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Screening of antimicrobial activity of flower extracts on human bacterial pathogens

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Abstract
Flowers are the wonderful gift of nature and act as a refresher and soothing agents. So they are used in Naturopathy and Aromatherapy. The present study deals with examining the antibiotic property of different selected flowers available in the winter season are *Michelia champaka, Michelia Alba, Nyctanthes arbor-tristis, Jasminum sambac, Jasminum trifoliatum, Artabotrys hexapetalus, Couroupita guianensis* and *Cananga odorata.* Various human bacterial pathogens were examined for sensitivity against flower extracts. Finally, activities of flower extracts were compared with the microbicidal activity of Ampicillin.

Keywords: *flower extract, Naturopathy, microbicidal activity.*

1. Introduction
Many of the plants used today were well known to the people of ancient cultures throughout the world and they were valued for their preservation and medicinal powers [1, 2]. The medicinal plants used as medicine were considered to be important due to a large number of factors such as unavailability, expensiveness and unwanted effects of the western medicines [3, 4]. The antimicrobial properties of plants have been investigated by a number of researchers worldwide [5, 6]. Since antiquity, plants have been used to treat common infectious diseases, the healing potential of many plants have been utilized in Indian traditional medicines like siddha, ayurveda and unani medicine [7]. Being nontoxic and easily affordable, there has been a resurgence in the consumption and demand for medicinal plants [8]. The therapeutic efficacies of many indigenous plants, for various diseases have been described by traditional herbal medicine practitioners [9].
In the past decade, there has been considerable change in opinion, regarding ethno-pharmacological therapeutic applications [10, 11, 12]. Presence of various life sustaining constituents in plants made researchers to have a view to determine potential wound healing properties [13, 14]. Extracts from the dried or wet flowers and leaves of plants are applied as a paste on wounds in some rural communities [15, 16]. The fresh juice of flowers for e.g., *Catharanthus roseus* was being added in the preparation of tea and also been used by Ayurvedic physicians in India for external use to treat skin problems such as dermatitis, eczema and acne [14, 17, 18]. With the rising prevalence of microorganisms showing resistance to antibiotics, there is an urgency to develop new antimicrobial compounds. So, the present study was to investigate the antimicrobial properties of the inflorescence [19].

2. Methodology
There are many parts of plants which give a variety of anti-microbial agents. Most of the studies are focused on root, leaves, and stems' extracts, but the present study was focused on garden-fresh and raw aromatic flowers available in winter season in and around Visakhapatnam region of Andhra Pradesh.
As a part of the work, eight aromatic flowers are collected during their blossoming time [20]. About 9 microorganisms; *Salmonella, Staphylococcus, Pseudomonas, Vibrio cholera, Streptococcus, Corynebacterium, Enterobacter aerogenes, Proteus mirabilis, Proteus vulgaris* and *Escherichia coli* were tested for their sensitivity towards the flower extracts and these pathogens were isolated from human specimens by following specific isolation techniques. The results were compared against the selected standard antibiotic-Ampicillin.

Table 1: Showing Plant scientific name, local name and sample code

S. No.	Plant Scientific Name	Common/ Local Name	Sample Code
1	*Michelia champaka*	Sampangi	P1
2	*Michelia alba*	Tella sampangi	P2
3	*Nyctanthes arbortristis*	Parijatham	P3
4	*Jasminum sambac*	Malli	P4
5	*Jasminum trifoliatum*	Sannajaji	P5
6	*Artabotrys hexapetalus*	Phala sampangi	P6
7	*Couroupita guianensis*	Nagamalli	P7
8	*Cananga odorata*	Aaku sampangi	P8

Michelia champaka

Michelia alba

Nyctanthes arbortristis

Jasminum sambac

Jasminum trifoliatum

Artabotrys hexapetalus

Couroupita guianensis

Cananga odorata

Fig 1: Showing selected flower species

2.1 Preparation of flowers' extracts

Sterile screw cap test tubes carrying 10 ml of n-butanol each were prepared. They are aseptically carried to the sample collection area. Flowers are plucked during their blossoming time (note: blossoming time varies with each flowering tree) and 5 g of each variety of flowers are weighed and directly suspended in 10 ml of n-butanol solvent in screw cap tube. Samples are transported to the laboratory and stored for extraction of compounds for 5 days at room temperature [21]. Butanolic extracts were collected by filtering the material using sterile cheese cloth [22]. Collected extracts were used for testing the antibacterial activity.

2.2 Agar diffusion test (Kirby–Bauer method)

The antimicrobial activity of flower extracts was identified by following Agar well diffusion method [23, 24, 25]. Microbial growth was determined by measuring the diameter of the zone of inhibition. For each bacterial strain, controls were maintained where pure solvent (n-butanol) was used instead of the extract. Occurrence of zone of inhibition around the wells is an indicator of sensitivity. The report can be attained by measuring the diameter of the zone of inhibition. The experiment was done thrice and the mean values were represented. Final report should be purely of zone of inhibition produced by extract which is obtained by subtracting zone of inhibition by control from zone formed by test sample against respective microorganism.

3. Results and Discussion

Identities of the test bacterial isolates were confirmed through biochemical characterization for catalase, indole production, Voges-Proskauer, methyl red, citrate utilization, H2S production and oxidase (Table 1).

Table 2: Identification of bacterial isolates by conventional biochemical tests

Organism	Cat	OX	IP	MR	VP	CU	HSP	Motility	Specific test
Salmonella typhi	-	-	-	+	-	-	+	**motile**	SIM test
Staphylococcus aureus	+	_	-	+	+	-	-	-	Coagulase
Pseudomonas	+	+	-	-	-	+	-	+	Pyocyanin Pigmentation
Vibrio cholera	+	+	+	+	-	+	-	+	Ornithinase
Streptococcus pyogenes	-	-	-				-	-	PYR test
Corynebacteria	+	-	-	-	-	-	-	-	Tellurite test
E. aerogenes	-	-	-	-	+	+	-	+	growth on EMB agar
Proteus vulgaris	-	-	+	+	-	-	+	+/-	PPA test
Escherichia coli	+	-	+	+	-	-	-	+	growth on EMB agar

Here, Cat = Catalase, IP= Indole Production, VP = Voges-Proskauer, MR = Methyl Red, CU = Citrate Utilization, HSP = H2S production, OX = Oxidase, + sign denotes positive and – sign denotes negative.

Table 3: Antibacterial effects of the flowers extracts collected during blossoming

S. No.	Microorganism	zone of inhibition in mm							
		P1	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**
1	*Salmonella typhi*	32	27	18	21	22	25	26	29
2	*Staphylococcus*	26	17	-	-	15	18	22	20
3	*Pseudomonas*	-	18	-	15	20	15	18	34
4	*Vibrio cholera*	28	21	8	18	18	25	20	22
5	*Streptococcus*	25	19	20	19	17	18	16	23
6	*Corynebacteria*	27	21	-	16	14	23	-	27
7	*E. aerogenes*	19	-	18	15	-	-	15	24
8	*Proteus vulgaris*	23	15	-	18	18	20	15	22
9	*Escherichia coli*	20	23	16	16	21	18	23	25

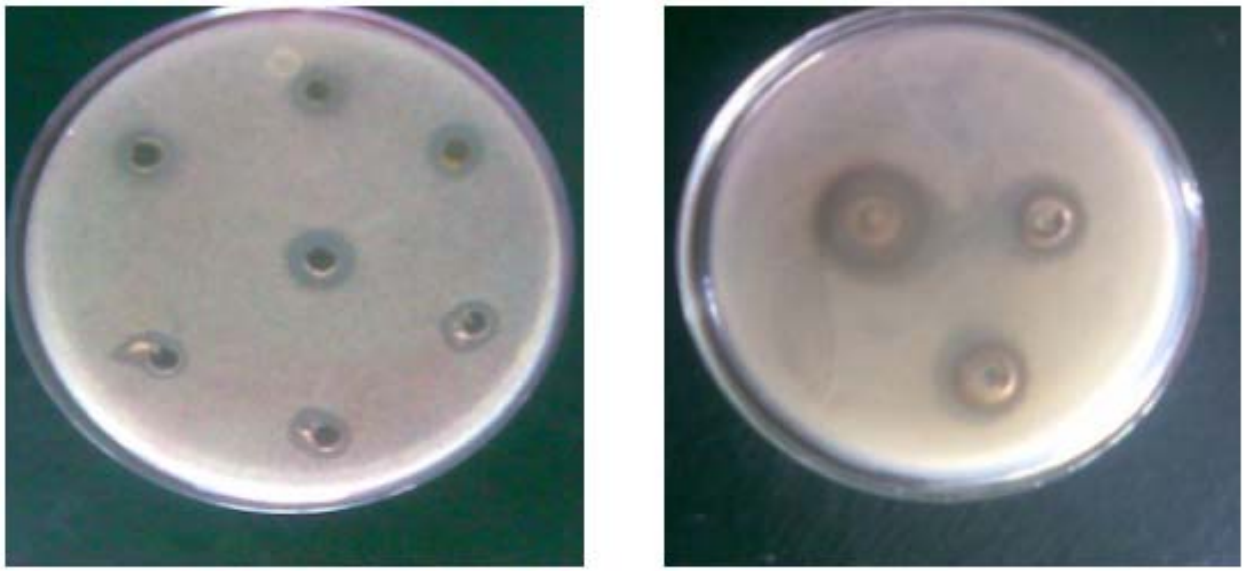

Fig 2: Zone of inhibition in mm (Kirby–Bauer method)

Table 4: Antibacterial effects of the flowers extracts collected after complete blossoming

S. No.	Microorganism	zone of inhibition in mm							
		P1	**P2**	**P3**	**P4**	**P5**	**P6**	**P7**	**P8**
1	*Salmonella*	23	17	18	14	14	18	15	24
2	*Staphylococcus*	17	-	-	-	12	16	12	12
3	*Pseudomonas*	-	11	-	-	17	11	9	20
4	*Vibrio cholera*	22	16	-	15	14	20	11	20
5	*Streptococcus*	23	14	7	14	13	13	15	21
6	*Corynebacteria*	22	15	-	12	10	16	-	23
7	*E. aerogenes*	15	-	10	-	-	-	10	12
8	*Proteus vulgaris*	19	-	-	14	14	17	8	19
9	*Escherichia coli*	15	17	11	13	13	15	17	22

The antimicrobial activity of butanol extract in during winter season flowers within during blossoming and complete blossoming of *Michelia champaka, Michelia alba, Nyctanthes arbortristis, Jasminum sambac, Jasminum trifoliatum, Artabotrys hexapetalus, Couroupita guianensis* and *Cananga odorata.* Against human pathogenic bacteria, *Salmonella, Staphylococcus, Pseudomonas, Vibrio cholera, Streptococcus, Corynebacterium, Enterobacter aerogenes, Proteus mirabilis, Proteus vulgaris* and *Escherichia coli* were measured by measuring the zone of inhibition in Agar diffusion test (Kirby–Bauer method). Against butanol extract, *Pseudomonas, Salmonella, Vibrio cholera, Corynebacterium, Staphylococcus, Streptococcus, Escherichia coli, Enterobacter aerogenes, Proteus vulgaris* showed a highest inhibition zone of 34, 32, 28, 27, 26, 25, 25, 24 and 22 during blossom and complete blossoming time. We found that the extracts of *Cananga odorata* flowers showed stronger antibacterial activity than that of *Nyctanthes arbortristis* (Table 2 and 3). The maximum zone of inhibition (34 mm) was observed against *Pseudomonas*, followed by *Salmonella* (32 mm) and *Vibrio cholera* (28 mm) the lowest against *Streptococcus* (7 mm) at the highest amount of flower extracts (Table 2).

4. Conclusion

Active compounds present in the butanol extracts show the antibacterial activity within the flower dependant manner. Extracts of flowers collected during blossoming time were found much effective when compared to extracts of flowers collected after complete blossoming. Extracts of *Michelia champaka, Michelia alba, Nyctanthes arbor-tristis, Jasminum sambac, Jasminum trifoliatum, Artabotrys hexapetalus, Couroupita guianensis* and *Cananga odorata* are used; out of which *Michelia champaka, Cananga odorata* have shown good activity. *Nyctanthes arbor-tristis extracts* have shown

inefficient inhibitory zones. Flower extracts of *Michelia champaka* and *Cananga odorata are* found to be effective when compared to Ampicillin antibiotic and also impose no side effects. Out of all the species that are tested against various microbes, *Michelia champaka, Michelia Alba, Artabotrys hexapetalus, Couroupita guianensis and Cananga odorata* gave distinct and different values. Antibiotics that may cause more side effects when compared to the use of natural therapeutic compounds.

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