Antimicrobial Activity of Some Flowers of Eastern Ghats, Tamil Nadu, India

Sevugaperumal Ganesan[*,1], Kumarasan Manimegalai[2] and Ramaiah Latha[3]

[1]*Department of Biology, St. Joseph University in Tanzania, Dar es Salaam, Post Box No - 11007, Tanzania, East Africa*

[2]*Centre for Research and PG Department of Botany, Thiagarajar College (Autonomous), Madurai – 625009, Tamil Nadu, India*

[3]*Department of Botany, Sri Meenakshi Govt. Arts College for Women, Madurai – 625002, Tamil Nadu, India*

Abstract: The bioactive compounds from the different parts of medicinal plants have shown many pharmacological activities but the screening for microbial activity from flower is very scanty, the purpose of the study is to discover the therapeutic ability of the flowers of ten ethnomedicinal plants found in the southern Eastern Ghats of Tamil Nadu with a destination of providing cheaper nature-based alternative medicine. Flower extracts of ethnomedicinal plants, *Abutilon indicum* (L). Sweet, *Calotropis gigantea* (L.) W.T. Aiton, *C. procera* (Aiton), *Catharanthus roseus* (L.) G. Don., *Martynia annua* L., *Memecylon umbellatum* Burm.f., *Ocimum tenuiflorum* L. *Ophiorrhiza mungos* Linn., *Scilla indica* Baker. and *Tecoma stans* (L.) Kunth. were examined using agar well diffusion method against the human pathogens, *Bacillus subtilis*, *Candida albicans*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Stephylococcus aureus* and *Streptococcus mutans*. Flowers were extracted using 80% ethanol, phytochemical screening of these flowers were performed for constituents like, Carbohydrates, tannin, steroids, terpenoids, saponins, flavonoids, alkaloids, essential/volatile oil and glycosides. The Minimum Inhibitory Concentration (MIC) of selected three flower extracts against the pathogens also tested in contingent. From these finding revealed the medicinal potential of these flowers to cure the various infectious diseases. Compare to reference antibiotics, the spectrum of antimicrobial activity of flower extracts also found be clearly superior. The broad spectrum of antimicrobial activity of *Martynia annua*, *Memecylon umbellatum* and *Calotropis gigantea* may help to discover new chemical classes of antibiotics substances.

Keywords: Ethnomedicinal plants, flower, antimicrobial activity, infectious diseases.

INTRODUCTION

Infectious diseases are the leading causes of death throughout the world that accounts for nearly one half of all death in the tropical countries, which are also becoming a serious problem in developed countries. It is calculated that infectious diseases are the main causes of death in 8% of the 9 deaths occurring in United States [1]. In addition, antibiotics are sometime associated with adverse effects including hyper-sensitivity, immunosuppressant and allergic reactions. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents.

The increase in prevalence of multiple drug resistance has showed down the development of new synthetic antimicrobial drugs and the new drug is necessary to search for new antimicrobial compound from alternative sources. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants. Phytochemicals from medicinal plants showing antimicrobial activities have the potential of filling this need because of structures are different from those of the more studied and thereof the more action may too very likely differ [2].

The ancient man through his own observation and experiences developed knowledge about the usage of plants for edible, medicinal and other purposes. Although in the modern era medicinal facilities and enough food are available to most of the people, still in several under developed and inaccessible areas of the country food deficiency and lack of medicinal facilities are prevalent. Plant parts like fruits, tubers, flowers, leaves etc. are consumed as principal or supple-mentary food and used as medicine [3-9].

In this growing interest, many of the Phytochemical bioactive compounds from the different parts of medicinal plants have shown many pharmacological activities [10-14] but the screening for microbial activity from flower is very scanty [15]. Keeping the facts in mind the purpose of the study is to discover the therapeutic ability of the flowers of ten ethnomedicinal plants found in the southern Eastern Ghats of Tamil Nadu, with a destination of providing cheaper nature-based alternative medicine.

*Address correspondence to this author at the Centre for Research and PG Department of Botany, Thiagarajar College (Autonomous), Madurai – 625009, Tamil Nadu, India; Tel: 91-9865070639; E-mail: sganesan76@yahoo.com

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MATERIALS AND METHODS

Collection of Plant Flower and Extraction

Fresh flowers of *Abutilon indicum* (L). Sweet, *Calotropis gigantea* (L.) W.T. Aiton, *C. procera* (Aiton), *Catharanthus roseus* (L.) G. Don., *Martynia annua* L., *Memecylon umbellatum* Burm.f., *Ocimum tenuiflorum* L. *Ophiorrhiza mungos* Linn., *Scilla indica* Baker. and *Tecoma stans* (L.) Kunth. were collected from in and around the (Piranmalai hill, Alagar hill and Sirumalai hill) Eastern Ghats of Tamil Nadu, India during the period of 2011-2012. The voucher plant specimen were collected and identified by referring standard local flora [16-19].

Fresh flowers were ground with 80% ethanol and kept in a shaker for 3 days and then filtered. The filtrates were evaporated in sand bath and the dried extract collected and stored in refrigerator for future use. The extract was re-suspended in the sterile distilled water at different concentrations depends upon the flower used.

Phytochemical Screening of Flower Extract

The preliminary phytochemical screening of flower extracts were carried out for alkaloids, saponin, carbohydrates, tannin, anthroquinone, steroids, terpenoids, flavonoids, essential oil, glycosides, and potassium salt according to the methods of Sofowora [20]; Trease and Evan [21] and Ganesan and Roopam [22].

Microbial Culture and Growth Condition

Test microorganisms (Human pathogens), *Bacillus subtilis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus mutans, Pseudomonas aeruginosa* and *Candida albicans* were obtained from the Microbiology Department of Thiagarajar College (Autonomous) and Lady Doak College (Autonomous), Madurai, Tamil Nadu, India and the stock culture of microbes were maintained on nutrient agar media.

Bioassay for Antimicrobial Activity

The agar well diffusion assay was used to determine the growth inhibition of microbes by plant extract. Bacteria and fungus were maintained at 4ºC on Nutrient Agar slant. Nutrient agar was prepared and 20ml of each was poured into sterile petriplates. Each petriplate inoculated with 0.2ml of different bacterial species and fungus (24 hrs culture) spread well using a sterile L-rod. Single hole (5mm) per plate was made by using sterile cork borer into the set agar containing the microbial culture. A total of 0.02ml of flower extract poured into the well. The plates were kept in incubator overnight and the zone diameter was then recorded.

Table 1: Enumeration of Ethno Medicinal Plants

S. No.	Plant Name	Vernacular Name	Family	Ethnomedicinal Uses
1	*Abutilon indicum*	Thutti	Malvaceae	Increasing the semen, leprosy, anti-inflammation.
2	*Calotropis gigantea*	Velerukku	Asclepiadaceae	Latex treated for wound activity, asthma and cough.
3	*C. procera*	Erukku	Asclepiadaceae	Asthma, cough
4	*Catharanthus roseus*	Nidyakalyani	Apocynaceae	Anticancer, diabetes, bee stings
5	*Martynia annua*	Thael kodukkukaai	Pedaliaceae	Asthma, leaf paste used to heal wounds and boils
6	*Memecylon umbellatum*	Kaaya	Melastomataceae	Burning sensation, pimple disappear, stomach pain, dysentery
7	*Ocimum tenuiflorum*	Thulasi	Laminaceae	Cough, fever, vomiting
8	*Ophiorrhiza mungos*	Keeripoondu	Rubiaceae	Ulcer, skin disease, poisonous bite
9	*Scilla indicum*	Narivengayam	Liliaceae	Broken bone, cardiac diseases
10	*Tecoma stans*	Thangaarali	Bignoniaceae	Diabetes, digestive

Table 2: Phytochemical Screening of Medicinal Plants

S. No.	Plant Name	Carbohydrate	Tannin	Steroids	Terpenoids	Saponins	Flavonoids	Alkaloids	Essential oil	Glycosides
1.	*Abutilon indicum*	+	-	+	-	+	+	-	+	+
2.	*Calotropis gigantea*	-	+	+	-	+	+	+	-	+
3.	*C. procera*	_	_	+	+	+	+	_	_	+
4.	*Catharanthus roseus*	_	_	+	+	_	_	+	+	_
5.	*Martynia annua*	+	+	_	_	+	+	-	+	+
6.	*Memecylon umbellatum*	+	+	+	+	+	+	-	+	+
7.	*Ocimum tenuiflorum*	+	+	+	+	-	+	+	+	+
8.	*Ophiorrhiza mungos*	+	+	_	+	+	+	+	_	+
9.	*Scilla indica*	+	_	_	_	_	+	_	_	+
10.	*Tecoma stans*	_	+	+	+	_	+	+	_	_

+ = Present; - = Absent

Table 3: Bioassay of Medicinal Plants for Antimicrobial Activity

S. No.	Plant Name	Zone of Inhibition (mm)*					
		B. subtilis	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*	*S. mutans*	*C. albicans*
1.	*Abutilon indicum*	18 ± 0.20	-	11 ± 0.10	11 ± 0.11	18 ± 0.10	9 ± 0.05
2.	*Calotropis gigantea*	12 ± 0.10	15 ± 0.15	17 ± 0.20	11 ± 0.15	20 ± 0.15	15 ± 0.10
3.	*C. procera*	8 ± 0.10	6 ± 0.05	11 ± 0.10	8 ± 0.10	7 ± 0.15	8 ± 0.05
4.	*Catharanthus roseus*	-	-	10 ± 0.05	12 ± 0.10	-	13 ± 0.15
5.	*Martynia annua*	28 ± 0.05	13 ± 0.10	24 ± 0.05	28 ± 0.05	16 ± 0.10	27 ± 0.10
6.	*Memecylon umbellatum*	21 ± 0.11	12 ± 0.10	21 ± 0.15	21 ± 0.05	17 ± 0.15	20 ± 0.10
7.	*Ocimum tenuiflorum*	9 ± 0.10	17 ± 0.10	20 ± 0.10	10 ± 0.10	14 ± 0.10	12 ± 0.05
8.	*Ophiorrhiza mungos*	20 ± 0.10	19 ± 0.10	14 ± 0.05	14 ± 0.05	11 ± 0.05	16 ± 0.10
9.	*Scilla indica*	-	-	13 ± 0.10	13 ± 0.05	6 ± 0.05	-
10.	*Tecoma stans*	16 ± 0.20	18 ± 0.05	17 ± 0.10	9 ± 0.15	17 ± 0.05	14 ± 0.10
11.	Tetracyclin (30µm)	15 ± 0.10	18 ± 0.05	24 ± 0.05	14 ± 0.10	22 ± 0.05	34 ± 0.05
12.	Amphicillin (10µm)	8 ± 0.10	9 ± 0.10	-	-	-	13 ± 0.10

± - Standard Error; * = Each value is a mean of Triplicate

Commercial products of Ampicillin (10µg) and Tetracycline (30 µg) were used as a standard drug (Himedia laboratories, Pvt. Ltd. Mumbai).

RESULTS AND DISCUSSION

Ethnomedicinal details and the result of the phytochemical screening of different flowers extract of *Abutilon indicum*, *Calotropis gigantea*, *Calotopis procera*, *Catharanthus roseus*, *Martynia annua*, *Memecylon umbellatum*, *Ocimum tenuiflorum*, *Ophiorrhiza mungos*, *Scilla indica* and *Tecoma stans* is present in Tables **1** and **2**. These classes such as alkaloid, carbohydrates, tannin, anthroquinone, steroids, terpenoids, saponins, flavonoids, essential oil, glycosides and potassium are known to have curative activity against several pathogens. The extracts were further subjected to antimicrobial studies. The susceptibility pattern against the test organisms are shown in Table **3**.

The results of the antimicrobial determinations for ethanolic extract of the wild flowers of *A. indicum*, *C. gigantea*, *C. procera, C. roseus*, *M. annua*, *M.*

umbellatum, *O. tenuiflorum*, *O. mungos*, *S. indica* and *T. stans* against the six microbial species showed significant reduction in microbial growth in term of zone of inhibition. The zone of inhibition increased on increasing the concentration of extract. This depicted the concentration dependent activity (Table **3**). The antimicrobial activity of the ethanol extract compared with that of two standard antibiotics (Ampicillin & Tetracycline).

Bioassay for the *Bacillus subtilis*, 28mm of zone length was observed with *M. annua*, it's a maximum level of inhibition compared with other flower extracts viz*., M. umbellatum* (21mm), *O. mungos* (20mm)*, A. indicum* (18mm), *O. tenuiflorum* (9mm) and *C. gigantea* (12mm).

In the case of *Candida albicans*, only *M. annua* showed high (27mm) level of inhibition followed by *M. umbellatum* (20mm), *O. mungos* (16mm), *C. gigantea* (15mm), T. stans (14mm), C. roseus (13mm), *O. tenuiflorum* (12mm), A*. indicum* (9mm) and *C. procera* (8mm). The maximum level of inhibition against *Pseudomonas aeruginosa* (24mm) and Staphylococcus aureus (28 mm) were also observed with 100% crude extract of *M. annua*, the remaining plants inhibited the pathogen ranged from 8mm to 21mm of zone length.

The flower extract of *O. mungos* inhibit the growth of *Klebsiella pneumoniae* at maximum (19mm) level compared with other flower extracts viz. *T. stans* (18mm), *O. tenuiflorum* (17mm), *C. gigantea* (15mm), *M. annua* (13mm), *M. umbellatum* (12mm) and *C. procera* (6mm), respectively.

C. gigantea exhibited maximum level (20mm*)* of antimicrobial activity against *Streptococcus mutans* followed by *A. indicum* (8mm), *M. umbellatum* (17mm), *T. stans* (17mm), *M. annua* (16mm), *O. tenuiflorum* (14mm), *O. mungos* (11mm), *C. procera* (7mm) and *S. indica* (6mm), respectively.

In the present investigation showed that the ethanolic extract of flower is highly effective against all tested microbes. The study also divulged that, the tested plants showed very interesting results, *Martynia annua*, *Memecylon umbellatum* and *Calotropis gigantea* appear to be the more active against the tested microbes (Table **3**). There are reports showed that flowers and different parts of the plant possess antimicrobial properties [23-30].

Martynia annua showed maximum level of antimicrobial activity against all the pathogens compared with other flower extracts, viz. *Bacillus subtilis* (28mm), *Candida albicans* (27mm), *Klebsiella*

Table 4: The MIC Regime of the Flower Extracts of *Calotropis gigantea*

S. No.	Concentration	Zone of Inhibition (mm)*					
		B. subtilis	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*	*S. mutans*	*C. albicans*
1.	2.58 $mgml^{-1}$	-	-	-	-	-	-
2.	3.87 $mgml^{-1}$	-	-	-	-	-	-
3.	6.45 $mgml^{-1}$	-	-	-	-	-	-
4.	12.9 $mgml^{-1}$	9 ± 0.1	-	10 ± 0.15	7± 0.15	7 ± 0.10	8 ± 0.10
5.	19.35 $mgml^{-1}$	11 ± 0.1	6 ± 0.05	12 ± 0.15	10 ± 0.10	9 ± 0.10	10 ± 0.15

± - Standard Error; * - Each value is a mean of Triplicate

Table 5: The MIC Regime of the Flower Extracts of *Martynia annua*

S. No.	Concentration ($mgml^{-1}$)	Zone of Inhibition (mm)*					
		B. subtilis	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*	*S. mutans*	*C. albicans*
1.	2.31 $mgml^{-1}$	10 ± 0.05	8 ± 0.05	7 ± 0.05	-	-	7 ± 0.05
2.	3.47 $mgml^{-1}$	11 ± 0.05	9 ± 0.15	9 ± 0.05	-	10 ± 0.05	8 ± 0.10
3.	5.79 $mgml^{-1}$	12 ± 0.10	9 ± 0.05	12 ± 0.10	10 ± 0.05	11 ± 0.05	9 ± 0.05
4.	11.58 $mgml^{-1}$	20 ± 0.10	10 ± 0.05	12 ± 0.10	13 ± 0.11	17 ± 0.10	10 ± 0.15
5.	17.37 $mgml^{-1}$	20 ± 0.10	10 ± 0.05	16 ± 0.05	21 ±0.10	16 ± 0.10	16 ± 0.05

± - Standard Error; * = Each value is a mean of Triplicate

Table 6: The MIC Regime of the Flower Extracts of *Memecylon umbellatum*

S. No.	Concentration ($mgml^{-1}$)	Zone of Inhibition (mm)*					
		B. subtilis	*K. pneumoniae*	*P. aeruginosa*	*S. aureus*	*S. mutans*	*C. albicans*
1.	4 $mgml^{-1}$	-	-	-	-	-	-
2.	6 $mgml^{-1}$	-	-	-	-	-	8 ± 0.10
3.	10 $mgml^{-1}$	7 ± 0.11	9 ± 0.10	7 ± 0.05	8 ± 0.15	8 ± 0.05	12 ± 0.10
4.	20 $mgml^{-1}$	11 ± 0.05	12 ± 0.20	9 ± 0.10	13 ± 0.10	12 ± 0	14 ± 0.10
5.	30 $mgml^{-1}$	9 ± 0.05	13 ± 0.10	13 ± 0.10	13 ± 0.05	14 ± 0.05	11 ± 0.10

± - Standard Error; * - Each value is a mean of Triplicate

pneumonia (13mm), *Pseudomonas aeruginosa* (24mm), *Staphylococcus aureus* (28mm) and *Streptococcus mutans* (16mm), respectively. The inhibition zone ranged between 13mm and 28mm with the extract concentration of 115.83mg/0.02ml.

The MICs of three selected flowers extract are *C. gigantea*, (12.9 to 19.35 $mgml^{-1}$); *M. annua*, (2.31 to 17.37 $mgml^{-1}$) and *M. umbellatum*, (6 to 20 $mgml^{-1}$).

M. annua, showed (MIC 2.31mgml-1) maximum level of inhibition against *B. subtilis* (10mm), followed by *K. pneumonia* (8mm), *P. aeruginosa* and *C. albicans* showed 7mm in each. For controlling *S. mutans* (10mm) the MIC is 3.47mgml-1 and for *S. aureus* (10mm) the MIC is 5.79$mgml^{-1}$ (Table **4**).

C. gigantea and *M. umbellatum* also showed very good level of inhibition with MICs (12.9 to 19.35$mgml^{-1}$ and 6 to 10$mgml^{-1}$), against all the microbes studied. The inhibition level ranged from 6 to 10mm (*C. gigantea)* and 7 to 12mm (*M. umbellatum)* (Table **5** and **6**).

From these experiments, it revealed that the medicinal potential of these flowers is effective on various infectious diseases. Compare to reference antibiotics, the spectrum of antimicrobial activity of flower extracts also found to be clearly superior. The broad spectrum antimicrobial activity of *Martynia annua*, *Memecylon umbellatum* and *Calotropis gigantea* may help to discover new chemical classes of antibiotic substances. The effect of these plant extracts on more pathogenic organism and further pharmacological evaluations are need to be carried out.

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