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ANTIMICROBIAL ACTIVITY IN ETHANOLIC EXTRACTS OF *BIXA ORELLANA* L., *SIMAROUBA GLAUCA* DC AND *OCIMUM TENUIFLORUM* L. COLLECTED FROM JNCH HERBAL GARDEN

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ABSTRACT

Medicinal plants contain several phytoconstituents that are used for treatment of common bacterial infections. Microbial activity cause serious damage to living organisms. In the present study ethanolic extracts were prepared from *Bixa orellana* leaves and fruit, *Simarouba glauca* leaves and *Ocimum tenuiflorum* leaves. The extracts prepared from these plants showed a wide spectrum of antibacterial activity against gram negative *Pseudomonas aeruginosa* and *Escherichia coli* and gram-positive *Streptococci* and *Staphylococci aureus* bacteria by disc diffusion method and growth inhibition assay.

KEYWORDS: Antimicrobial, *Streptococci* and *Pseudomonas aeruginosa.*

INTRODUCTION

The existence of microbes was found by mankind long back. The idea that some plants have therapeutic ability that is currently distinguished as antimicrobial principles and was very well accepted. Since ancient times, man has treated ordinary infectious diseases with the help of plants, and some of these customary medications are still included as part of the habitual therapy for various disease conditions. The uses of plants in the native society of developing countries are numerous and diverse.[2,19] The higher plants are a potential source of newly developed anti-infective agents, as shown by the screening of plant extracts and natural

antimicrobial products, as well as helping to discover drug from natural products for lead compounds.[14]

The use of medicinal plants as a potential source in therapy can be tracked down back over five millennia in the written papers on the premature human development in China, India and Near-east, but it was undoubtly an art as ancient as human race. Indian medicinal plants are regularly used in various systems of medicine because of less side effects and cost effectiveness.[2]

Nowadays, due to lower side effects and easier availability of medicinal plants, herbal remedies have become more popular.[7,11] Among the approximate 250,000-500,000 plant, only a short percent has been examined phytochemically and the fragment submitted to biological or pharmacological screening is even smaller. On global basis, at least 130 drugs, all single chemical structure extracted from higher plants, or improved further synthetically, are presently in use, even so for economic causes some of them are now being made synthetically.[21]

A rich source of antimicrobial agents is represented by medicinal plants.[20] The antimicrobial activities of many plants have been reported by many researchers.[8] In different countries, plants are used medicinally and are a source of many strong and powerful drugs. A broad span of medicinal plant parts is used for extract as raw drugs and they hold diverse medicinal properties. Root, stem, flower, fruit, twigs exudates and modified plant organs are the different parts that are used in various therapeutic purposes.

Even today, local communities and folk healers collected some of these raw drugs in smaller quantities for local use, many other raw drugs are manufactured in larger amounts and sold in the market as the raw materials for many herbal industries. Although, for antimicrobial properties, hundreds of plant species have been examined, many of them have not been appropriately evaluated and there can be substantial changes may occur based on the geographical locations in the antimicrobial activity. On the whole, the vast ability of plants as sources for antimicrobial agents, an organized investigation was tackled to screen the local flora of Madhya Pradesh, India for antimicrobial activity from *Ocimum tenuiflorum* L., *Simarouba glauca* DC. and *Bixa orellana* L.

MATERIALS AND METHODS

In the present study plant extracts were prepared and evaluated for anti-microbial activity by Disc diffusion method and Growth inhibition assay. The plants selected for the study were *Bixa orellana* L., *Simarouba glauca* DC and *Ocimum tenuiflorum* L.

Collection of plants

Fresh leaves of all the plants and fruit of *B. orellana* were collected from the Madan Mohan herbal garden at Jawaharlal Nehru Cancer Hospital and Research Centre, Idgah hill, Bhopal, India.

Preparation of plant extract

The leaves collected from four plants were properly cleaned from both the sides and kept aside for few minutes to dry at room temperature. The leaves were minced in small pieces using scissor. The scissor was cleaned and sterilized every time using methanol. The hydroalcoholic (50%) extracts was prepared using ethanol and double distilled water in the ratio of 1:1. The minced leaves were soaked in 40 ml of 50% ethanol in fresh beaker for 24 h. After 24 h the extract was centrifuged at 1000 rpm for 15 minutes. The prepared extract was used for further experiments and rest of the extract is stored at 4℃ in a refrigerator.

Antimicrobial activity

The antimicrobial activity was screened by disc diffusion method and growth inhibition assay using different bacterial strains.

Bacterial strains

The bacterial strains used for the present study were isolated in Department of Research, Jawaharlal Nehru Cancer Hospital and Research Centre. The strains used for the study were two-gram positive bacteria *Streptococci* and *Staphylococci aureus* and two-gram negative bacteria namely *Pseudomonas aeruginosa* and *Escherichia coli*.

Disc diffusion method

Disc diffusion method for antimicrobial activity was performed to evaluate the antibacterial activity of the plant extracts. In this method, discs were prepared of Whatman's filter paper using punch machine. The discs were taken in different petri plates and loaded with extracts and air dried and subsequent loading was done with drying. After this, the discs were taken in

glass vials, labeled and autoclaved. The discs for negative control were prepared in ethanol only.

The bacterial strains were inoculated on Mueller Hinton plates using stirrer of high quality stainless steel. After inoculation, the extract discs were placed using sterile forceps. The negative and positive control discs were also placed and the plates were incubated at 37℃ for 24 hours in the incubator. The plates were observed after 24 h of treatment and zone of inhibition were calculated. For positive control standard disc containing the contents of table 3 and 4 were placed separately for gram positive and gram negative bacteria.

Growth inhibition Assay

The nutrient agar media was prepared and poured in plates with 500 µl of ethanolic extract and mixed using stirrer. The media was allowed to solidify and after solidification bacterial strains were inoculated using swab. Incubation of plates was done at 37℃ for 24 h, the plates were observed after incubation and the number of bacterial colonies was counted.

RESULTS

In the present study three plants were selected which includes *Bixa orellana, Simarouba glauca* and *Ocimum tenuiflorum*. The extracts showed potent antimicrobial activity by disc diffusion method and growth inhibition assay.

Disc diffusion Assay

The ethanolic crude plant extracts was loaded on discs and placed on Mueller Hinton plates showed potent antimicrobial activity against all strains used for the study. The strains used for the study were *Streptococci, Pseudomonas aeruginosa, Staphylococcus aureus* and *Esterichia coli.* ***Ocimum tenuiflorum*** both the species showed less zone of inhibition against bacterial strains used. Whereas, *Simarouba glauca* gave zone of inhibition of 6 mm against gram negative bacteria *Pseudomonas aeruginosa* and no zone of inhibition found against gram positive bacterial strains. *Bixa orellana* showed considerable potent activity as compared to other plant extracts and gave zone of inhibition of 7 mm in both leaves and fruit extract against gram negative bacteria *P. aeruginosa*. The crude ethanolic extract of *B. orellana* leaves showed 6 mm zone of inhibition against gram negative bacteria *E. coli.* The results of Disc diffusion are given below in tabular form. The standard disc as positive control was used for the study for gram positive and gram negative bacteria given in tabular form for comparative study.

Growth Inhibition Assay

The nutrient agar plates were taken along with 500µl of extract and inoculated with bacterial strains. The extracts which inhibited the growth of bacteria showed less or no growth in culture plates. The ethanolic extracts of *O. tenuiflorum*, *B. orellana* and *S. glauca* leaves inhibited the growth of *Pseudomonas aeruginosa*, whereas extract of *Bixa orellana* fruit inhibited the growth of *Streptococci* bacteria.

Table 1: Plants Name and its description.

COMMON NAME	BOTANICAL NAME	FAMILY	PLANT PART USED
Kapoor tulsi	*Ocimum tenuiflorum*	Lamiaceae	Leaves
Shyama tulsi	*Ocimum tenuiflorum*	Lamiaceae	Leaves
Lakshmi taru	*Simarouba glauca*	Simaroubaceae	Leaves
Sindur	*Bixa orellana*	Bixaceae	Leaves
Sindur	*Bixa orellana*	Bixaceae	Fruits

Table 2: The zone of inhibition of ethanolic plant extracts against *Streptococci*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. The diameter of zone of inhibition (mm). No zone of inhibition indicated by NZ.

S. No.	Plant Name	Botanical Name	Zone of Inhibition (in mm)			
			Streptococci	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Escherichia coli*
1	Kapoor Tulsi	*Ocimumtenuiflorum*	NZ	NZ	NZ	NZ
2	ShayamaTulsi	*Ocimumtenuiflorum*	NZ	NZ	NZ	NZ
3	Lakshmi Taru	*Simarouba glauca*	NZ	6 mm	NZ	NZ
4	Sindur leaf	*Bixaorellana*	NZ	7 mm	NZ	6 mm
5	Sindur fruit		NZ	7 mm	NZ	NZ

Table 3: List of Standard Drug used for Gram positive bacteria. No zone of inhibition indicated by NZ. The diameter of zone of inhibition (mm).

S. No.	Drug Name	Zone of Inhibition (mm)	
		Streptococci	*Staphylococcus aureus*
1.	Cefotaxine	NZ	-
2.	Ceftriaxome	NZ	-
3.	Ceftazidine	NZ	-
4.	Nitrofurantoin	NZ	-
5.	Gentamicin	NZ	-
6.	Amikacin	NZ	-
7.	Cefuroxime	20 mm	-
8.	Cefixime	NZ	-
9.	Nalidixic acid	NZ	-
10.	Cefdinir	NZ	-
11.	Cephalexin	18 mm	-

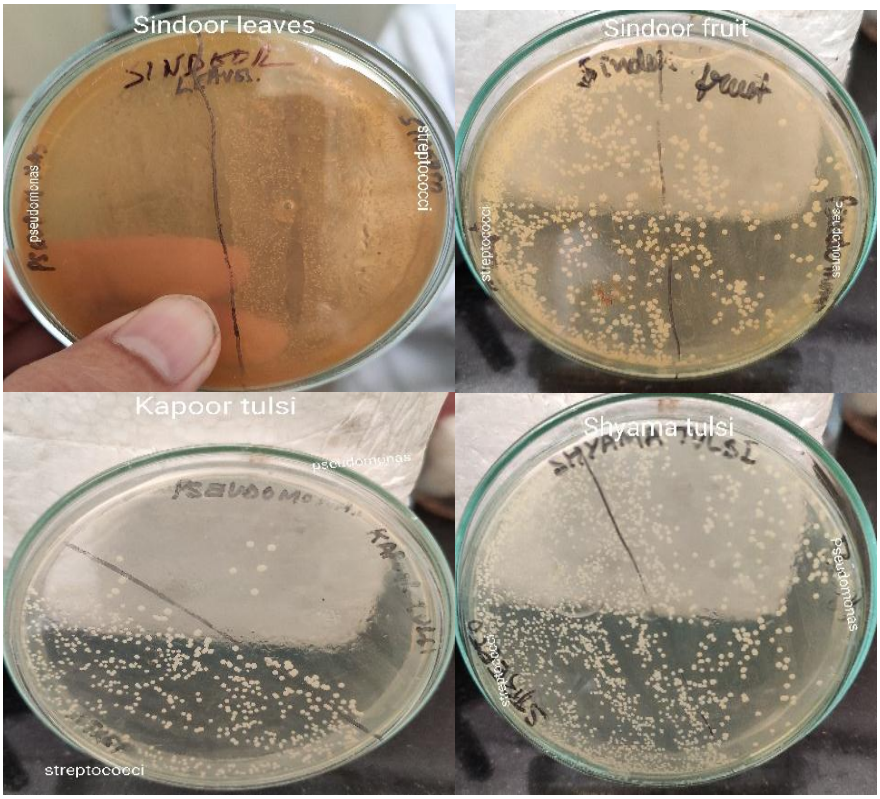
12.	Tetracyclin	10 mm	-
13.	Piperacillin	30 mm	-
14.	Pinicillin G	16 mm	-
15.	Cefazolin	16 mm	-
16.	Chloramphenicol	14 mm	-
17.	Ciprofloxacin	7 mm	-
18.	Erythromycin	13 mm	-
19.	Azithromycin	12 mm	-
20.	Cotrimoxazol	18 mm	-
21.	Amoxicillin clavulanic acid	25 mm	-
22.	Amoxicillin	25 mm	-

Table 4: List of Standard Drug used for Gram negative bacteria. No zone of inhibition indicated by NZ. The diameter of zone of inhibition (mm).

S. No.	Drug Name	Zone of Inhibition (mm)	
		Pseudomonas aeruginosa	***Escherichia coli***
1.	Cefotaxine	23 mm	-
2.	Ceftriaxome	20 mm	-
3.	Ceftazidine	12 mm	-
4.	Nitrofurantoin	18 mm	-
5.	Gentamicin	12 mm	-
6.	Amikacin	10 mm	-
7.	Cefuroxime	18 mm	-
8.	Cefixime	8 mm	-
9.	Nalidixic acid	8 mm	-
10.	Cefdinir	20 mm	-
11.	Cephalexin	-	-
12.	Tetracyclin	-	-
13.	Piperacillin	-	-
14.	Pinicillin G	-	-
15.	Cefazolin	-	-
16.	Chloramphenicol	-	-
17.	Ciprofloxacin	-	-
18.	Erythromycin	-	-
19.	Azithromycin	-	-
20.	Cotrimoxazol	-	-
21.	Amoxicillin clavulanic acid	-	-
22.	Amoxicillin	-	-

Table 5: The Growth inhibition assay of ethanolic plant extracts against *Streptococci, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*.

S. No.	Plant Name	*Streptococci*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Escherichia coli*	*Streptococci*
1	Kapoor Tulsi	Moderate growth of bacteria	No growth	No inhibition	No inhibition	No inhibition
2	ShayamaTulsi	No inhibition	Scanty growth of bacteria	No inhibition	No inhibition	No inhibition
3	Lakshmi Taru	Moderate growth of bacteria	No growth	No inhibition	No inhibition	No inhibition
4	Sindur leaf	No growth	No growth	No inhibition	Scanty growth of bacteria	Scanty growth of bacteria
5	Sindur fruit	No inhibition	Scanty growth of bacteria	No inhibition	No inhibition	No inhibition



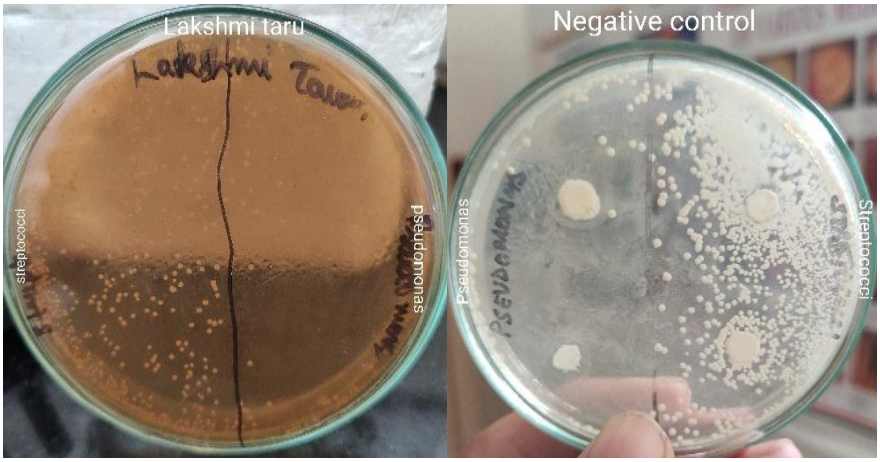


Figure 1: Growth inhibition of different plant extracts against *Pseudomonas aeruginosa* and *Streptococcus* (A) Sindur leaves (B) Sindur fruit (C) Kapoor Tulsi (D) Shyama Tulsi (E) Laxmi Taru (F) Control.

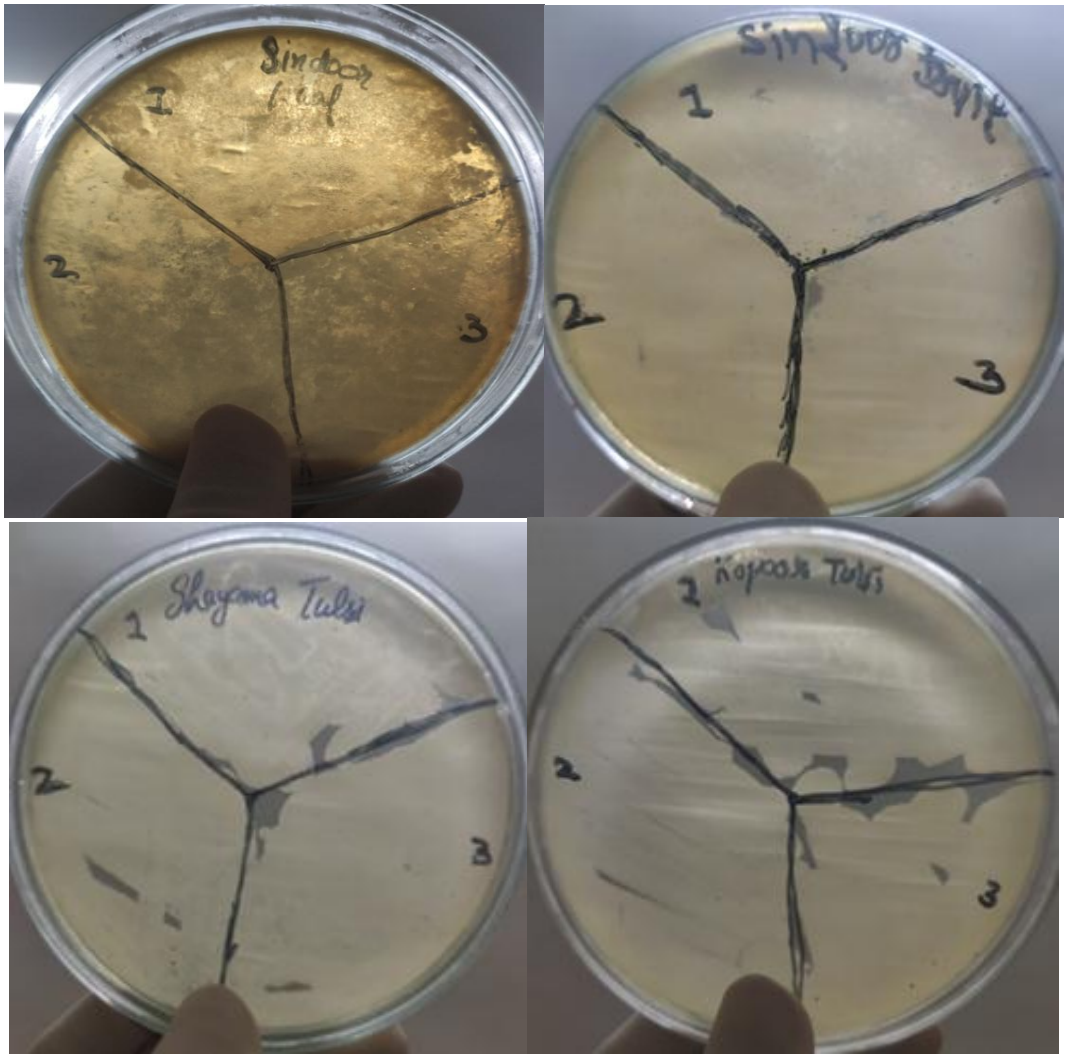


Figure 2: Growth inhibition of different plant extracts against *Eschirichia coli* and *Staphylococcus aureus* (A) Sindur leaves (B) Sindur fruit (C) Kapoor Tulsi (D) Shyama Tulsi (E) Laxmi Taru.

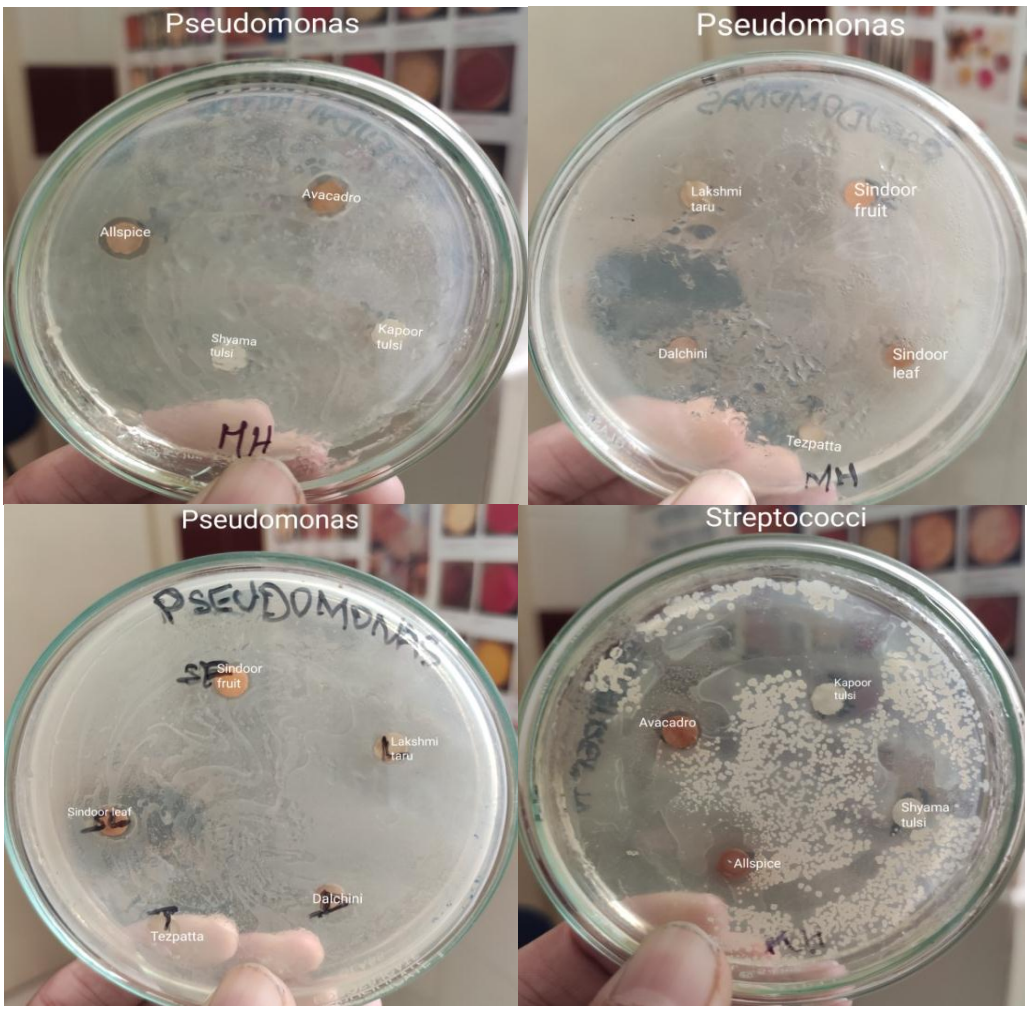


Figure 3: Zone of inhibition (mm) of different plant extracts against *Pseudomonas aeroginosa* and *Streptococcus* by disc diffusion method.

DISCUSSION

Although, many plant species have been examined for antimicrobial properties, however, the majority of them have not still been analytically assessed.[22] Our work illustrates a rigorous explanation of the antimicrobial activity of *Bixa orellana*, *Ocimum tenuiflorum* and *Simarouba glauca.*

Bixa orellana

Bixin, a red-colored carotenoid, is the pigment present in high concentration in the annatto seed aril.[13] Numerous researchers have reported antimicrobial activity in *Bixa orellana* leaves extract. In one of the studies done by Fleischer *et al* (2002)[17] showed antimicrobial activity in *B. orellana* ethanolic extracts of leaves and seeds against gram positive and gram-negative bacteria. The ethanolic leaves extract gave zone of inhibition 19.5 mm and 19 mm against *Streptococcus pyogenes* and *Pseudomonas aeruginosa* respectively. Whereas, ethanolic seeds extract gave zone of inhibition of 17 mm and 19 mm against *S. pyrogenes* and *P. aeruginosa* respectively. In another study, ethanolic leaves extract of *B. orellana* showed effective antimicrobial activity by standard method against *E. coli*, *B. subtilis*, *P. aeruginosa* and *S. aureus* with zone of inhibition 25 mm, 17 mm, 17 mm and 14 mm respectively.[15] The methanolic extract of *B. orellana* gave in vitro antimicrobial activity against *S. aureus* with MIC values of 15.62 µg/ml in leaves extract and 62.5 µg/ml in seeds extract (Tamil, 2011).[4] Irobi and his team (1996)[16] reported inhibitory effect of the leaf extract of Annatto against different test organisms. The methanolic extract of *B. orellana* demonstrated *in vitro* antibacterial effect against *S. mutans* and *S. sanguinis* strains (Flores *et al*, 2016).[18] In the

present study ethanolic extract of *B. orellana* leaves showed 6 mm zone of inhibition against gram negative bacteria *E. coli* and inhibited the growth of bacteria *Pseudomonas aeruginosa*.

Ocimum tenuiflorum

Ocimum tenuiflorum species was selected for the present study, the common name were Kapoor tulsi and Shyama tulsi. Both the plants were reported for its antimicrobial activity. Naik *et al* (2015)[19], reported hexane, acetone and methanolic extracts of leaves of *O. tenuiflorum* showed antimicrobial activity by disc diffusion method against gram negative (*Pseudomonas putida*, *Klebsiella pneumonia* and *Escherichia coli)* and gram positive (*Staphylococcus aureus* and *Bacillus subtilis)* bacteria. A study exhibited potent antimicrobial activity in finished textiles of cotton, polyester, bamboo and a blend of polyester and cotton using *O. tenuiflorum* against gram positive and gram negative bacterial strains.[21] Other study showed antimicrobial activity in essential oils distilled from Australian-grown *O. tenuiflorum*. The growth of *S. aureus* and *E. coli* was completely inhibited at the concentration of 4.5% and 2.25% essential oil extracted from *O. tenuiflorum*, whereas the growth of *P. aeruginosa* was partially inhibited at the same concentration.[19] One of the studies showed potent antimicrobial activity of the different leaf extracts (Ethanol, Methanol, Ethyl acetate and chloroform) of *O. tenuiflorum* also known as *Ocimum sanctum*, against three human pathogens *E. coli, S. aureus* and *C. albicans*. In the present study *O. tenuiflorum* inhibited the growth of *Pseudomonas aeruginosa* strain.[5]

Simarouba glauca

Simarouba glauca commonly known as Sindur in India and known for its dye obtained from fruits of the plant also reported for its antimicrobial activity. Jain and his co-workers (2016)[1] showed antimicrobial activity in aqueous extracts of leaves of *S. glauca* against *E. faecalis* by agar diffusion method. The crude methanolic and ethanolic extracts prepared from dried and fresh leaves of *S. glauca* inhibited the growth of *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli*.[3] Varghese and his team (2016)[6] also reported antimicrobial components from the leaf of *S. glauca* against gram positive and negative bacteria. Apart from antimicrobial, antioxidant, hemolytic and thrombocytic activities were also reported.[1] Jangale[3] and co-workers reported the crude ethanol and methanol extracts from dried and fresh leaves of *Simarouba glauca* were tested for their inhibitory activity against two food borne pathogenic microorganisms (*Staphylococcus aureus* and *Escherichia coli*) and two food spoilage microorganism (*Bacillus Subtilis* and

Pseudomonas aeruginosa) for antimicrobial activity using well diffusion assay and found that the plant showed inhibition against entire tested microorganisms.[9]

CONCLUSION

The ethanolic extracts prepared from all the three plants showed potent antimicrobial activity against two gram positive and gram negative bacteria. The extract of *Bixa orellana* showed highest inhibition as compared to *Ocimum tenuiflorum* and *Simarouba glauca* extracts. The plant can be further studied and investigated. The investigation and research on medicinal plants might bring to the scientific world numerous capable remedies for the treatment and cure of human being sufferings. It can be concluded that additional research on these plants may helpful for future researchers to expand some new pharmacophores.

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REFERENCES

1. Jain M, George R, Theruvil R, Padavil TC, Tomy L and Kurian A. Antibacterial Activity of Leaf Extract of *Annona muricata* and *Simarouba glauca* on *Enterococcus faecalis*. J Contemp Dent Pract, 2016; 17(8): 650-653.
2. Rajurkar BM. A Comparative study on antimicrobial activity of *Clerodendrum infortunatum, Simarouba glauca* and *Psoralea corylifolia.* IJRRPAS, 2011; 1(4): 278-282.
3. Jangale BL, Ugale TB, Aher PS, Toke NR, Shivangikar AN and Sanap NT. Antibacterial activity of *Simarouba glauca* leaf extracts against food borne spoilage and pathogenic. IJPSR, 2012; 3(2): 497-500.
4. Tamil SS, Dinesh MG, Satyan RS, Chandrasekaran B and Rose C. Leaf and seed extracts of *Bixa orellana* L. exert anti-microbial activity against bacterial pathogens. J Applied Pharm Sci, 2011; 01(09): 116-120.
5. Gomathinayagam S, Brij B. Tewari and Rekha G. Studies of Antimicrobial Properties of Different Leaf Extract of Tulsi (Ocimum tenuiflorum) against Human Pathogens. American Int J Contemporary Res, 2014; 4(8): 149-157.

6. Varghese J, Rajamani S and Daniel B. Antimicrobial Effect of *Simarouba glauca* (Lakshmi Taru) on Opportunistic Pathogens in HIV/AIDS patients. IOSR J Pharmacy and Biological Sci, 2016; 11(6): 32-39.
7. Ramya KS. GC–MS analysis and antimicrobial activity of various solvent extracts from *Simarouba glauca* leaves. J Pharma Phytochemistry, 2019; 8(2): 166-171.
8. Mahalingam R, Ambikapathy V, Panneerselvam A. and Prince L. Biological activities of some medicinal plants against *Setophaeriaro strata* causing seedling blight disease in sugarcane. Asian J Plant Sci Res, 2011; 1(2): 92-95.
9. Antony J, Thomas A, Gnanasekaran D and Elizabeth H. Review study on Pharmacological importance of *Simarouba glauca*. IJNTR, 2016; 2: 59-62.
10. Udayaprakash NK, Ranjithkumar M, Deepa S, Sripriya N, Al-Arfaj AA and Bhuvaneswari S. Antioxidant, free radical scavenging and GC–MS composition of *Cinnamomum iners* Reinw. ex Blume. Ind Crops Prod, 2015; 69: 175-9.
11. Nishaa S, Vishnupriya M, Sasikumar JM and Gopala krishnan VK. Phyto chemical Screening and GC-MS Analysis of Ethanolic Extract of Rhizomes of *Maranta arundinacea* L. RJPBCS, 2013; 4(2): 52-59.
12. Vilar DA, Vilar MSA, Moura TFAL, Raffin FN, Oliveira MR, Franco CFO, Filho PFA, Diniz MFFM and Filho JMB. Traditional Uses, Chemical Constituents and biological Activities of *Bixa orellana* L.: A review The Scientific World J, 2014; ISSN 23566140.
13. Khazir J, Mir BA, Mir SA and Cowan D. Natural products as lead compounds in drug discovery. J Asian Nat Prod Res, 2013; 15(7).
14. Erdogrul OT. Antibacterial activities of some plant extracts used in folk medicine. Pharma biol, 2002; 40(4): 269-273.
15. Rajendra SB. Antibacterial activity of the ethanolic leaves extract of *Bixa orellana*. World J Pharm Pharma Sci, 2014; 3(9): 389-394.
16. Irobi ON, Moo-Young M and Anderson WA. Antimicrobial activity of Annatto (*Bixa orellana*) extract. Int J Pharmacognosy, 1996; 34: 87-90.
17. Fleischer TC, Ameade EPK, Mensah MLK and Sawer IK. Antimicrobial activity of the leaves and seeds of *Bixa orellana*. Elsevier Science, 2002; 74(1-2): 136-138.
18. Flores DM, Urizar GU, Colarossi RC, García SC, Tovalino FM and Mendoza1 JV. Antibacterial activity of *Bixa orellana* L. (achiote) against *Streptococcus mutans* and *Streptococcus sanguinis.* Asian Pac J Trop Biomed; 2016; 6(5): 400-403.

19. Naik LS, Shyam P, Marx KP, Baskari S and Devi VR. Antimicrobial Activity and Phytochemical Analysis of *Ocimum tenuiflorum* Leaf Extract. Int J Pharm Tech Res, 2015; 8(1): 88-95.
20. Yamani HA, Pang EC, Mantri N and Deighton MA. Antimicrobial Activity of Tulsi (*Ocimum tenuiflorum*) Essential Oil and Their Major Constituents against Three Species of Bacteria. Frontiers in Microbiology, 2016; 7(681).
21. Divisha R, Ranganathan V, Vijayakaran K and Elamaran A. Evaluating *Ocimum basilicum* and *Ocimum tenuiflorum* leaf extracts for the presence of phenolic compounds. J Pharma Phytochemistry, 2018; 7(6).
22. Ijaz M, Maqsood M and Naveed M. Evaluation of antimicrobial activity of *Ocimum tenuiflorum* leaves on mechanical behavior of fabrics. Pure and Applied Biol, 2019; 9(1): 538-544.