PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIMICROBIAL ACTIVITY OF *Gmelina asiatica L.* BARK

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Abstract

Present study has been designed to carry out the phytochemical screening, FT-IR analysis, HPTLC finger printing and *in vitro* antimicrobial activity of aqueous extract of *Gmelina asiatica* bark. Preliminary phytochemical screening confirmed the presence of carbohydrates, coumarins, glycosides, phytosterols, tannins, phenols etc., FT-IR analysis revealed the presence of functional groups such as alcohol, alkane, fatty acids, alkyne, nitrite, amide, acid, nitro, amine, alkyl halide and ether. HPTLC analysis was performed and the amount of quercetin in ethanolic extract of *Gmelina asiatica* was found to be 0.0640%w/w. *In vitro* antimicrobial activity of plant extracts (100,200 and 300 µg/ml) was also performed by using disc diffusion method against the pathogenic bacteria such as *Escherichia coli, Bacillus subtilis, Staphylococus aureus*, and *Pseudomonas aeruginosa,* pathogenic fungi such as *Candida albicans* and *Trichoderma viride.* Among the three concentrations, 300µg of bark extract was active against both the bacterial and fungal species. Standard antibiotic ampicillin and amphotericin were also subjected for antibacterial and antifungal activities respectively in order to compare the antimicrobial efficacy of plant extract. Based on the present study, bark extract of *Gmelina asiatica* possess remarkable antimicrobial activity against several pathogens tested and thus can be a good source of natural antimicrobial agent which may be due to the presence of flavonoids like quercetin.

Keywords: Antimicrobial, Bark extract, FT-IR, *Gmelina asiatica*, HPTLC, Quercetin Phytochemical.

INTRODUCTION

Infectious diseases, also known as contagious diseases or transmissible diseases, caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite the tremendous progress in human medicine. Pathogenic microorganisms have always been considered as a major cause of morbidity and mortality in humans, particularly in developing countries. Resistance to antimicrobial agents has become an increasingly important and pressing global problem. Now a day's various medicinal plants have been used in daily life to treat infectious disease all over the world. Therefore, pharmaceutical companies have been motivated to develop new antimicrobial drugs in recent years, especially due to the constant

emergence of microorganisms resistant to conventional antimicrobials [1] Keeping this in view, medicinal plant *Gmelina asiatica* was selected to evaluate its antimicrobial potential. Present study has also been designed to study the FT-IR and HPTLC fingerprinting of *Gmelina asiatica* bark. *Gmelina asiatica* L. (Syn: *Gmelina parvifolia* Roxb.), is a deciduous large sized bush or shrub belonging to the family Verbenaceae is commonly called "Asiatic Bush Beech" and "Nilakumizh" in Tamil. The whole plant is medicinally important and well documented as a source of bioactive components with medicinal properties. Roots, seeds and leaves are the reported plants part used from ancient times.

The major phytoconstituents found in *Gmelina asiatica* are the retinoid, an isoquinoline derivate as well as terpenoids, steroids, phenolic compounds, alanine, alpha-amyrine, arabinose, beta-amyrins, oleanolic acid, trophan, stigmasterol, beta-D-glycoside, urolic acid, mirablisoic acid, mirabalisol, trigonellin and antiviral protein C-methylabronisflavone, tartaric acid, betanin, brassicasterol, brtalanic acid. *G.asiatica* have been reported to possesses anti-inflammatory [2], antioxidant [3], antihyperglycemic and hypoglycemic [4], hepatoprotective [5] and antipyretic [6], anticancer and antiproliferative [7] activities.

MATERIALS AND METHODS

Collection of plant materials

The plant material used in the present study was the bark of *Gmelina asiatica*. The bark was collected from in and around Pattukottai, Thanjavur district, Tamil Nadu, India. The collected samples were carefully kept in polythene bags. The bark were dried in shade and stored in air tight containers until further studies.

Preparation of extracts

The dried bark material is coarsely powered using grinder; 100 gm of powdered bark was packed evenly in the soxhlet extractor and subjected to extraction with 70% methanol. After extraction, the solvent was distilled off and the extracts were concentrated on water bath to a dry residue and kept in a dessicator. The crude extract was used for the present study.

Preliminary phytochemical screening

The extract was subjected to preliminary phytochemical investigation to determine the different phytoconstituents [8].

Fourier Transform Infrared (FT-IR) Spectrum analysis

10mg of the dried extract powder was encapsulated in 100mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of extractwas loaded in FTIR spectroscope (Shimadzu,

Japan), with a scan range from 400 to 4000cm^{-1} with a resolution of 4 cm^{-1}. All the analysis was repeated thrice and mean ± SD was recorded [9].

HPTLC analysis

The complete CAMAG TLC equipment consists of a fully automatic sample Linomat V sample applicator, a developing chamber. Finally, a CAMAG TLC scanner is available allowing densitometric evaluation. The extract of the *Gmelina asiatica* L. bark was taken and dissolved in respective solvents and the volume was made up to 1ml in a standard flask (1000ug/ml). Standard Quercetin (1mg) was taken and dissolved in water. This was transferred into a standard flask and the volume was made up to 10 ml to prepare 10 ug/0.1 ml solutions. Silica gel 60 F254 and HPTLC aluminum sheets were used as absorbent (stationary phase). The extracts were applied point-wise from 1000 ug/ml sample solution, 5 and 10 ul of the sample was applied on HPTLC aluminum sheets as different tracks in the form of 6 mm wide bands by using Acamag semi-automatic Linom at 5 spotter at a distance of 12 mm. Nitrogen gas was also supplied for simultaneous drying of bands and then using drier for completely drying of bands. To saturate the chamber, 10 ml mobile phase (toluene: ethyl acetate: formic acid (5:4:1)) was placed in each flat- bottomed CAMAG twin trough TLC chamber, 30 min before the development of the TLC plate. The developed plates were then dried and scanned using a TLC scanned 3 with wincats software under 364 nm. All plates were visualized directly after drying and a fingerprint profile was photo documented using a CAMAG Reproster-3 under 254 nm. The digital images of the chromatograms were evaluated with the programme CAMAG Video scan. The captured image was subjected a visual infection on the computer screen. The differences found, are specified by the HPTLC system in which the difference is detected and by the RF value (and colour) of a compound in the system [10].

Antimicrobial activity

Selection of microorganism

Four pathogenic bacteria such as *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis* and two pathogenic fungi such as *Candida albicans, Trichoderma viride* were selected for antimicrobial activity.

Assay of antimicrobial activity

Antimicrobial activity test was carried out following the modification of the method originally described by Bauer *et al.,* (1966) [11]. Muller Hinton agar for bacteria and Potato Dextrose Agar (PDA) for fungi were prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45ºC. The cooled media was poured onto sterile petriplates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The discs of Whatmann filter paper prepared which had been impregnated with a series of plant extracts (100, 200 and 300 µg/ml) were placed on respective medium. Control and standard (Gentamicin (10 µg) for Bacteria and Ketoconazole (10 µg) for fungi) were also performed in same manner. The bacterial plates were incubated at 37ºC for 24 hrs and fungal plates

were incubated at 28ºC for 72 hrs. After incubation period, the diameter of the zone formed around the paper disc were measured and expressed in mm.

RESULTS AND DISCUSSION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural source. Interest towards traditional natural products has increased on a larger scale [12]. In the traditional system of Ayurvedic treatment, medicines consisting of plant products either single or in combination with others are considered to be less toxic and free from side effects when compared to synthetic drugs [13]. Medicinal plants are plants that have at least one of their parts (leaves, stem fruit have barks or roots) used for therapeutic purpose [14]. In the present study, the medicinal plant *Gmelina asiatica* was selected and subjected to phytochemical screening, FT-IR, HPTLC analysis and *in vitro* antimicrobial activity. The obtained results were discussed in this chapter.

Phytochemicals are natural bioactive compounds found in plants and these are divided into two groups; primary and secondary compounds. These classes perform functions in plant metabolism. Amino acids, sugars, proteins and chlorophyll are known as primary compounds while secondary compounds consists of alkaloids, terpenoids, phenolic compounds etc, [15]. Many of these phytochemicals have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases.

In the present study, phytochemical analysis of bark extract of *Gmelina asiatica* was carried out and result revealed the presence of carbohydrates, coumarins, glycosides, phytosterols, tannins, phenols and absence of alkaloids, anthraquinones, cardiac glycosides, flavonoids, saponins, terpenoids, triterpenoids, phlobatannins and quinines (table 1). The presence of glycosides indicated that the plant has ability in curing cardiac insufficiency, cough and circulatory problems and may acts as good sedatives and have antispasmodic properties [16]. Alkaloids were absent in bark extract which was supported by some other works [5, 17, 18]. Phytosterols was found in the extracts studied which is indirectly and directly inhibit the growth and metastasis of prostate cancer (PC-3) cells [19].

Table1: Qualitative phytochemical screening of bark extract of *Gmelina asiatica*

S.No.	Phytochemicals	Results
1.	Alkaloids	_
2.	Anthraquinones	_
3.	Carbohydrates	+
4.	Cardiac glycosides	_
5.	Coumarins	+
6.	Glycosides	+
7.	Flavonoids	_
8.	Saponins	_
9.	Phytosterols	+
10.	Tannins	+
11.	Terpenoids	_

12.	Triterpenoids	_
13.	Phenols	+
14.	Phlobatannins	_
15.	Quinones	_

+ - indicates presence

- - indicates absence

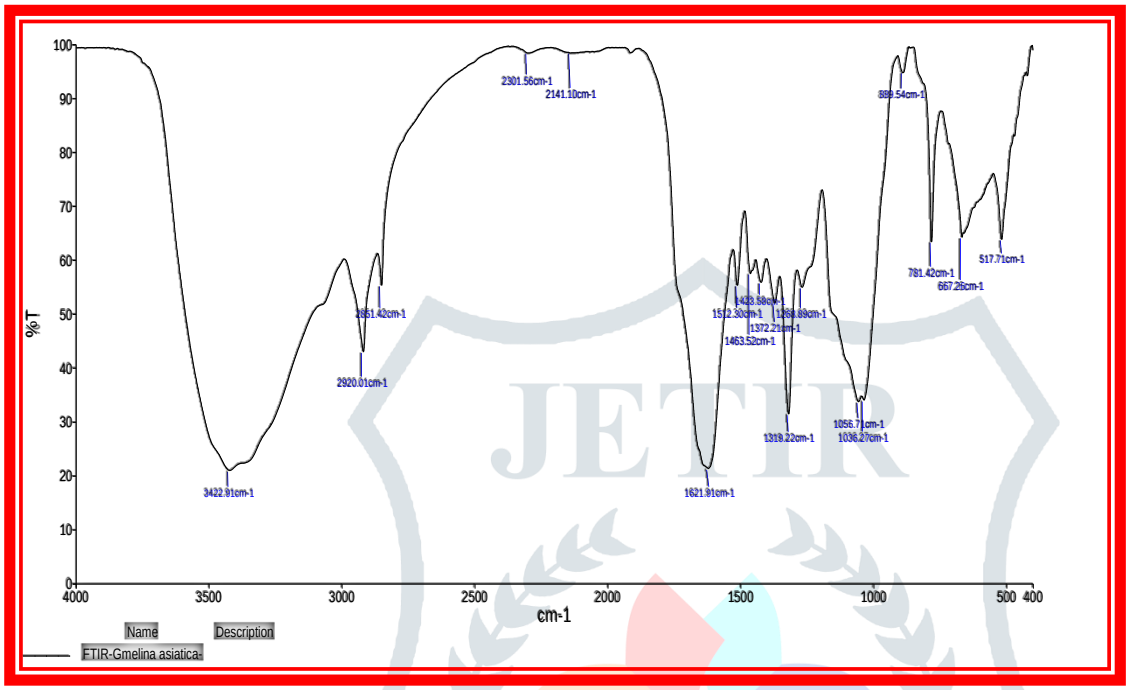
FT-IR spectroscopy has demonstrated to be a reliable and sensitive method for finding out the functional groups present in plant samples which were determined with the help of IR region in the range of 400-4000cm-1. For most common plant compounds, the spectrum of an unknown compound can be identified by comparison to a library of known compounds [20]. In the current study, FT-IR spectrum analysis was carried out in order to determine the functional group present in the bark extract of *Gmelina asiatica* which was shown in table 2 and figure 1. Spectrum profile revealed the presence of functional groups such as alcohol (3422.91, 1056.71), alkane (2920.01, 1463.52, and 617.26), fatty acids (2851.42), alkyne (2130.56), nitrite (2141.10), amide (1621.91), acid (1512.30), nitro (1423.58), amine (1372.21), alkyl halide (1319.22, 781.42, and 517.71), amine (1268.89), ether (1036.27) and alkene (889.54). The bands between 3000 and 2800 cm- 1 represent C-H stretching vibrations that are mainly generated by lipids [21, 22] (Wolkers and Hoekstra, 1995; Wei *et al*., 2009). Florence and jeeva, (2015) [23] reported the presence of functional groups such as carboxylic acids, aldehydes, aromatics, alkenes, phenols or tertiary alcohols, alkanes, aliphatic bromo compounds and alkynes might be responsible for the various medicinal properties of *G. asiatica* leaves.

Table 2: FT-IR spectral analysis of bark extract of *Gmelina asiatica*

S. No.	Peak value	Bond /Stretch	Functional groups
1.	3422.91	O-H Stretch	Alcohol
2.	2920.01	C-H Stretch	Alkane
3.	2851.42	C-H Stretch	Fatty acids
4.	2130.56	C-H Stretch	Alkyne
5.	2141.10	CN Stretch	Nitrile
6.	1621.91	C=O Stretch	Amide
7.	1512.30	C-H Stretch	Acid
8.	1463.52	C-H Stretch	Alkane
9.	1423.58	N-O Stretch	Nitro
10.	1372.21	C-N Stretch	Amine
11.	1319.22	C-F Stretch	Alkyl Halide
12.	1268.89	C-N Stretch	Amine
13.	1056.71	C-O Stretch	Alcohol
14.	1036.27	C-O Stretch	Ether
15.	889.54	=C-H Stretch	Alkene

16.	781.42	C-F Stretch	Alkyl Halide
17.	617.26	=C-H Stretch	Alkane
18.	517.71	C-F Stretch	Alkyl halide

Figure 1: FT-IR spectral analysis of bark extract of *Gmelina asiatica*



HPTLC is playing an important role in today's analytical world. It is used in quality control and in purity checks, in the detection and identification of pharmaceutical raw materials, drugs and their metabolites in biological media. HPTLC method is also a very powerful tool for identification of the presence of adulterants in herbal products based on the characteristic image produced and much useful for determining the presence and the quantification of both inadvertent substitution as well as intentional adulteration of prescription drugs [24].

In HPTLC analysis of present study, the TLC plate was visualized at 254 and 356nm after derivatization (figure 3 and 4). A photograph of a TLC plate after chromatography of quercetin standard and bark extract of *G. asiatica* and the identity of quercetin bands in the sample chromatogram were confirmed by the chromatogram obtained from the sample with that obtained from the reference standard solution (figure 5) and by comparing retention factor of quercetin from sample and standard solution. The calibration graph of quercetin was linear in the range of 100ng to 800ng (Figure 6). The peak corresponding to quercetin from the sample solution had same retention factor as that from quercetin standard (R_f 0.63). The amount of quercetin in bark extract of *Gmelina asiatica* was found to be 0.0640%w/w.

Figure 3: HPTLC Quantification of Quercetin in bark extract of *Gmelina asiatica*

Photo Documentation under UV

AT 254nm

S1 S2 S3 S4 S5 S6 S7 S8 T1 T2 T3 T4 T5

AT 366nm

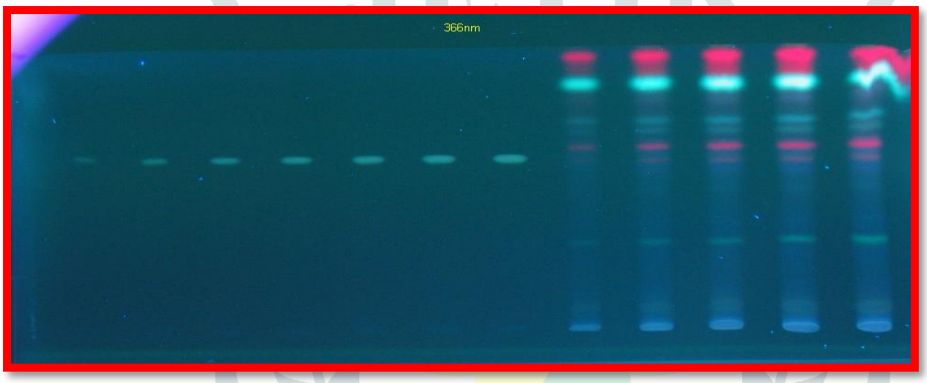


Figure 4:3D Display at 254nm

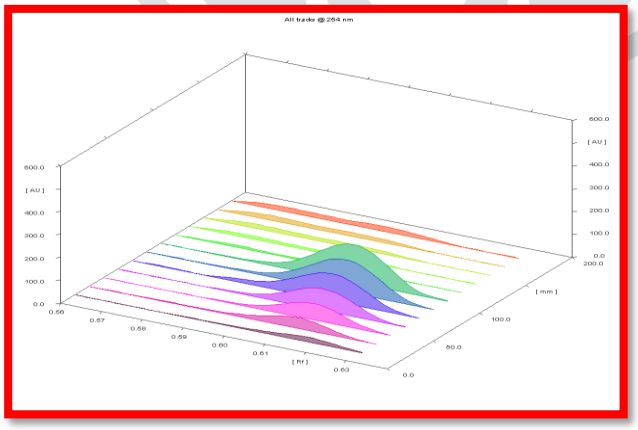
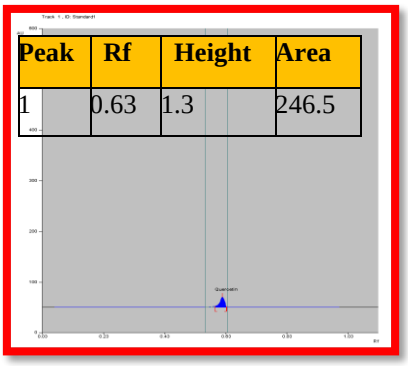


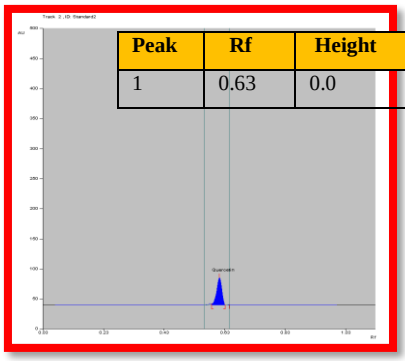
Figure 5: HPTLC chromatogram of standard (different concentrations) and plant extract

1µl



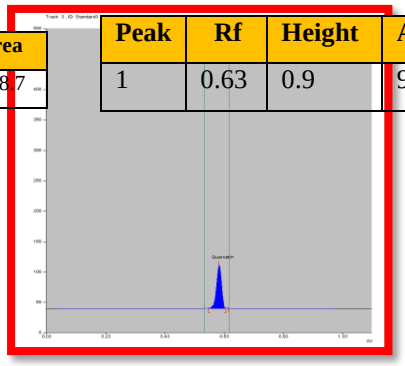
Peak	Rf	Height	Area
1	0.63	1.3	246.5

1.5µl



Peak	Rf	Height	Area
1	0.63	0.0	638.7

2.0µl

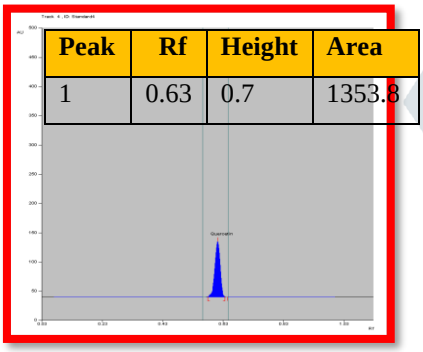


Peak	Rf	Height	Area
1	0.63	0.9	992.8

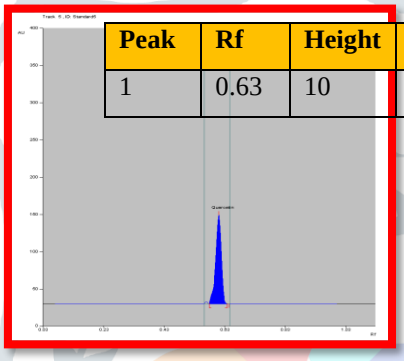
2.5µl

3.0µl

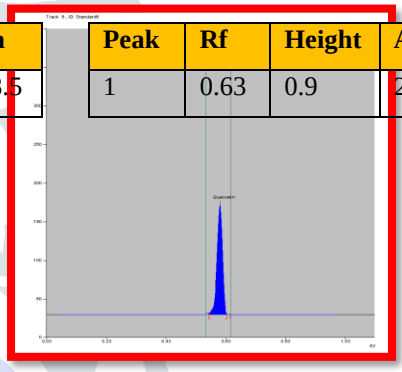
3.5µl



Peak	Rf	Height	Area
1	0.63	0.7	1353.8



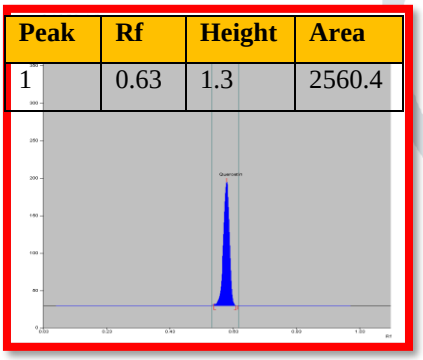
Peak	Rf	Height	Area
1	0.63	10	1888.5



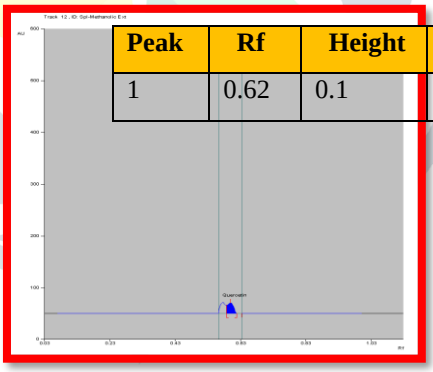
Peak	Rf	Height	Area
1	0.63	0.9	2187.8

4.0µl

***Gmelina asiatica* (25µl)**

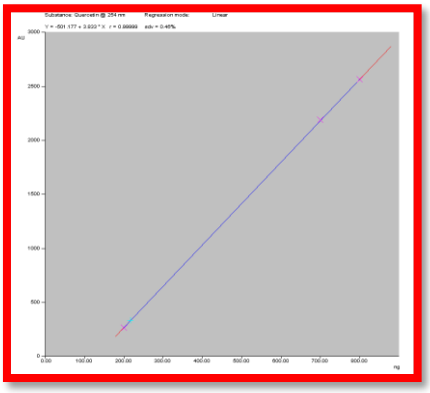


Peak	Rf	Height	Area
1	0.63	1.3	2560.4



Peak	Rf	Height	Area
1	0.62	0.1	332.4

Figure 6: Calibration plot obtained by chromatography of marker compound



Human infections particularly those involving microorganism i.e. bacteria, fungus, viruses; they causes serious infections in tropical and subtropical countries of the world. In recent years, multiple drug resistance in human pathogenic microorganism has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of such diseases [25, 26]. Plants are the richest source of natural antimicrobial agents. Traditional healers claim that some medicinal plants are more efficient to treat infectious diseases than synthetic antibiotics [27]. Phytochemicals are one of the alternatives for the control of these antibiotic resistant human pathogens [28].

Hence in the present study, bark extract of *Gmelina asiatica* was subjected to analyse its antimicrobial activity against selected pathogenic bacteria such as *Escherichia coli*, *Bacillus subtilis*, *Staphylococus aureus*, and *Pseudomonas aeruginosa* and pathogenic fungi such as *Candida albicans* and *Trichoderma viride* by disc diffusion method.

Three different concentrations (100,200, and 300µg/ ml) of plant extracts were selected, for antibacterial activity which were found to be effective against both gram positive and gram negative bacterial strains (table 3 and plate 1). Maximum zone of inhibition was observed in 300µg of plant extract against *Pseudomonas aeruginosa* (22.3mm), followed by *Bacillus subtilis* (19mm), *E.coli* (9.6mm), and *Staphylococcus aureus* (9mm). Moderate results were obtained in 200µg of plant extract against *Pseudomonas aeruginosa* (21.3mm), followed by *Bacillus subtilis* (13.3mm), *E.coli* (8.6mm), and *Staphylococcus aureus* (8.3mm) and least activity was observed in 100µg of plant extract against *E.coli* (14.3mm), followed by *Bacillus subtilis* (14mm), *Staphylococcus aureus* (13mm), and *Pseudomonas aeruginosa* (10mm).

Antibacterial activity of standard antibiotic ampicillin was also performed and the maximum zone of inhibition were 18 mm for *E.coli*, followed by 17.3 mm for *B.subtilis*, 14.6mm for *S.aureus* and 8 mm for *P.aeruginosa.* Antibacterial activity of plant extract was highest in *Pseudomonas aeruginosa* and *Bacillus subtilis* and moderate activity was observed in *E.coli* and *Staphylococcus aureus* when compared to antibiotic ampicillin. The results shows in agreement with previous study which indicated that plant extracts were more active against Gram positive bacteria than those of Gram negative bacteria [17, 29]. Different species of plants influence its activity against microbe tested due to the difference microbe cell wall compound [30].

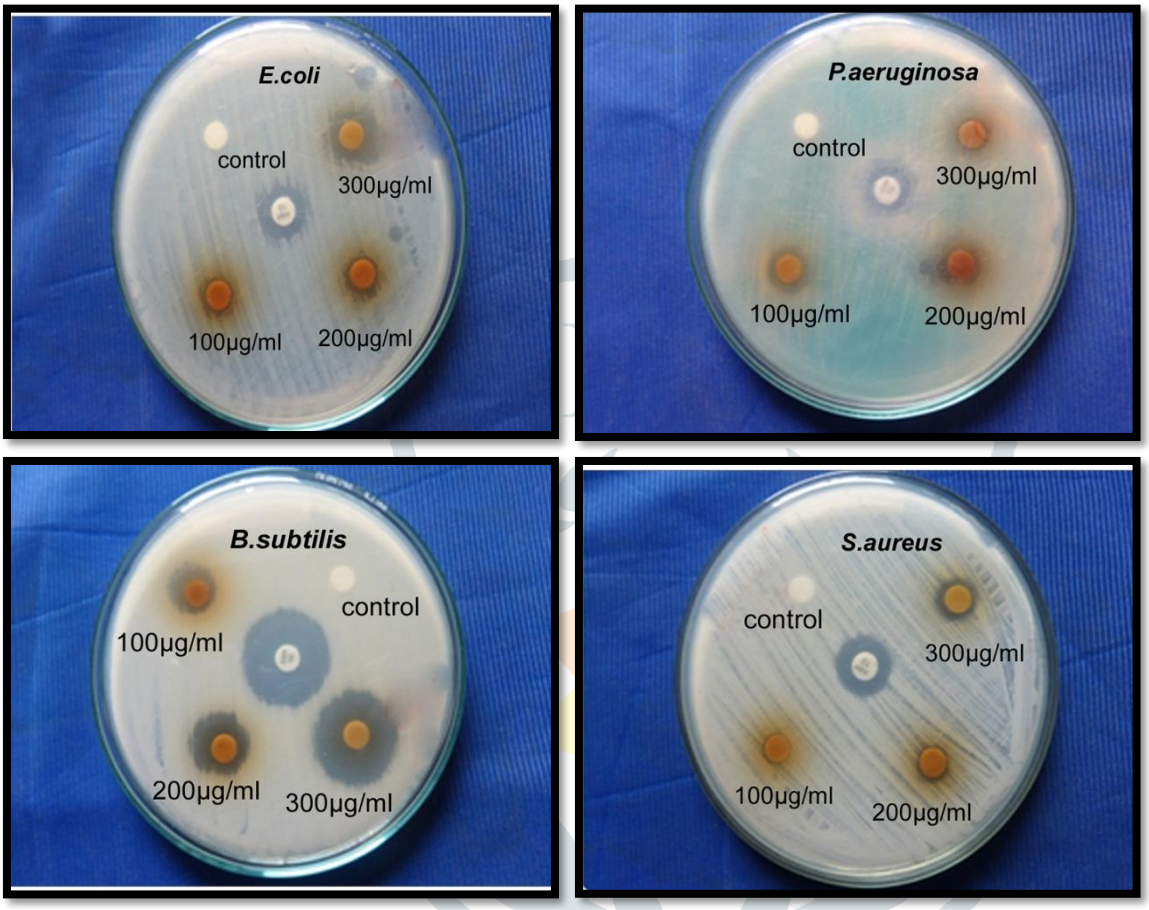
Table 3: Antibacterial activity of bark extract or *Gmelina asiatica*

S.No.	Bacterial strains	Zone of Inhibition (mm in diameter)			
		Plant extract (µg/ml)			Ampicillin (10 µg)
		100	200	300	
1.	*Escherichia coli*	14.3±1.69	8.6±1.88	9.6±1.69	18±0.82

2.	*Pseudomonas aeruginosa*	10±4.08	21.3±2.05	22.3±8.05	8±1.63
3.	*Bacillus subtilis*	14±1.63	13.3±0.82	19±6.18	17.3±5.25
4.	*Staphylococcus aureus*	13±0.82	8.3±1.69	9±0.82	14.6±3.68

All values are expressed as mean± SD for three determinations

Plate 1: Antibacterial activity of bark extract or *Gmelina asiatica*



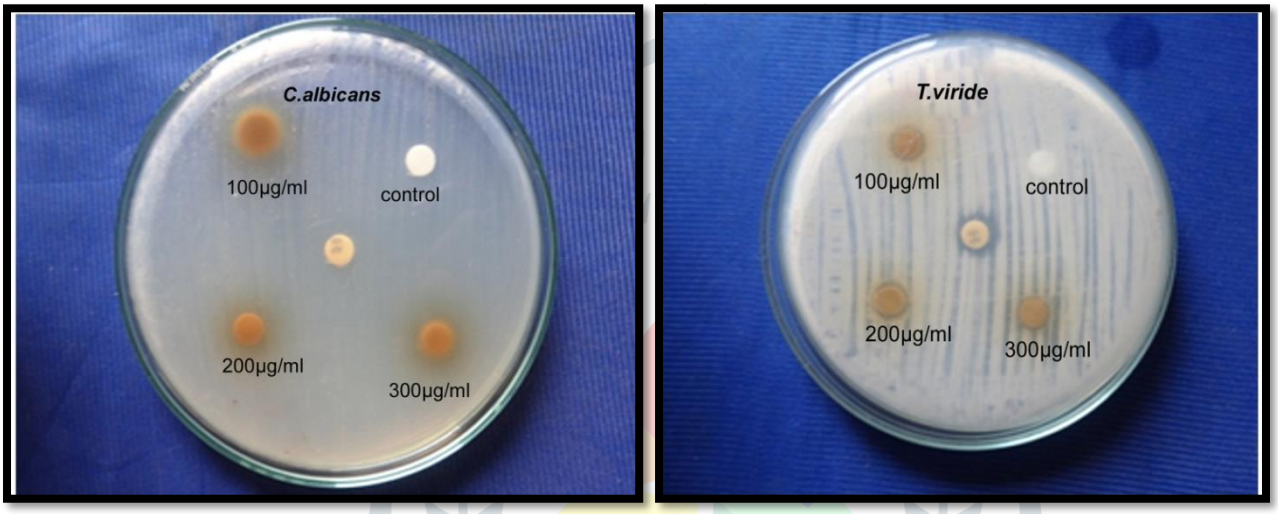
Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants [31]. Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings. In the present study, *in vitro* antifungal activities of three different concentration of (100,200,300 µg) bark extract of *Gmelina asiatica* have been evaluated against fungal strains *Trihoderma viride* and *Candida albicans* (table 4; plate 2). Maximum zone of inhibition was observed in 300 µg of plant extract against *Trichoderma viride* (19.6mm) followed by *Candida albicans* (14 mm). Moderate results were obtained in 200 µg of plant extract in the order of *Trichoderma viride* and *Candida albicans* and their zone of inhibition in mm were 12.3 and 11 respectively. Similarly, least result of 12.3 and 11mm was observed in 100 µg of plant extract against *Trichoderma viride* and *Candida albicans*. Standard antibiotic was also performed for its antifungal activity and the inhibitory zone which were 20.3 mm for both *Candida albians* and *Trichoderma viride.*

Table 4: Antifungal activity of bark extract or *Gmelina asiatica*

S.No.	Fungal strains	Zone of Inhibition (mm in diameter)			
		Plant extract (µg/ml)			Ketoconazole (10 µg)
		100	200	300	
1.	*Candida albicans*	11±0.82	11±0.82	14±0.82	20.3±1.25
2.	*Trichoderma viride*	12.3±2.05	13.6±1.25	19.6±6.85	20.3±1.25

All values are expressed as mean± SD for three determinations

Plate 2: Antifungal activity of bark extract or *Gmelina asiatica*



Plant extract exhibited moderate antifungal activity against all tested pathogenic fungi when compared with antibiotic. Among the two studied pathogens, maximum antifungal activity of plant extract was observed in *Trichoderma viride* followed by *Candida albians*. Our current study aqueous bark extract exhibited remarkable antifungal activity against *Candida albicans.* Our study correlated with previous report [32]. Present study indicated the studied plant part is an important source of antimicrobial compound that provide a vital sources of useful antifungal drugs against infections in humans. Results obtained from this study, indicated that, the plant extracts showed the strongest antimicrobial activity.

CONCLUSION

From the above results we can conclude bark extract of *Gmelina asiatica* has remarkable antimicrobial activity as compare to antibiotic activity which might be due to the presence of flavonoids like quercetin. Thus this plant could be utilized as an alternative source of useful antimicrobial drugs. Further studies are needed to isolate, characterize the lead compound of this plant for antimicrobial drug formulation.

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