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Evaluation of antibacterial activity of some selected Angiosperm flower extract

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Abstract: The methanol extracts of the flower *Quisqualis indica, Calotrophis gigantea* and *Polianthes tuberose* showed significant antibacterial activity against the microbes *Klebsiella pneumoniae, Pseusdomonas aeruginosa, Proteus mirabilis, Esherichia coli*, Methicillin Resistant Staphylococcus aureus and *Bacillus subtilis*. The flower extracts were prepared both in dry and wet forms. In the present study wet flower extracts of *Calotrophis gigantea* and *Polianthes tuberose* showed no antibacterial activity while the dry flower extracts of all flowers showed antimicrobial activity against all the microbes studied but the concentration required for the impact varies from flower to flower. Among the three flowers studied the dry flower extract of *Quisqualis indica* showed antimicrobial activity even in low concentration (10µg/ml) in almost all organisms studied. The *Calotrophis gigantea* dry extract showed antimicrobial activity on all organisms studied except *Bacillus subtilis* at higher concentrations (40µg/ml). The flower extract of both dry and wet flowers of *Polianthes tuberosa* showed no impact in all organisms studied except *Proteus mirabilis* and *Esherichia coli* at higher concentrations only (> 50µg/ml). The study result concluded that the dry flower extract of *Quisqualis indica* showed best antimicrobial property than other flowers studied.

Key words: Antibacterial activity, dry & wet flower extracts, bacterial strains, well cut method.

INTRODUCTION

Nature has been as source of medicinal agent for thousands of years and an impressive number of modern drugs have been isolated form natural sources; many based on their uses in traditional medicine. Various medicinal plants have been used as a source of medicine for years in daily life to treat diseases all over the world. The widespread use of herbal remedies and health care preparations, such as those described in ancient Vedas and the Bible, has been traced to the occurrence of natural products with medicinal properties. In fact, plant produces a diverse range of bioactive molecules, making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times[1]. Over 50% of all modern clinical drugs are of natural product origin[2] and natural products play an important role in drug development programs in the pharmaceutical industry[3].

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [4]. A wide range of medicinal plant part extracts used as raw drugs and they possess varied medicinal properties and these raw drugs are collected in smaller quantities by the local communities and folk healers for local use, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries[5]. Although hundreds of plant species have been tested

for antimicrobial properties, the vast majority of have not been adequately evaluated [6].

Relatively lower incidence of adverse reactions to plant preparations than modern conventional pharmaceuticals, coupled with their reduced cost, is encouraging both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs. Plants with possible antimicrobial activity have been identified by [7-11] number of researchers in different parts of the world [1 &12]. Much work has been done on ethnomedicinal plants in India[13-15]. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoral and antimicrobial agents [3&16]. The selection of crude plant extracts of screening programs have the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products[17]. The present study was planned to evaluate the antimicrobial property of some angiosperm flower such as *Quisqualis indica, Calotrophis gigantea* and *Polianthes tuberosa* against selected bacterial strains.

MATERIAL AND METHODS

Flower material

The flowers of *Quisqualis indica, Calotrophis gigantea* and *Polianthes tuberosa* were collected from near by villages of Thoothukudi and brought to the laboratory. Then the flowers were rinsed twice with distilled water and air dried on a clean sheet for one week at room temperature. It was made into small pieces using sharp sterile scissors and powdered using sterile mortal and pestle.

Preparation of plant extract

Extraction was done at room temperature by simple extraction method[18]. Ten grams of air dried powder was placed in 100ml of methanol in a conical flask, plugged with cotton and then kept on a rotary shaker at 190-220rpm for 24 hour. After 24 hours it was filtered through 8 layers of muslin cloth and centrifuged at 5000xg for 15min. The supernatant was collected and the solvent was evaporated to make the final volume one- fourth of the original volume and it was stored at 4^{O}C in air tight bottles for further studies.

Test Microorganisms

Identified microbial strains were obtained form the Department of Microbiology, Bharathidasan University, Thichy, TamilNadu, India. The bacterial strains were *Klebsiella pneumoniae, Pseusdomonas aeruginosa, Proteus mirabilis, Escherichia coli*, Methicillin Resistant Staphylococcus aureus and *Bacillus subtilis*. They were cultured in nutrient broth for 24 hours and fresh inoculums were taken for the experiments.

Determination of antibacterial activity by agar well diffusion method

Antibacterial activity of the methanolic extract of selected flower was evaluated by agar well diffusion method[19]. The inocula with respective tested bacteria were homogenously seeded onto the 90mm Petri dishes containing 20ml of cooled molten MH agar medium using a sterile swab in such a way as to ensure thorough coverage of the plates and a uniform thick lawn of growth following incubation[20]. Wells were dug in the medium with the help of a sterile cork borer. Different concentration of extract containing 10, 20, 30, 40, 50, 60, 70, 80, 90, 100µg/ml were prepared using sterile DMSO from this 100 µl of each dilution was added to their respective wells with a sterile pipette.

The plates were kept for 1h at room temperature for the diffusion of the extract into the agar. Subsequently, all the plates were incubated at 37^{O}C for 18-24h. Following incubation the plates were examined for signs of microbial growth. Bacterial growth inhibition was determined as the diameter of the inhibition zones around the wells. Each experiment was carried out in triplicates.

RESULT AND DISCUSSION

The antimicrobial activity of wet flower extracts of *Calotrophis gigantea* and *Polianthes tuberosa* showed no antibacterial activity while the dry flower extracts of all flowers showed a significant level of antimicrobial activity against all the microbes studied but the concentration required for the impact varies from flower to flower. Among the three flowers studied the dry flower extract of *Quisqualis indica* showed a significant antimicrobial activity even in low concentration (10µg/ml) in almost all organisms studied. The *Calotrophis gigantea* dry extract showed antimicrobial activity on all organisms studied at high concentration alone (>40µg/ml) except *Bacillus subtilis*. The flower extract of both dry and wet flowers of *Polianthes tuberosa* showed no impact in all organisms studied except *Proteus mirabilis* and *Escherichia coli* at very higher concentrations only (> 50µg/ml). The study result concluded that the dry flower extract of *Quisqualis indica* showed best antimicrobial property than other flowers studied (Table 1 to 6).

Table No: 1 Antimicrobial activity of the flower extracts against *Klebsiella pneumonia* - Zone of inhibition in diameter (mm)

Flowers	Extract	Concentrations (µg/ml)									
		10	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
Quisqualis indica	Dry	-	11.66 ±1.52	14±2	15.33 ±1.52*	16 ±2*	17.33 ±1.52*	17.66 ±1.52*	19.33 ±1.52*	20.33 ±1.52*	21.33 ±1.52*
	Fresh	-	-	-	11.66 ±1.52	14 ±2	15.33 ±1.52*	16 ±2*	17.33 ±1.52*	17.66 ±1.52*	19.33 ±1.52*
Calotrophis gigantea	Dry	-	11.33 ±1.52	14 ±2	16 ±2*	18.33 ±1.52*	20.66 ±2.08*	22 ±1*	24 ±2*	26 ±1*	28.33 ±1.52*
	Fresh	-	-	-	11.33 ±1.52	14 ±2	16 ±2*	18.33 ±1.52*	20.66 ±2.08*	22 ±1*	24 ±2*
Polianthes tuberose	Dry	-	-	-	-	-	-	-	-	-	-
	Fresh	-	-	-	-	-	-	-	-	-	-

Control – Nil (*- significant at 0.05% level)

Table No: 2 Antimicrobial activity of the flower extracts against *Pseudomonas aeruginosa* - Zone of inhibition in diameter (mm)

Flowers	Extract	Concentrations (µg/ml)									
		10	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
Quisqualis indica	Dry	11.33 ±1.52	12.66 ±1.52	14.33 ±1.52	15 ±1*	16.33 ±1.52*	17 ±2*	17.66 ±1.52*	19 ±1*	19.66 ±1.52*	20.33 ±2.08*
	Fresh	-	11.33 ±1.52	12.66 ±1.52	14.33 ±1.52	15 ±1*	16.33 ±1.52*	17 ±2*	17.66 ±1.52*	19 ±1*	19.66 ±1.52*
Calotrophis gigantea	Dry	-	-	-	-	-	13 ±1	13.66 ±1.52	15 ±2	16 ±1*	17.33 ±1.52*
	Fresh	-	-	-	-	-	-	-	-	-	13±1
Polianthes tuberose	Dry	-	-	-	-	-	-	-	-	-	-
	Fresh	-	-	-	-	-	-	-	-	-	-

Control – Nil (*- significant at 0.05% level)

Table No: 3 Antimicrobial activity of the flower extracts against *Proteus mirabilis* - Zone of inhibition in diameter (mm)

Flowers	Extract	Concentrations (µg/ml)									
		10	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
Quisqualis indica	Dry	11.33 ±1.52	12.66 ±1.52	14.33 ±1.52	15 ±1*	16.33 ±1.52*	17 ±2*	17.66 ±1.52*	19 ±1*	19.66 ±1.52*	19.66 ±1.52*
	Fresh	-	11.33 ±1.52	12.66 ±1.52	14.33 ±1.52	15 ±1*	16.33 ±1.52*	17 ±2*	17.66 ±1.52*	19 ±1*	19.66 ±1.52*
Calotrophis gigantea	Dry	-	-	-	-	-	14 ±1*	15 ±1*	16.33 ±1.52*	17.33 ±1.52*	18.33 ±1.52*
	Fresh	-	-	-	-	-	-	-	-	-	14 ±1*
Polianthes tuberose	Dry	-	-	-	-	-	16 ±1*	17.33 ±1.52*	17.66 ±1.52*	19 ±2*	20 ±1*
	Fresh	-	-	-	-	-	-	-	-	-	16 ±1*

Control – Nil (*- significant at 0.05% level)

Table No: 4 Antimicrobial activity of the flower extracts against *Esherichia coli* - Zone of inhibition in diameter (mm)

Flowers	Extract	Concentrations (µg/ml)									
		10	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
Quisqualis indica	Dry	11.33 ±1.52	12.66 ±1.52	14.33 ±1.52	15 ±1*	16.33 ±1.52*	17 ±2*	17.66 ±1.52*	19 ±1*	19.66 ±1.52*	21.33 ±1.52*
	Fresh	-	11.33 ±1.52	12.66 ±1.52	14.33 ±1.52	15 ±1*	16.33 ±1.52*	17 ±2*	17.66 ±1.52*	19 ±1*	19.66 ±1.52*
Calotrophis gigantea	Dry	-	-	-	11.33 ±1.52	12.33 ±1.52	13 ±1	14.33 ±1.52	15 ±1*	16.33 ±1.52*	17.33 ±1.52*
	Fresh	-	-	-	-	-	-	-	11.33 ±1.52	12.33 ±1.52	13 ±1
Polianthes tuberose	Dry	-	-	-	12.33 ±1.52	13 ±1	14.33 ±1.52	15 ±1*	16.33 ±1.52*	17.33 ±1.52*	18.33 ±1.52*
	Fresh	-	-	-	-	-	-	-	12.33 ±1.52	13 ±1	14.33 ±1.52

Control – Nil (*- significant at 0.05% level)

Table No: 5 Antimicrobial activity of the flower extracts against MRSA - Zone of inhibition in diameter (mm)

Flowers	Extract	Concentrations (µg/ml)									
		10	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
Quisqualis indica	Dry	-	11.33 ±1.52	14.33 ±1.52	15 ±1*	16.33 ±1.52*	17 ±2*	17.66 ±1.52*	19 ±1*	19.66 ±1.52*	21.33 ±1.52*
	Fresh	-	-	-	11.33 ±1.52	14.33 ±1.52	15 ±1*	16.33 ±1.52*	17 ±2*	17.66 ±1.52*	19 ±1*
Calotrophis gigantea	Dry	-	-	-	-	-	15.66 ±1.52*	17 ±2*	18 ±1*	19.33 ±1.52*	20.33 ±1.52*
	Fresh	-	-	-	-	-	-	-	-	-	15.66 ±1.52*
Polianthes tuberose	Dry	-	-	-	-	-	-	-	-	-	-
	Fresh	-	-	-	-	-	-	-	-	-	-

Control – Nil (*- significant at 0.05% level)

Table No: 6 Antimicrobial activity of the flower extracts against *Bacillus subtilis* - Zone of inhibition in diameter (mm)

Flowers	Extract	Concentrations (µg/ml)									
		10	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**100**
Quisqualis indica	Dry	11.33 ±1.52	12.33 ±2.-8	14.33 ±1.52	15 ±1*	16.33 ±1.52*	17 ±2*	17.66 ±1.52*	19 ±1*	19.66 ±1.52*	21.33 ±1.52*
	Fresh	-	11.33 ±1.52	12.33 ±2.08	14.33 ±1.52	15 ±1*	16.33 ±1.52*	17 ±2*	17.66 ±1.52*	19 ±1*	19.66 ±1.52*
Calotrophis gigantea	Dry	-	-	-	-	-	-	-	-	-	-
	Fresh	-	-	-	-	-	-	-	-	-	-
Polianthes tuberose	Dry	-	-	-	-	-	-	-	-	-	-
	Fresh	-	-	-	-	-	-	-	-	-	-

Control – Nil (*- significant at 0.05% level)

Nair reported that the bark extract of *T. chebula* was bacteriostatic against few Gram positive and Gram negative bacteria such as *Bacillus substilis*, *P. florescence* and *E. coil*[21].Sunil and Mohashine found the antibacterial effects of barks of *A. leucophloea* and *A. indica* against selective microbes[22&23].Abbas found the antimicrobial activity in the petroleum ether extract of *Mimusops elengi* [24]. *Datura metel* is also known for its antibacterial activity against burn pathogens[25] and antifungal activity against phytopathogens[(26&27)]. The antibacterial activity of methanolic extracts showed better performance than others. This indicated that most of the active components are extracted from the materials with the help of methanol several reports supports this view. Shirsat reported that the anti – phytopathogenic activity of methanol extract of leaves, stem bark, seed and dry fruit of *Terminalia thorelli*, against four phyto pathogens[28]. Ghosh evaluated the antibacterial potentiality of hot aqueous and methanol solvent extract of mature leaves of *Polyalthia longifolia* against six different bacteria species[29].

In the present study the flower *Quisqualis indica* extract prepared both in dry and wet form showed maximum inhibitory activity against all organisms studied even in very low concentrations. This indicated that this flower contain enormous antimicrobial inclusions than the other flower studied. In the present study only crude extracts are used and hence further investigation and identification of components are needed.

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