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A Preliminary Study on the Evaluation of Antibacterial Potential of Ethanolic Extracts of Some Common Ornamental Flowers and Their Leaves

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Abstract

Leaves and flowers of few ornamental plants of family viz. *Catharanthus rosea* white and pink variety (*alba* and *Rosea*), *Hibiscus* (red variety), *Campsis*, *Portulaca*, *Plumeria*, *Jasminum*, *Cascabela*, grown in the premises of VISM, Gwalior, MP, India were chosen to evaluate the antibacterial effect, if any, against three bacterial pathogens (*Stphylococcus aureus*, *E.coli* and *Salmonella typhi*).Saline extract and ethanolic extracts were prepared using dry powder leaves and flowers of the above plants. Results clearly suggested that ethanolic extract of leaves of *Catharanthus roseus* (white-*alba* variety) is a good potential agent in inhibition of growth of the above pathogenic microorganisms.

Keywords: *Catharanthus roseus*, ethanolic extracts, pathogenic microorganisms

INTRODUCTION

Antibiotics are known as remedial measures against many microbial infections. Of late, however, antibiotic resistances are creating problems in the cure of the diseases. Bacterial resistance to antibiotic is major therapeutic problem (Russell, 2002) .Both clinicians and patients are in a fix to find out the avenues. Plant products are, however, are coming up with promising effect. According to World Health Organization medicinal plants with various life sustaining constituents would be the best source to obtain a variety of potential, safe and novel drugs (Natarajan *et al*., 2003).Thus, the search for novel antimicrobial agents is of the utmost importance (Gootz *et al*., 1990). Efforts are being made by various workers to search out the better/ best among them. One such thing was envisaged to work with some of the ornamentals flowers, mostly come across in the gardens of dwelling houses/ offices. A few of them was selected for this work.

Different parts of plants are good source of potent and powerful drugs and are used in medicine in different countries. Their extracts are used as raw medicine and believed to be the important source of new chemical substances with potential therapeutic properties. Phytochemicals, produced by plants could be used directly for the production of new drugs. The phytomedicines are believed to be more acceptable by the human body, as compared to modern synthetic drugs (Chandra, 2013).

Catharanthus roseus

C.roseus is an important medicinal plant of family Apocynaceae.This plant has been mostly studied with respect to its anticancer, anti hypertension and anti diabetic properties. It is cultivated mainly for its alkaloids, which are having anticancer activities (Jaleel *et al.,* 2009). Till date, very little studies have been done on the anti microbial properties of the plant extracts (Prajakta and Ghosh, 2010). Antibacterial potential in crude extracts of different parts (viz., leaves, stem, root and flower) of C. roseus against clinically significant bacterial strains are reported by Muhammad *et al*. (2009).

Extraction of complete phytoactive compounds from plant material is mostly dependent on the type of solvents being used in the extraction procedure. Mostly used organic solvents are ethanol, acetone, and methanol to extract phytocompounds from plants (Wendakoon *et al*, 2012).

Hibiscus rosa-sinensis

It is considered as an auspicious flower in India, it is used in many rituals and offerings to the God. Apart of this, there are a lot of medicinal uses of Hibiscus leaves and flower. It is used as an anti ageing agent and reduces blood pressure.Oil extract is used in wound healing caused by cancer in its initial stage. The crude protein in the ethanol extract of *H. rosa-sinensis* flower showed maximum inhibitory zone of inhibition, as observed against *Salmonella* sp. and *E. coli* (Ruban and Gajalakshmi, 2012).

Campsis grandiflora

C. grandiflora, commonly known as the Chinese trumpet vine. The flowers and the whole plant are blood tonic, carminative, depurative diuretic and febrifuge. They are used in the treatment of women's complaints. It has been reported thatit did not have any antibacterial activity and no report is, however, available about *Campsis grandiflora*.

Portulaca grandiflora

Leaf, stem and root of Portulaca oleracea Linn *has been used as a folk medicine in many countries as diuretics, febrifuge, antiseptic, antispasmodic and vermifuge.* Boiled water extracts of the roots were found to kill *Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Shigella sonnei, Pseudomonas aeruginosa*. It could kill both the bacteria and amoeba E. *histolytica* (Metta Ongsakul *et al*, 1992-

1993).No information is available about the antibacterial effect of ***Portulaca grandiflora*.**

Plumeria alba

Methanolic extract of this flower has showed antimicrobial activity against *Bacillus anthracis* and *Pseudomonas aeruginosa* (Amani *et al*, 1998). High concentrations of the Frangipani extract produced an inhibitory zone towards the Escherichia coli resembling the antibiotic, Streptomycin (Syakira and Brenda ,2010).

***Jasminum Sambac* (malligai)Mogra**

Methanol extracts of *Jasminum sambac* leaves was found to have more antibacterial effect against ***Staphylococcus aureus***, *Streptococcus mutans*, *S. pyogenes*, *S. sobrinus*, *S. sanguinis* and *Lactobacillus acidophilus and Candida albicans (fungi)* causing dental infections (Sanjay Kumar*et al*.,2015; Al-Hussaini and Mahasneh,2011).

Gowdhami *et al* (2015), however, concluded that the ethanol extract of *J.sambac* plants showed the maximum antimicrobial activity in comparison to other extracts against *Escherichia coli, Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa* and *Candida albicans and Aspergillus niger*(fungi) in ethyl acetate and petroleum ether extracts whereas extracts of chloroform was found inactive against *Escherichia coli, and Pseudomonas.*

Cascabela thevetia

Cascabela thevetia (L.) belongs to the family Apocynaceae and commonly called as yellow oleander. Sowjanya *et al* (2013) reported that methanol leaf extract showed highest antibacterial activity against *Staph.aureus, Enterococcus fecalis, Pseudomonas aeruginosa and C.albicans (fungi) and chloroform extract against E.coli and P. aeruginosa.*Solomon *et al* (2016) reported that the compound isolated from ethyl acetate fraction of flowers shown antimicrobial activity against human pathogenic bacteria and fungi. Effect aggravated with increasing quantity. No information is, however, available about crude ethanolic extracts.

Escherichia coli (E.coli)

Today the most common infection in women is Urinary tract infection (UTI) with *E. coli*, affecting throughout their lifespan (Sharma *et al,* 2009). It could even affect the kidneys, ureters, bladder or urethra and considered as one of the most common infections in humans especially in women (Geetha *et al.,* 2011).

Salmonella typhi (S.typhi)

S.thpyi is an enterobacterial organism very common in India affecting people of all age group and is commonly spread by contaminated food and potable water (Dutta *et al*, 2001; Kumar and Kumar, 2016).

Staphylococcus aureus (Staph.aureus)

Skin infections are the most common form of *S. aureus* infection. This can manifest in various ways, including small benign boils, folliculitis, impetigo, cellulitis, and more severe, invasive soft-tissue infections (Tong, *et al.*, 2015). *Staph. aureus* is also responsible for food poisoning. It is capable of generating toxins that produce food poisoning in the human body.

The present work was undertaken to study the antibacterial properties of crude ethanolic extract of some common ornamental plants(as above), being grown in small gardens, even in tubs, attached to the residential complexes against *Escherichia coli, Staphylococcus aureus and Salmonella typhi,* isolated from patients.

MATERIAL AND METHODS

(i)Bacterial cultures:

Staphylococcus aureus, Escherichia coli and Salmonella typhi, all collected from patients earlier, well characterized, were obtained from the department of Biotechnology of VISM, Gwalior. According to Koneman *et al* (1998) bacterial colonies were analyzed by physiological and biochemical tests for conformation.

Briefly, before undertaking the work, selective agar medium like MacConkey and EMB were used broadly for further identification of *E. coli* and *Salmonella*. *Salmonella* being non lactose fermentor and *E.coli* as lactose fermentor grew with pink colonies, as well as *E.coli* with metallic shining on EMB agar. Gram staining was done to differentiate between gram positive and gram negative organisms. After phenotypic identification, further confirmation was done on the basis of their biochemical characterization *viz.* amylase production, hydrolysis of gelatin, IMViC test, catalase test, skim milk test, carbohydrate fermentation tests,etc**.**Indole and M.R were positive with *E. Coli* ,and M.R. and Citrate with *Salmonella.* Biochemical characterization of other orgs.was undertaken to fulfill their criteria.

	Staph.aureus	*E.coli*	*S.typhi*
Indole	-	+	-
M.R	+	+	+
Coagulase	+	-	+
Catalase	+	+	+
VP	+	-	-
Citrate utilization	+	-	-
Urease	+	-	-
Glucose	+	+	+
Lactose	+	+	-
Sucrose	+	+	-
Xylose	-	+	+

Collection of plant materials:

Fresh leaves and flowers of some ornamental plants of family viz. Catharanthus white and pink variety (*alba* and *rosea*),Hibiscus (red variety), Campsis, Portulaca, Plumeria, Jasminum, Cascabela, were randomly collected from the garden area of Vijayaraje Institute of Science and Management (VISM), Gwalior (M.P.) ,India during their growing seasons.

1. (a) Catharanthus roseus flowers and leaves white variety (*alba*)

(b)*Catharanthu roseus* flowers and leaves red variety (*Rosea*)

2. *Hibiscus* flowers and leaves

3. *Campsis grandiflora*

4.*Portulacagrandiflora*

5.*Plumeria Alba*

6. *Nyctanthes arbor-tristis* 7.*Cascabela thevetia*

8. ***Jasminum sambac*** **(malligai)** (Mogra)

(ii)Preparation of plants extracts from leaves and flowers of the above samples:-

Fresh plants parts namely leave and flowers were washed 4 to 5 times with tap water and then distilled water, and then dried under shade at 28°C for about 10 days. The dried plants samples were pulverized into a fine powder in a mixer grinder.

(a)**. Preparation of saline extract:**

5gm of dry powder of each of the samples were suspended in 100ml of saline (0.85%) separately in conical flasks and autoclaved under running steam for 20 min.They were then allowed to cool and centrifuged at 7000 rpm for 20 min.The clear supernatants were collected, labeled and stored at 4°C.These material were taken out, looked for clarity and used for testing for their antibacterial effect against the selected organisms as above.

(b). **Preparation of ethanolic extract**:

The plant parts namely leaves and flowers were washed with distilled water, dried in air and pulverized as above. Twenty grams of the material was used for extraction with 99.9% ethanol of analytical grade (Changshu Yangyuan Chemical).The samples were packed in Soxhlet apparatus. Extraction was continued at the temperature of

35°C till clear solvent was observed in thimbles .The extracts were kept overnight in oven at 50°C to evaporate the excess amount of ethanol. Hundred milligram of residue of ethanolic extract was dissolved in 10 ml of ethanol to get final concentration of 1 % and kept in air tight containers.

(iii)Testing of antimicrobial activities:
Antimicrobial activity was studied by well agar plate diffusion method according to Pandey *et al* (2011). Antimicrobial activity test of ethanolic extract of the leaves and flowers of some ornamental plants of family *Catharanthus roseus*(white and pink variety), Hibiscus (red variety), *Campsis*, *Portulaca*, *Plumeria*, *Jasminum*, *Cascabela*, were collected from the garden area of VISM, Gwalior (M.P.), India was carried out using nutrient agar plates.
Briefly-

(1) Each of the organisms (*Staphylococcus aureus*, *Encolpia and S.typhi*) were grown separately in nutrient broth and incubated overnight at 37°C.

2. Next day the bacterial growths in nutrient broth were poured on the nutrient agar plates and allowed to stand for a minute. The excess cultures were removed after a while.

3. Wells were cut of the size of 8mm dia. to a depth of 8mm in this nutrient agar with the help of microtips (usually used with micropipette of 100 to 1000 micro litre). Five wells were cut at equal distance in each plate. The bottom of the wells were sealed with a drop of fresh molten nutrient agar and allowed to stand for some time so that it solidified. The wells were charged fully with the different preparations and incubated at 30°Cfor 24 hours.

5. Next day the plates were observed for the inhibition of bacterial growth with clear zones around the wells. Diameter the clear zones were measured with the help a ruler and results recorded.

The test was also performed along with chosen antibiotics against *Escherichia coli* and *Staphylococcus aureus* and *S.typhi* for their comparative study.

RESULTS

Antibacterial activity of Extracts:

Observations were made for the antibacterial activity of saline extract and eathnolic extracts of leaves and flowers of *Catharanthus roseus* white and pink variety (alba and Rosea), *Hibiscus* (red variety), *Campsis*(pink), *Portulaca* (white and pink), *Plumeria*(white), *Jasminum*(white serrated petals), and *Cascabela*(Yellow). Results of few of these as representative are presented in the tabular form as given below. Since

most of others are negative, not included in the table.

Table-1:

Well No.	Saline Extract	*Staphylococcus aureus*	*E.coli*	*S.typhi*
1	Flowers of *Catharanthus roseus* white variety(*alba*)	Negative	Negative	Negative
2	Flowers of *Catharanthus roseus* pink variety(*Rosea*)	Negative	Negative	Negative
3	Leaves of *Nycthanthes arbour-tristis*	Negative	Negative	Negative
4	Leaves of *Catharanthus* roseus white variety (*alba*)	Negative	Negative	Negative
5	Leaves of *Catharanthus roseus* pink variety(*Rosea*)	Negative	Negative	Negative
6	Leaves of *Hibiscus* (Red)	Negative	Negative	Negative
10	Leaves of *Punica granatum**	Negative	Negative	Negative

*Not an ornamental flowering plant but a fruit, finds out of place

Table 2:

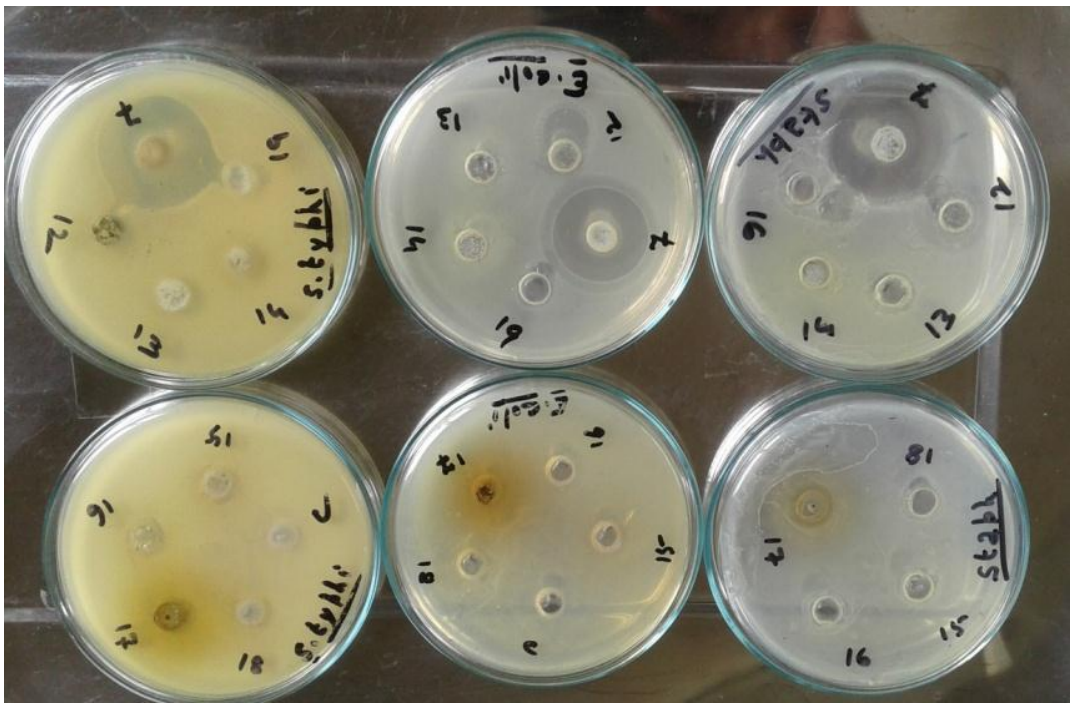
Well No.	Ethanolic Extract	*Staphylococcus aureus*	*E.coli*	*S.typhi*
7	Leaves of *Catharanthus* roseus white variety(*alba*)	Positive (35 mm dia.)	Positive (28 mm dia.)	Positive (33 mm dia.)
8	Flowers of *Hibiscus* (Red)	Negative	Negative	Negative
9	Leaves of *Nyctanthes arbour-tristis*	Negative	Negative	Negative
11	Leaves of *Hibiscus* (Red)	Negative	Negative	Negative
12	Leaves of *Catharanthus roseus* pink variety(*Rosea*)	+ (20mm dia.)	± (13mm dia.)	± (12mm dia.)
13	Flowers of *Catharanthus roseus* pink variety(*Rosea*)	Negative	Negative	Negative
14	Flowers of *Catharanthus roseus* white variety(*alba*)	Negative	Negative	Negative
15	Flowers of *Plummer alba*(white)	± (11mm dia.)	± (11mm dia.)	± (11mm dia.)
16	Flowers of *Cascabela thevetia* (Yellow)	± (15mm dia.)	± (12mm dia.)	± (11mm dia.)
17	Flowers of *Portulaca granduflora*(White)	± (15mm dia.)	+ (20mm dia.)	± (12mm dia.)
18	Flowers of *Campsis grandiflora* (Pink)	Negative	Negative	Negative
19	Flowers of *Jasminum Sambac* (Malaga)	Negative	Negative	Negative
20	*Ethanol* alone (as control)	Negative	Negative	Negative

Positive (+): Inhibition of bacterial growth and Negative (-): No Inhibition of bacterial growth around the well. ±: Showing lesser inhibition (≥20mm).

The present study reveals that there was no inhibitory effect of the saline extracts of the leaves and flowers of *Catharanthus roseus* (*alba* and *rosea* variety), *Hibiscus* (red variety), *Campsis* (pink), *Portulaca* (white and pink), *Plumeria* (white), *Jasminum* (white serrated petals), and *Cascabela* (Yellow) on the growth of *Staphylococcus, E.coli and S.typhi.*

Ethanolic extract of the flowers of the above all also did not show any activity. The ethanolic extract of the leaves of *Catharanthus roseus (alba)* showed very strong antimicrobial activity against these microorganisms. Leaves of *Catharanthus* roseus pink variety (*Rosea*), flowers of *Portulaca granduflora* (White), flowers of *Cascabela thevetia* (Yellow), flowers of *Plumeria alba* (white) others were, however, 2nd,3rd,4th and 5th next in order. The strongest inhibitory activity was observed against *Staphylococcus aureus* (zone of inhibition with 35 mm dia.) followed by *S.typhi* and *E.coli* with 33mm dia. and 25mm in dia. Respectively(Table 2 and Figure 1).

Figures 1: Showing positive results with **ETHANOLIC EXTRACTS** as given below



Plates with negative results were, however, not shown.

Antibiotics:

Figure 2: Showing zone of inhibition of bacterial growth, presented below for comparison:

Results were compared with antibiotics discs viz. Ciprofloxacin(CIP), Erythromycin(E), Gentamicin(GEN), Lincomycin(L), Penicillin-G(P), Vancomycin (VA), present in HX090(Hexa G-plus 18) of HIMEDIA, mostly works against Gram positive and Gram negative organisms (Table 3 as below and Fig.2 above).

Table 3:

Discs	**Antibiotics**	***Staphylococcus aureus*** (mm dia.)	***E.coli*** (mm dia.)	***S.typhi*** (mm dia.)
CIP	Ciprofloxacin	32	18	32
GEN	Gentamicin	23	14	24
E	Erythromycin	13	25	20
VA	Vancomycin	12	R	13
P	Penicillin-G	25	R	R
L	Lincomycin	20	12	22

R=Resistant (without any zone of inhibition of bacterial growth).

DISCUSSION

Literature showed that plant kingdom contain many species of plants harboring substances of medicinal value that have yet to be discovered; though large numbers of plants are constantly being screened for their antimicrobial effects (Pankaj *et al*.2008). Medicinal plants are very important and widely available resources for primary healthcare and complementary healthcare systems. These plants show that they are

very rich source of compounds with possible antimicrobial activities, but more pharmacological investigations are necessary.

The present study deals with *in vitro* evaluation of the antimicrobial activity of saline and ethanolic extracts of leaves and flowers of *Catharanthus roseus* (white and pink variety) and certain other ornamental plants. These extracts were tested against three organisms viz. *Staphylococcus aureus*, *E.coli* and *Salmonella typhi*, all are human pathogenic bacteria. The ethanolic extract of the leaves of *Catharanthus roseus* white variety (*alba*) showed very strong antimicrobial activity against these microorganisms. The antimicrobial activity was evaluated by measuring the zone of inhibition. The strongest inhibition activity of the leaf extract was observed against *Staphylococcus aureus* (35 mm dia. zone of inhibition) followed by *S.typhi* with 33mmdia. and *E.coli* with 25mm dia. The results of this work are in agreement with those of Shanmugaraju and Bhakayaraj (2016). The ethanolic extract of *Catharanthusrose's* pink variety (*rosea*) was, however, second in order and others viz. flowers of *Portulaca granduflora* (White), flowers of *Cascabela thevetia* (Yellow), flowers of *Plumeriaalba* (white) otherswere3rd, 4th and 5th next in order. Our observations are supported with findings of Amax Khalil (2012).Prajakta and Ghosh (2010), however, reported that the extracts of C. roseus did not exhibit antibacterial activity against *Staphylococcus aureus*, and it could be due to the plant of different geographic origin with different climates and vegetations. Certain variations in observations in this study vis-à-vis others findings may be attributed to the above statements.

Saline extracts of the chosen leaves and flowers did not show any inhibitory effect. Our observation is supported with the statement of Thongson *et al*. (2004) who suggests that the extraction of antimicrobial substances by organic solvents is better as compared to aqueous extracts.

The present study shows that the ethanol extracts have inhibitory activity against the selected microorganisms. Inhibition of the growth of these organisms *in vitro* by the extracts may be due to the presence of some active constituents in the extracts. These active principles may have acted alone or in combination to inhibit the growth of the bacterial organisms. The problem of microbial resistance is growing and the outlook for use of antimicrobial drug in the future is still uncertain. Therefore, action must be taken to develop research to better understand the genetic mechanisms of resistance and to continue studies to develop new drug, either synthetic or natural. In the present study it was observed that *Catharanthus roseus* have brought about the possibility of utilization of plant extract, which has provided scientific evidence for the development of antibacterial products. As the ultimate goal is to offer appropriate and

efficient antimicrobial drugs to the patient, it can be used in the treatment of bacterial infection in future. The variation in findings,as different to others may be attributed to concentration dependence, different strain of the plants,different geographical origin,etc as claimed by Prajakta and Ghosh(2010) as above.

SUMMARY AND CONCLUSION

Leaves and flowers of few ornamental plants of family viz. *Catharanthus roseus* (white and pink variety), *Hibiscus* (red variety), *Campsis*, *Portulaca*, *Plumeria*, *Jasminum*, *Cascabela*, grown in the premises of VISM Gwalior were chosen to evaluate the antibacterial effect, if any, against three bacterial human pathogens (*Stphylococcus*, *E.coli* and *Salmonella typhi*).Saline extract and ethnolic extract were prepared using dry powder leaves and flowers of the above the plants. *Staphylococcus aureus, E.coli* and *Salmonella typhi* cultures were obtained from the Biotechnology dept. of VISM.These were grown in nutrient broth and poured in nutrient agar plates. Wells were cut equidistantly on these plates. The above preparations were added in the wells. The plates were observed after 24 hours in incubation 37°C .Clear zone of inhibition around the wells were measured with the help of ruler. The strongest inhibitory activity was observed against *Staphylococcus aureus* with zone of inhibition of 35 mm dia. followed by *S.typhi* with 33mm dia. and *E.coli* 25mm in dia. The results of the present study clearly suggested about the importance of *Catharanthus roseus* and its possible usage in treatment against infectious diseases caused by pathogenic microorganisms. However, the therapeutic potential of the plant should be checked when used in combination with other herbal drugs. It may help to discover new chemical classes of antibiotics that could serve as selective agents for the maintenance of animal or human health and provide biochemical tools for the study of infectious diseases.

Further, the biomolecules present in the extract which are active against these microbes needs to be characterized. Use of natural products has been encouraged due to less or no side effects, cost effectiveness and development of resistance to conventional synthetic antibiotics. Hence, this study holds importance in using ornamental plants as an alternative source for treating various diseases.

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