging and trolox for ABTS radical cation scavenging were found to be 18.91µg/mL and 20.12µg/mL; 14.81µg/mL and 21.83µg/mL; 19.17µg/mL and 21.18µg/mL and 18.16g/mL and 21.16µg/mL respectively. IC_{50} values of Benzene extract of *T. acuminata* seed and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging and trolox for ABTS radical cation scavenging were found to be 16.55µg/mL and 20.12µg/mL; 19.34µg/mL and 21.83µg/mL; 22.96µg/mL and 21.18µg/mL and 17.34µg/mL and 21.16µg/mL respectively. IC50 values of Ethyl acetate extract of *T. acuminata* seed and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging and trolox for ABTS radical cation scavenging were found to be 23.16µg/mL and 20.12µg/mL; 20.13µg/mL and 21.83µg/mL; 19.54µg/mL and 21.18µg/mL and 22.16µg/mL and 21.16µg/mL respectively. IC_{50} values of Methanol extract of *T. acuminata* seed and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging and trolox for ABTS radical cation scavenging were found to be 14.18µg/mL and 20.12µg/mL; 19.16µg/mL and 21.83µg/mL; 18.56µg/mL and 21.18µg/mL and 13.84µg/mL and 21.16µg/mL respectively. IC_{50} values of Ethanol extract of *T.acuminata* seed and standard ascorbic acid for DPPH, hydroxyl, superoxide radical scavenging and trolox for ABTS radical cation scavenging were found to be 22.41µg/mL and 20.12µg/mL; 14.16µg/mL and 21.83µg/mL; 22.13µg/mL and 21.18µg/mL and 20.16µg/mL and 21.16µg/mL respectively (Table 3).

Table 3. IC_{50} values of different solvent extracts of seed of *T. acuminata*[a]

Solvent	IC_{50} (µg/mL)			
	DPPH	Hydroxyl radical	Superoxide	ABTS
Petroleum ether	18.91	14.81	19.17	18.16
Benzene	16.55	19.34	22.96	17.34
Ethyl acetate	23.16	20.13	19.54	22.16
Methanol	14.18	19.16	18.56	13.84
Ethanol	22.41	14.16	22.13	20.16
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* seed 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* seed against all the tested bacteria with varied activity. The petroleum ether extract of seed of *T. acuminata* illustrated the zone of inhibition against only five pathogens viz; *Streptococcus faecalis*(10mm), *Pseudomonas aeruginosa* (10mm), *Bacillus thuringiensis* (8mm), *Enterococcus faecalis* (7mm) and *Proteus mirabilis* (6mm each). The benzene extract of *T. acuminata* seed showed the highest zone of inhibition against five pathogens viz; *Salmonella paratyphi-B* (15mm), *Serratia marcescens* (13mm), *Staphylococcus aureus* (12mm), *Pseudomonas aeruginosa* and *Salmonella paratyphi* (11mm each). The ethyl acetate extract of *T. acuminata* seed showed the highest zone of inhibition viz; *Pseudomonas aeruginosa*

Table 4. Antibacterial activity of different extracts of seed of *T. acuminate*

Microorganisms	Name of the extract/ Zone of Inhibition (mm)					
	Petroleum ether	Benzene	Ethyl acetate	Methanol	Ethanol	AB
1.*Bacillus thuringiensis*	8	9	12	11	16	20
2.*Bacillus subtilis*	-	9	11	13	15	21
3.*Streptococcus faecalis*	10	9	14	17	19	35
4.*Staphylococcus aureus*	-	12	9	9	10	20
5.*Staphylococcus pyogenes*	-	7	11	17	16	29
6. *Staphylococcus aureus* (Methicillin sensitive)	-	-	8	9	11	25
7.*Enterococcus faecalis*	7	9	20	13	17	25
8. *Salmonella paratyphi A*	-	6	12	-	-	20
9.*Salmonella paratyphi-B*	-	15	17	14	24	32
10. *Salmonella paratyphi*	-	11	15	-	8	20
11.*Proteus mirabilis*	6	9	7	22	30	22
12. *Serratia marcescens*	-	13	14	-	-	24
13.*Klebsiella pneumonia*	-	-	13	-	12	24
14. *Proteus vulgaris*	-	-	19	10	9	21
15.*Pseudomonas aeruginosa*	10	11	9	-	14	22
16.*Pseudomonas aeruginosa* (ESBL)	-	-	25	15	19	25
17.*Mycobacterium smegmatis*	-	7	9	15	10	20
18. *Escherichia coli*	-	8	20	10	13	25
19. *Escherichia coli* (ESBL)	-	-	10	-	9	25

(ESBL) (25mm), *Escherichia coli, Enterococcus faecalis* (20mm each), *Proteus vulgaris* (19mm), *Salmonella paratyphi-B* (17mm), *Salmonella paratyphi* (15mm) and *Streptococcus faecalis*(14mm). The methanol extract of *T. acuminata* seed illustrated the highest zone of inhibition against eight pathogen viz; *Proteus mirabilis* (22mm each), *Staphylococcus pyogenes, Streptococcus faecalis* (17mm each), *Mycobacterium smegmatis*, *Pseudomonas aeruginosa* (ESBL) (15mm each), *Salmonella paratyphi-B* (14mm), *Bacillus subtilis* and *Enterococcus faecalis* (13mm each). While the ethanol extract of *T. acuminata* seed demonstrated maximum zone of inhibition against the pathogen *Proteus mirabilis* (30mm), *Salmonella paratyphi-B* (24mm), *Pseudomonas aeruginosa (ESBL), Streptococcus faecalis*(19mm each), *Enterococcus faecalis* (17mm), *Staphylococcus pyogenes, Bacillus thuringiensis* (16mm each), *Bacillus subtilis* (15mm) and *Pseudomonas aeruginosa* (14mm) (Table 4).

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 (**Jeeva *et al.*, 2011; Kiruba *et al.*, 2011; Sukumaran *et al.*, 2011; Anitha *et al.*, 2012; Johnson *et al.*, 2012; Jeeva and Johnson, 2012**). In the present investigation, seed 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 (**Lutterodt *et al.*, 1999; El astal *et al.*, 2005; Pretorius and Watt, 2001**).

The FT-IR has proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plant extract (**Eberhardl *et al.*, 2007; Egwaikudi *et al.*, 2009**). The observed presence of bonded OH stretching of phenols in the plant suggests that the plant has a displayed potential medicinal property. Some phenolic compounds are believed to be cancer preventives (**Shachi, 2012**), these are compounds that may decrease the risk of developing cancer (**Del Rio *et al.*, 2010**), which is believed to become the main cause of death in worldwide and also play role in the prevention of cardiovascular diseases (**Sharma and Sharma, 2013**). Phenolic compounds found in plants may also have antioxidant effects by reacting with and capturing dangerously reactive compounds before it can react with other biomolecules and cause serious damage. On the other hand, several studies claim that the phenolic compounds present in spices and herbs might also play a major role in their antimicrobial effects (**Masih *et al.*, 2012**).

Phenolic compounds are known as powerful chain breaking antioxidants, may contribute directly to antioxidative action (**Duh *et al.*, 1999**). These compounds are very important constituents of plants and their radical scavenging ability is due their hydroxyl groups (**Hatano *et al.*, 1989**). It is well known that phenolic compounds are constituents of many plants and they have attracted a great deal of public and scientific interest because of their health promoting effects as antioxidants (**Hollman and Katan, 1999**). The phenolic compounds exhibit considerable free radical scavenging activities, through their reactivity as hydrogen or electron donating agents, and metal ion chelating properties (**Rice-Evans *et al.*, 1996**). The interests of phenolics are increasing in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. The phenolic compounds in herbs act as antioxidants due to their reducing agents, hydrogen donors, free radical quenchers and metal chelators (**Rao *et al.*, 2011**). Therefore, it would be valuable to determine the total phenolic and flavonoid contents of the plant extracts. Flavonoids are a group of more than 4000 polyphenolic compounds that occur naturally in foods of plants origin. Flavonoids are a large class of phytochemicals which are omnipresent in human diets,

found for example in fruit, vegetables, tea, chocolate and urine, and to which a number of beneficial effects on human health, such as antioxidant, antiinflammatory, antiallergic, antiviral and anticarcinogenic activities; while some flavonoids exhibit potential for anti-human immunodeficiency virus functions (**Yao *et al.*, 2004**). Flavonoids are important for human health because of their high pharmacological activities as radical scavengers. These compounds possess a common phenylbenzopyrone structure (C_6-C_3-C_6), and they are categorized according to the saturation level and opening of the central pyran ring, mainly into flavones, flavanols, isoflavones, flavonols, flavanones and flavononols (**Jing *et al.*, 2010**). Flavonoids have been known for their antibiotic activities and recently for immunoregulatory functions (Haslam, 1996). Also tannins have been reported to have antiviral, antibacterial and antiparasitic effects (**Zhang and Wang, 2001**). Alkaloids are known to exhibit emetic amoebicides, expectorant, amaesthetics, antipyuretics, analgesics, antileminthic and can be used for the treatment of stomach problems (**Clere *et al.*, 2011**).

In vitro antioxidant activity of the petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts of seed 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. The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple colour). When Antioxidants react with DPPH, which is a stable free radical becomes paired off in the presence of a hydrogen donor and is reduced to the DPPHH and as consequence the absorbance's decreased from the DPPH. Radical to the DPPH-H form, results in decolorization (yellow colour) with respect to the number of electrons captured (**Mohammad *et al.*, 2009**). More the decolorization higher is the reducing ability. This test has been the most accepted model for evaluating the free radical scavenging activity of any new drug. In the present study, the percentage of scavenging effect on the DPPH radical was concomitantly increased with the increase in the concentration of *T. acuminata* seed extracts from 50-800µg/mL. Among the solvent tested, methanol extract of *T. acuminata* seed exhibited maximum DPPH radical scavenging activity.

The •OH radical is an extremely reactive in biological systems and has been implicated as highly damaging species in free radical pathology, capable of damaging biomolecules of the living cells. These radical combines with nucleotides in DNA and cause strand breakage leading to carcinogenesis, mutagenesis and cytotoxicity. Hydroxyl radical (•OH) scavenging capacity of an extract is directly related to its antioxidant activity (**Khan *et al.*, 2012**). Among the tested solvents, ethanol extract of *T. acuminata* seed exhibited the strongest hydroxyl radical scavenging activity (88.13% at 800µg/mL). The radical scavenging capacity may be attributed to phenolic compounds in seed extracts with the ability to accept electrons, which can combine with free radical. Superoxide is biologically important as it can form singlet oxygen and hydroxyl radical. Overproduction of super oxide anion radical contributes to redox imbalance and associated with harmful physiological consequences. Super oxide anion are generated in PMS-NADH system by the oxidation of NADH and assayed by the reduction of NBT resulting in the formation of blue formazan (**Rajesh and Natvar, 2011**). In the present study, among the tested solvents, methanol extract of *T. acuminata* seed showed the maximum superoxide inhibition at the concentration of 800µg/mL. This extract exhibited higher ability in scavenging superoxide radical. 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 cation 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 (**Paulpriya and Mohan, 2013**). In the present study, among the tested solvents, methanol extract of *T. acuminata* seed showed higher ABTS radical scavenging activity. This study indicated that the extracts have the hydrogen donating ability and could serve as free radical scavenging by acting as primary antioxidants.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (**Meir *et al.*, 1995**). The presence of reductants such as antioxidant substances causes the reduction of Fe3+/Ferric cyanide complex to Fe2+/ ferrous form. Therefore, the reducing power of the sample could be monitored by measuring the formation of Perl's Prussian blue at 700nm (**Manian *et al.*, 2008**). Samples with higher reducing power have better abilities to donate electrons. Free radicals form stable substances by accepting the donated electrons, resulting in the termination of radical chain reactions. It has been widely accepted that the higher the absorbance at 700nm, the greater reducing power. In the present study, the results revealed the dose dependent reducing ability for all the extracts. Among the tested solvents, methanol extract of *T. acuminata* seed exhibited greater reducing ability. In the present study, *in vitro* antibacterial activity of *T. acuminata* seed of petroleum ether, benzene, ethyl acetate, methanol and ethanol extracts was quantitatively assessed on the basis of zone of inhibition. Plant part studied in the present investigation exhibited varying degree of inhibitory effect against the selected bacterial human pathogens.

It has been shown that when solvents like ethanol, hexane and methanol are used to extract plants most of them are able to exhibit inhibitory effect of both gram positive and gram negative bacteria (**Shymala Gowri and Vasantha, 2010**). **Eloff (1998)** reported that methanol was the most effective solvent for plant extraction than hexane and water. In the present study we used petroleum ether, benzene, ethyl acetate, methanol and ethanol for extraction. The present study confirmed the Eloff observations with maximum activity. *T. acuminata* seed ethyl acetate extract shows highest activity (19/19) against the bacterial pathogens, followed by ethanol extract of *T. acuminata* seed (17/19) and methanol extract of *T. acuminata* seed (16/19). In the present study, we observed the antibacterial activity against nineteen pathogenic bacteria. But there is no report on *T. acuminata* seed extracts. Thus, the present study shows the presence of antibacterial activity in *T. acuminata* seed extracts for the first time. The presence of antibacterial activity in a particular part of a particular species may be due to the presence of one or more bioactive

compounds such as alkaloids, glycosides, flavonoids, steroids, saponins etc (**Raja *et al.*, 2011**). Recently, a number of plants have been reported for antibacterial properties across the world (**Olowosulu and Ibrahim, 2006; Chendurpandy et al., 2010; Murugan and Mohan, 2013; Tresina and Mohan, 2014**). In the present study, ethyl acetate extracts of *T. acuminata* seed showed highest activity against *E. faecalis, E. coli* and *P. vulgaris* whereas ethanol extract showed highest activity against *Salmonella paratyphi B, P. mirabilis* and *P. aeruginosa* (ESBL). Methanol extract of *T. acuminata* seed exhibited highest activity against *P. mirabilis*. Among the pathogens, *P. vulgaris* is known to cause urinary tract infections and wound. *S. typhi* is known to cause fever and food borne illness. In general, *E. coli*, *P. vulgaris* and *P. mirabilis* are known to cause gastroenteritis, food borne illness, urinary tract infections, wound, septicemia, pneumonias and form mild superficial skin infections to life-threatening systemic diseases.

E. faecalis can cause endocarditis, as well as bladder, prostate and epididymal infections, nervous system infections are less common (**Anitha *et al.*, 2012**). *P. aeruginosa* have been implicated as causative agents of brils, sores and wounds. *E. coli* can cause gastroenteritis, urinary tract infections and neonatal meningitis. In rare cases, virulent strains are also responsible for hemolytic- uremic syndrome, peritonitis, mastitis, septicemia and gram negative pneumonia (**Todar, 2007**). In the present study, the extracts of seed of *T. acuminata* detected the inhibitory activity against the above said pathogens. It suggests that the plant can be used to treat urinary tract infections, food borne illness, fever and neonatal meningitis in the future. It is hoped that this study would direct to the establishment of some compounds that could be used to invent new and more potent antibacterial drugs of natural origin. Further work will emphasize the isolation and characterization of active principles responsible for bio-efficacy and bioactivity.

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