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Antibacterial efficacy of some Indian medicinal plants against human commensal pathogens

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Abstract

Background and Aim: The present study aimed to evaluate the antibacterial efficacy of the total flower extracts of five medicinal plants widely used in folk medicine in India, against some Gram positive and Gram negative bacteria. **Methodology:** The present work designed to assess the *in vitro* antibacterial activities of five medicinal plants (flowers of *Bauhinia purpurea, Clitoria ternatea*, *Millingtonia hortensis*, *Nyctanthes arbortristis* and *Combretum indicum*) against panel of Gram positive bacteria and Gram negative bacteria using disk diffusion assays. **Results:** Among the plants screened, *Combretum indicum* showed effective and broad spectrum antibacterial activity with zones of inhibition ranging from 10.33±0.57 to 23.33±1.52mm. Among the other four plants, only *Clitoria ternatea* showed moderate antibacterial activity at high concentration. The other three did not show any antibacterial activity. The phytochemical analysis of the active crude extract from *C. indicum* revealed the presence of steroids, terpenoids, phenols, flavonoids, quinonoids, alkaloids, glycosides and saponins. Moreover, *C. indicum* showed the highest activity against the tested pathogens with minimum inhibitory concentration (MICs) values ranging from 125-1000µg/mL. The qualitative chemical characterization by using high performance liquid chromatography (HPLC) revealed the presence of compounds such as beta-sitosterol, gallic acid, lupeol, rutin and quercetin which could be responsible for its significant broad spectrum activity. **Conclusion:** The results of this study suggest that the *C. indicum* flower extract could be probed further for possible use to control bacterial infections.

***Keywords*:** Antibacterial; *Combretum indicum;* bioactive; phytochemicals

1. Introduction

The increase in bacterial resistance to drugs and the rapid emergence of new infections are posing a great challenge to treat pathologies caused by certain microorganisms. Microbial infectious agents create huge health-hazards to populations where they cause high morbidity and mortality[1-4]. The persistent use of common antibiotics leads to the development of drug resistant pathogenic bacteria; this is globally considered a major medical problem and thus leading to big threat to human society[5]. This situation evokes an urgent need for the development of new antibacterial agents, preferably from natural sources [6,7].

Natural products are considered important sources of therapeutic agents against bacterial and fungal diseases, cancer, lipid disorders and immunomodulation[8-11]. From the beginning of human existence, plants have been used for medicinal purposes and are the primary source of phytochemicals present in conventional medicaments. Ethnobotanical studies have described and explained the relationships between cultures and the traditional use of plants. These studies are of great importance and provide essential information that allows the development of scientific research more oriented to explore and prove the therapeutic potential of plants.

Plants are utilized as therapeutic agents since time immemorial in both organized (Ayurveda, Unani) and unorganized (folk, tribal, native) form [12]. Therefore, it is worthwhile to search for such compounds from plant resources showing potent antibacterial activity against

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pathogenic organisms. The search for new antibacterial compounds which have different mechanisms of action from those in current use is an alternative way for solving this problem. Keeping the above mentioned importance of medicinal plants in view, the current study was undertaken to evaluate the broad spectrum antibacterial activity of total flower extracts of five medicinally important plant species belonging to Bignoniaceae (*Millingtonia hortensis*), Combretaceae (*Combretum indicum*), Fabaceae (*Bauhinia purpurea* and *Clitoria ternatea*), Oleaceae (*Nyctanthes arbortristis*) families.

2. Methodology

Plant material

The flowers of *Bauhinia purpurea, Clitoria ternatea, Millingtonia hortensis*, *Nyctanthes arbortristis* and *Combretum indicum* were collected from in and around Chennai, Tamilnadu, India. The plants used in the study were identified by Dr V. Duraipandiyan, and the voucher specimens (No.ERI/ETHPH/TA236-240) were deposited at the herbarium of Entomology Research Institute, Loyola College, Chennai for future reference.

Preparation of methanolic extract

The dried flowers of the plants (100g) were macerated individually and soaked in 500mL methanol separately and extracted three times by cold percolation method of Edeoga et al. (2005)[13]. The filtrates were concentrated under reduced pressure at 50ºC. The yields of the crude methanolic extracts were: *Bauhinia purpurea* (7.05g), *Clitoria ternatea* (5.63g), *Millingtonia hortensis* (8.05g), *Nyctanthes arbortristis* (10.00g) and *Combretum indicum* (9.90g). The extracts were stored in a refrigerator at 4–8 ºC for use in subsequent experiments.

Antibacterial activity assays

Test Microbes

Gram positive bacteria such as *Staphylococcus aureus* MTCC 96, *Micrococcus luteus* MTCC 106, *Bacillus subtilis* MTCC 441, *Staphylococcus epidermis* MTTC 3615 and Methicillin resistant *staphylococcus aureus* (MRSA) and Gram negative bacteria such as *Klebsiella pneumoniae* MTCC 109, *Enterobacter aerogenes* MTCC 111, *Vibrio parahaemolyticus* MTCC 451, *Yersinia enterocolitica* MTCC 840, *Shigella flexneri* MTCC 1457, *Proteus vulgaris* MTCC 1771 and *Salmonella typhimurium* MTCC 1251 were used as test pathogens. The reference bacterial cultures were obtained from the Institute of Microbial Technology (IMTECH), Chandigrah, 160 036, India.

Preparation of inoculums

Bacterial inoculums were prepared by growing cells in Muller Hinton broth (MHB) Himedia for 24h at 37 ºC.

Antimicrobial activity

The *in vitro* antibacterial susceptibility of the total flower extracts was tested by disc diffusion method[14]. 25 mL of agar medium was poured into the plates to obtain uniform depth and was allowed to solidify. Briefly, 0.1mL (1×10^5 CFU/mL) of standard inoculum suspension was swabbed on the freshly prepared Muller Hinton agar to ensure the confluent growth of the organism and the plates were allowed to dry for 5 min. After drying, the discs with concentrations of 5.0, 2.5 and 1.25mg/disc were placed on the surface of the plate with sterile forceps and gently pressed to ensure contact with the inoculated agar surface. Streptomycin was used as positive control; 5 % DMSO was used as a solvent control. Finally the inoculated plates were incubated overnight at 37ºC. The inhibition zones and the diameter of the discs were measured in millimeters. All the experiments were done in triplicates and the results were presented as mean±SD.

Phytochemical analysis

Primary qualitative phytochemical analysis of the active extract was done according to standard procedure. Analysis of some phytochemicals such as steroid, terpenoid, phenol, flavonoid, quinonoid, alkaloid, glycoside, and saponin were done[13].

High performance liquid chromatography (HPLC)

The active antibacterial compounds of *C. indicum* methanolic extract was carried out on a Waters Alliance 2695 separations Module with photodiode array detector following the method of Raj et al., (2012)[15]. The HPLC profile of *C. indicum* methanol extract was compared with that of standard compounds, beta-sitosterol, lupeol gallic acid, rutin and quercetin.

Determination of minimal inhibitory concentration

The evaluation of MIC was performed for the active extract of *C. indicum*. It was tested using the micro-dilution methodology described by the Clinical and Laboratory Standards Institute (CLSI, 2007)[16]. The bacterial cell number was adjusted to approximately $1x10^5$ CFU (colony forming unit)/mL (0.5 on the McFarland scale). The crude methanolic extract (1000µg/mL) and standard drug streptomycin 50µg/mL) were serially two-fold diluted to obtain the following concentrations (µg/mL): 1000, 500, 250, 125, 62.5, 31.3, and 15.6. They were added to 96 well micro-titer plate containing 0.1 ml broth. The 3mL of log phase culture was introduced into respective wells and the final inoculum size was $1x10^5$cfu/mL. The plates were

incubated at 37°C for 18h. Negative and solvent controls (DMSO) were also included. Streptomycin was included in the assay as positive control. 5mL of the test broth was introduced on plain Mueller Hinton agar plates to observe the viability of the organism. MIC was determined as the lowest concentration which inhibited complete growth [17].

3. Results

The plant based traditional medicine system continues to play an essential role in health care; about 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care. Many reports are available on the antiviral, antibacterial, antifungal, antihelmintic, antimolluscal and anti-inflammatory properties of plants based molecules[18-24]. The continuous development of antibiotic resistant microbial pathogens, such as methicillin resistant, penicillin-resistant and vancomycin resistant strains, is a growing problem and it is therefore extremely important to discover and develop new antibacterial compounds with no side effects[25]. There is an ever increasing demand for plant based therapeutics in both developing and developed countries due to a growing recognition that they are natural products, non-narcotic and in most cases, easily available at affordable price with no side effects.

The purpose of this study was to investigate the antibacterial potential of the crude methanolic flower extracts of *B. purpurea* (Leguminosae), *C. ternatea* (Fabaceae), *M. hortensis* (Bignoniaceae), *N. arbortristis* (Oleaceae) and *C. indicum* (Combretaceae) which have been used for thousands of years in traditional medicine in India to treat various infections and ailments. Based on traditional knowledge, the plants materials were extracted only with methanol for better solubility of bioactive phytoconstituents having antibacterial activity[26].

In the initial screening 20% of the plant extracts showed activity against *S. aureus* MTCC 96, *M. luteus* MTCC 106, MRSA, *K. pneumoniae* MTCC 109, *E. aerogenes* MTCC 111, *Y. enterocolitica* MTCC 840, *S. flexneri* MTCC 1457, *P. vulgaris* MTCC 1771 and *S. typhimurium*; 40% of the plant extracts showed activity against *B. subtilis* MTCC 441 and *S. epidermis* MTTC 3615 and 60% of the plant extracts showed activity against *V. parahaemolyticus* MTCC 451.

Among the plants screened *C. indicum* showed significant antibacterial activity with the zones of inhibition ranging between 10.33±0.57 to 23.33±1.52mm against the tested Gram positive and Gram negative bacterial pathogens at 5, 2.5 and 1.25 mg/disc concentrations. At 5mg/disc concentration the total extract of *C. indicum* registered the highest inhibition zone of 23.33±1.52mm against *M. lutues* followed by 21.66±1.15mm against *S. flexneri*, 15.00±1.00 against *S. typhimurium*, 13.66±1.52mm against *S. aureus,* MRSA, *E. aerogens*, 12.00±1.00mm against *B. subtilis, S. epidermis, K. pneumonia, P. vulgaris*, 11.66±0.57mm against *V. parahaemolyticus* and lowest zone of inhibition 10.33±0.57mm was recorded against *Y. enterocolitica*. The extract of *C. indicum* showed varying degrees of antibacterial activity depending on the strains and also largely depending upon the concentrations of the extract (**Table 1**). [27]Kumar et al. (2015) reported that the methanol extracts of *C. indicum* at the concentration range of 100µL/well inhibited the growth of selected Gram positive and Gram negative bacterial pathogens with zones of inhibition in the range of 20-15 mm but in this study, *C. indicum* showed better activity at the tested concentrations and the results were also compared with standard drug streptomycin. This variation of antibacterial activity could be due to the environmental factors and strains tested. However, *C. ternatea* showed weak activity and other extracts from *B. purpurea*, *M. hortensis*, and *N. arbortristis* showed no antibacterial activity against the tested pathogens. The significant antibacterial activity of *C. indicum* encouraged us to determine the presences of various phytoconstituents which are responsible for their broad spectrum antibacterial activity. The qualitative phytochemical analysis revealed the presence of alkaloids, flavonoids, glycosides/sugars, quinonoids, phenols/tannins, saponins, steroids and terpenoids (**Table 2**)[28].

The antibacterial activity was further validated by determining its minimum inhibitory concentration (MIC) using broth dilution assay. The results of MIC are represented in Table 3. The methanol extract of *C. indicum* exhibited activities depending on bacterial strains with the MIC values in the range of 125-1000µg/mL. The extract exhibited significant activity against *M. lutues* and *S. flexneri* with the MIC value of 125µg/mL followed by MRSA, *S. typhimurium* (250µg/mL), *S. aureus, B. subtilis*, *S. epidermis* and *K. pneumonia* (500µg/mL), *E. aerogenes*, *V. parahaemolyticus*, *Y. enterocolitica* and *P. vulgaris* (1000µg/mL). This activity of *C. indicum* extract further encouraged us to investigate the bioactive constituents using two different monitoring wavelengths namely 210nm for beta-sitosterol and lupeol and 254nm for gallic acid, rutin and quercetin. The qualitative fingerprint chromatograms of the methanolic flower extract with standards of beta-

Table I: Antibacterial activity of methanol extracts of some medicinal plants

Bacteria	*C. indicum* (MeOH)			*C. ternatea* (MeOH)			Streptomycin
Gram positive	**5mg/disc**	**2.5mg/disc**	**1.25mg/disc**	**5mg/disc**	**2.5mg/disc**	**1.25mg/disc**	**(25µg/disc)**
S. aureus	13.00 ± 1.00	10.66 ± 0.57	10.33 ± 0.57	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	17.66 ± 1.15
M. lutues	23.33 ± 1.52	18.33 ± 0.57	14.66 ± 1.15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	19.33 ± 0.57
B. subtilis	12.33 ± 0.57	10.33 ± 0.57	10.33 ± 0.57	10.33 ± 0.57	0.00 ± 0.00	0.00 ± 0.00	20.66 ± 1.52
S. epidermis	12.00 ± 1.00	10.66 ± 1.15	0.00 ± 0.00	10.33 ± 0.57	0.00 ± 0.00	0.00 ± 0.00	18.66 ± 1.52
MRSA	13.66 ± 1.52	11.66 ± 1.15	10.33 ± 0.57	11.66 ± 1.15	0.00 ± 0.00	0.00 ± 0.00	22.33 ± 1.52
Gram Negative							
K. pneumoniae	12.33 ± 0.57	10.66 ± 0.57	10.33 ± 0.57	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	31.33 ± 1.15
E. aerogens	13.00 ± 1.00	11.33 ± 0.57	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	13.00 ± 1.00
V. parahaemolyticus	11.66 ± 0.57	10.66 ± 0.57	11.00 ± 1.00	10.33 ± 0.57	0.00 ± 0.00	0.00 ± 0.00	22.00 ± 1.00
Y. enterocolitica	10.33 ± 0.57	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	22.00 ± 1.00
S. typhimurium	15.00 ± 1.00	13.00 ± 1.00	11.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	24.33 ± 0.57
S. flexneri	21.66 ± 1.15	17.33 ± 1.15	13.00 ± 1.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	10.33 ± 0.57
P. vulgaris	12.66 ± 1.15	10.33 ± 0.57	11.33 ± 1.15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	22.00 ± 1.00

sitosterol (Fig.1a), gallic acid (Fig.1b), lupeol (Fig.1c), rutin (Fig.1d) and quercetin (Fig.1e) were successfully characterized based on retention time (Fig. 2a and 2b). Our results are in agreement with the previous report of Bairagi et al. (2012)[29]. The comparative standard extract

Table II: Antibacterial activity of methanol extracts of some medicinal plants

Class of compound	*C. indicum*
Steroid	+
Terpenoid	-
Phenol/Tannin	+++
Flavonoid	+++
Quinonoid	+
Alkaloid	++
Glycosides/Sugars	+
Saponin	+

+++; Strongly positive, ++; moderately positive, +; weakly positive, -; Negative

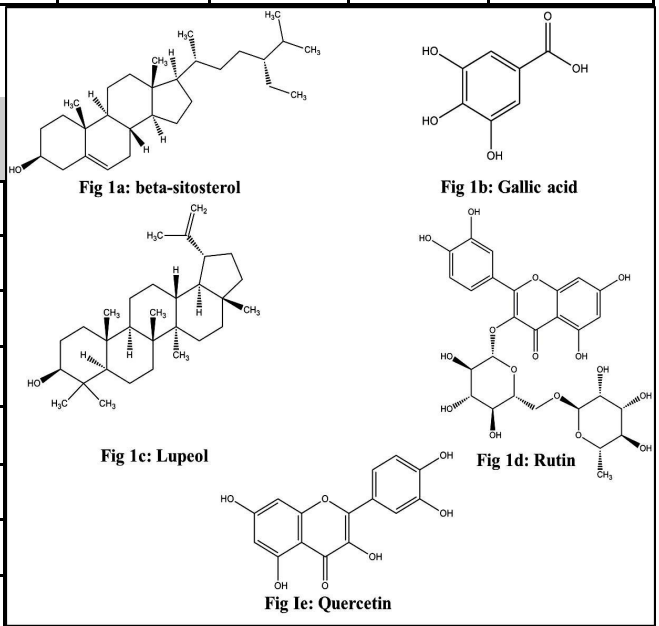

Fig 1a: beta-sitosterol

Fig 1b: Gallic acid

Fig 1c: Lupeol

Fig 1d: Rutin

Fig 1e: Quercetin

Fig I (a-e) : Compounds detected in methanolic flower extract

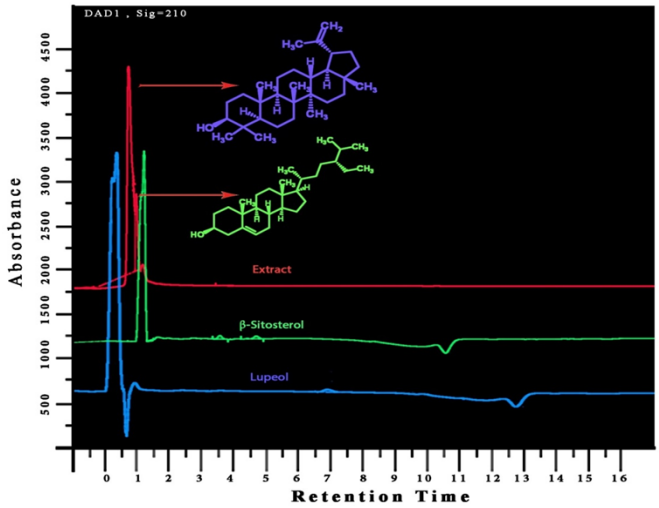

Fig 2a: Qualitative HPLC chromatogram of the active extract of the flowers of *C. indicum* with the standards of beta-sitosterol and lupeol HPLC

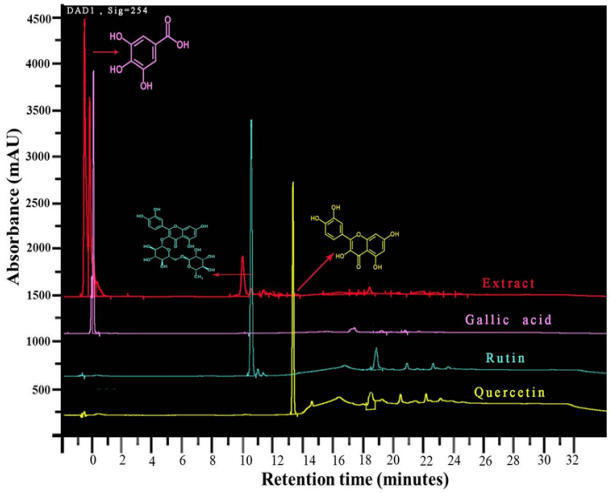

Fig 2b: Qualitative HPLC chromatogram of the active extract of the flowers of *C. indicum* with the standards of gallic acid, rutin and quercetin

Bacteria	*C. indicum*	Streptomycin
Gram positive		
S. aureus	>500 µg/mL	>6.25µg/mL
M. lutues	>125 µg/mL	>6.25µg/mL
B. subtilis	>500 µg/mL	>6.25µg/mL
S. epidermis	>500 µg/mL	>6.25µg/mL
MRSA	>250 µg/ml	>25µg/ml
Gram negative		
K. pneumoniae	>500 µg/mL	>25µg/mL
E. aerogens	>1000 µg/mL	>25 µg/mL
V. parahaemolyticus	>1000 µg/mL	>6.25 µg/mL
Y. enterocolitica	>1000 µg/mL	>30µg/mL
S. typhimurium	>250 µg/mL	>30 µg/mL
S. flexneri	>125 µg/mL	>30µg/mL
P. vulgaris	>1000 µg/mL	>25 µg/mL

Table III: Minimum inhibitory concentrations of the crude extract of *C. indicum*

analysis demonstrated the presence of various bioactive phytochemicals in the investigated extract which was responsible for antibacterial activity.

4. Conclusion

The present study highlights the potential of methanolic flower extract of *Combretum indicum* in the treatment of bacterial infections. The qualitative analysis of the active extract revealed the presence of major phtyochemicals including beta-sitosterol, lupeol, gallic acid, rutin and quercetin confirming their major role in antibacterial activity. This antibacterial potential of this extract may contribute to support the traditional use against bacterial infections and this extract can be probed further for pharmaceutical use at the industrial scale.

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Conflict of interest

The author's declares none.

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