Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN 2320-3862
JMPS 2016; 4(3): 283-290

Received: 26-03-2016
Accepted: 29-04-2016

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Antimicrobial activity of ethno-medicinal plants against cariogenic pathogens

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Abstract
Medicinal plants have become part of complementary medicines worldwide and have been explored for centuries because of their potential health assistance. Recent years have revived interest in medicinal plants for drug discovery. Presently, infectious diseases caused by fungi, bacteria, viruses and parasites are becoming a major threat to public health. Among these dental caries and periodontal diseases are the most important and most common global oral health problems. Dental caries are caused by tooth enamel demineralization by acidophilus microorganisms growing as a biofilm or plaque. Hence, extracts from different parts of six plants viz. *Bougainvillea spectabilis, Cordia obliqua, Dahlia, Hibiscus rosa-sinensis, Bambusa arundinacea, Bombax ceiba*, were investigated for their antimicrobial activity against oral pathogens. Extracts were prepared using dried plant powder dissolved in three different solvents (distilled water, methanol and ethanol). The resulting solutions were lyophilized and the powder thus obtained was dissolved in 10% DMSO. The antibacterial activity of these extracts was assessed against some cariogenic bacteria (*Streptococcus mutans* (MTCC No: 890), *Staphylococcus aureas* (MTCC No: 3160), *Candida albicans* (MTCC No: 183), *Lactobacillus acidophilus* (MTCC No: 10307), *Streptococcus gordonii* (MTCC No: 2695) using agar well diffusion assay. Kanamycin (100µg/ml) was used as positive control and DMSO (10%) as negative control in agar well diffusion assay. Microbial growth inhibition was determined by measuring the diameter of zone of inhibition, excluding the diameter of well. Methanolic extract of *Dahlia* showed antimicrobial activity against all the cariogenic bacteria used in the study. Distilled water extract of *Hibiscus rosa-sinensis* showed the largest zone of inhibition against *Candida albicans* and that of *Bougainvillea* against *Candida albicans and Streptococcus mutans.* Ethanolic extract of *Dahlia* showed activity against oral microbes but zone of inhibition was comparatively smaller.

Keywords: Cariogenic Pathogens, *ethno-medicinal*

Introduction
Oral diseases including dental caries, gingival inflammation, periodontal disease, and tooth loss are affecting overall health significantly. Among these, dental caries is the most common chronic oral disease which affects 60% to 90% of young population (Petersen, 2003). Dental caries or cavities, more commonly known as tooth decay, are caused by plaque formation that leads to demineralization of the tooth enamel. Dental plaque is a biofilm consisting of microorganisms generally present on the tooth surface that plays an important role in the development of dental caries and periodontal diseases (Koo *et al*., 2000). The main microorganisms associated with dental caries are, in order of frequency: 1. *Streptococcus mutans* and to a lesser extent *S. sobrinus* and *S. gordonii* (Loesche, 1986) and 2. *Lactobacillus* and *Actinomyces* species (Marcotte *et al.*1998).
Streptococci especially *Streptococcus mutans* have been implicated as the primary causative organism as acidic oral environment is well tolerated by *S. mutans* and *Lactobacillus acidophilus*. *S. mutans* mainly initiate tooth decay by dissolving tooth structures in the presence of fermentable carbohydrates such as sucrose, fructose, and glucose (Kleinberg, 2002) while *Lactobacillus acidophilus* makes it progress to dental cavity-like lesion (Swift *et al*., 2002). Antimicrobial mouth rinses such as triclosan and chlorhexidine may be used to limit these two plaque-related oral infections (Baca *et al*., 2009). But these mouth rinses may have undesirable side effects such as tooth staining, taste alteration and development of hypersensitivity reactions (Chang *et al*., 2001). Although antibiotics are used routinely to prevent systemic infections originating from the oral cavity but they are not recommended because of the risk that bacteria will develop resistance to them. For these reasons, alternative

Herbal medicines have been used as traditional treatment for numerous human diseases for centuries in many parts of the world. Antibacterial chew sticks (miswak), which are plant based alternatives for oral health has been successfully promoted and they have been advocated by health agencies (Tichy and Novak, 1998). The different plant parts such as seed, fruit, root, bark, stem, leaf and even the whole plant were extracted using different solvents like ethanol, methanol, petroleum ether, chloroform, acetone, alcohol and ethyl acetate. These extract were tested by diffusion method against gram positive, gram negative bacteria and fungi to assess their antimicrobial activity. For example, *Bougainvillea*, an ornamental plant, has been used in traditional medicine as anti-inflammatory, antidiabetic, antibacterial and antiviral agent. *B. spectabilis* showed antimicrobial activity against Gram positive bacteria like *Bacillus subtilis and streptococcus faecalis* and Gram negative bacteria like *Shigellaa flexneri, Vibrio cholerae, Salmonella typhii, Serratia marcescens* and *Klebsillea pneumonia* (Umamaheswari *et al.*, 2008). Similarly, *Hibiscus rosa-sinensis*, an evergreen, sweet, astringent, cooling herb that checks bleeding, soothes irritated tissues and relaxes spasms (Uddin *et al.*, 2010).

Materials and Methods

Plant Materials

Different plant parts (leaves, bark, twigs and flowers) from six different plant species were collected during winter season from the campus of Guru Jambheshwar University of Science and Technology, Hisar and Herbal Garden, Hisar.

Table 1: List of plants used in the study

Sr. No.	Plants	Parts of plant used
1	*Bougainvillea spectabilis*	Flowers
2	*Cordia oblique*	Twigs
3	*Dhalia*	Flowers
4	*Hibiscus rosa-sinensis*	Leaves
5	*Bambusa arundinacea*	Leaves
6	*Bombax ceiba*	Flowers

Test Microorganisms

Pure cultures of all experimental microbes were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. The pure microbial cultures were maintained on nutrient agar medium and yeast culture on Yeast Extract Peptone Dextrose medium (YPED). Each bacterial and yeast culture was further maintained by sub-culturing regularly on the same medium and stored at 4^0C before use in experiments.

Table 2: List of Pure Microbial Cultures Used For Study

Sr. No.	Culture	MTCC No.	Media
1	*Streptococcus mutans*	890	Brain Heart Infusion
2	*Staphylococcus aureus*	3160	Nutrient Agar
3	*Candida albicans*	183	Yeast Extract Peptone Dextrose
4	*Lactobacillus acidophilus*	10307	MRS
5	*Streptococcus gordonii*	2695	Brain Heart Infusion

Plants Extract Preparation (Handa *et al*., 2008)

Plant materials were grinded using pestle and mortar, air dried and 20g of that coarse powder was poured in a glass stoppered conical flask and macerated with 100ml of analytical grade solvents (distilled water, methanol and ethanol). The mixture was kept on continuous shaking at 100 rpm for 72 hours at 30°. After incubation the mixture was allowed to stand for 24 hours and then supernatant was taken out carefully and transferred into other tubes followed by filtration through Whattman No. 1 filter paper. Filtrate was transferred to flat-bottom dish and solvent was evaporated using a Lyophilizer. Dry extracts were dissolved in 10% DMSO in the concentration 50mg/ml.

Determination of Antibacterial Activity

Agar Well Diffusion Assay (Schillinger and Lucke, 1989)

To make activated culture of bacteria, 100µl of bacterial suspension was inoculated in 100ml of Nutrient Broth and incubated for 18hr at 30°C in shaking incubator at 120rpm. 25ml of sterile Mueller-Hinton Agar No.2 (Hi-Media), inoculated with 100µl of bacterial culture, was poured into sterile autoclaved petri plates and allowed to solidify completely. The wells were prepared with the help of sterile 6mm diameter cork-borer. Then 100µl of each plant extract from stock (25mg dry powder extract/ml) solution was poured into the wells. Kanamycin (100µg/ml) was used as a positive control and 10% DMSO as negative control. The plates were then incubated at 37°C for 24 hours. Microbial growth inhibition was determined by measuring the diameter of the zone of inhibition, excluding the diameter of well.

Results and Discussion

In vitro antibacterial activity of plant extracts and their potency was assessed qualitatively as well as quantitatively by determining diameter of zone of inhibition. The analysis of six plants extracts which showed positive inhibitory activity against almost every strain in agar well diffusion assay is given below:

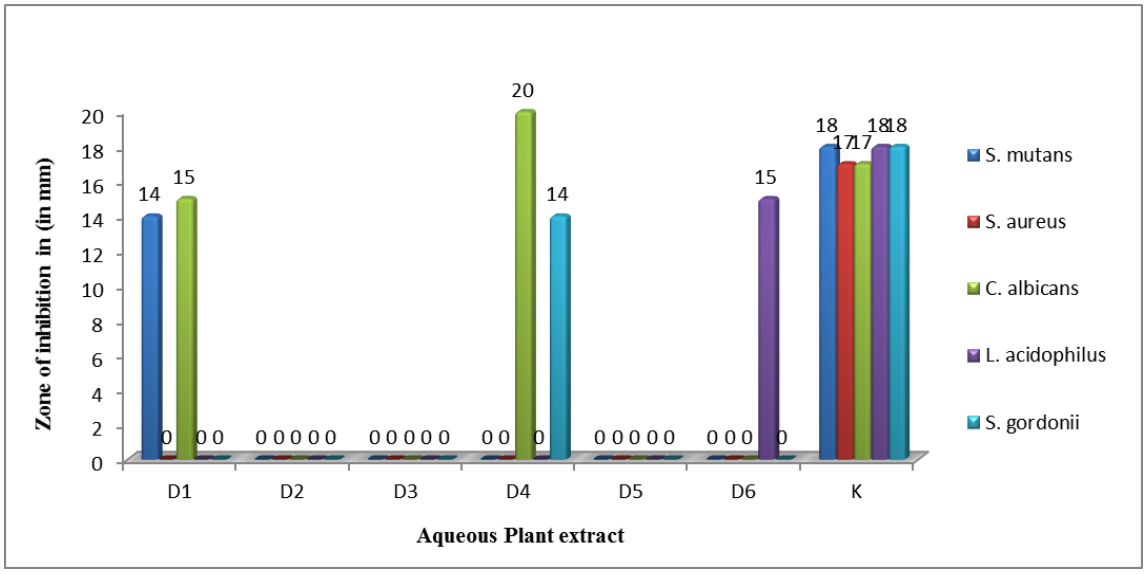
Table 3: Detail of coding of plants as per the type of extraction solvent

Sr. No.	Plant Name	Aqueous extraction	Ethanol extraction	Methanol extraction
1	*Bougainvillea spectabilis*	D1	E1	M1
2	*Cordia obliqua*	D2	E2	M2
3	*Dahlia*	D3	E3	M3
4	*Hibiscus rosa-sinensis*	D4	E4	M4
5	*Bambusa arundinacea*	D5	E5	M5
6	*Bombax ceiba*	D6	E6	M6

M3 and E3 (*Dahlia*) showed positive antimicrobial activity against all the five strains and the largest zone of inhibition Was observed in aqueous extract of *Hibiscus rosa-sinensis* against *Candida albicans.*

Table 4: Antimicrobial activity of aqueous plant extract (in mm)

Sample	*S. mutans*	*S. aureus*	*C. albicans*	*L. acidophilus*	*S. gordonii*
D1	14	-	15	-	-
D2	-	-	-	-	-
D3	-	-	-	-	-
D4	-	-	20	-	11
D5	-	-	-	-	-
D6	-	-	-	15	
K	18	15	17	20	15

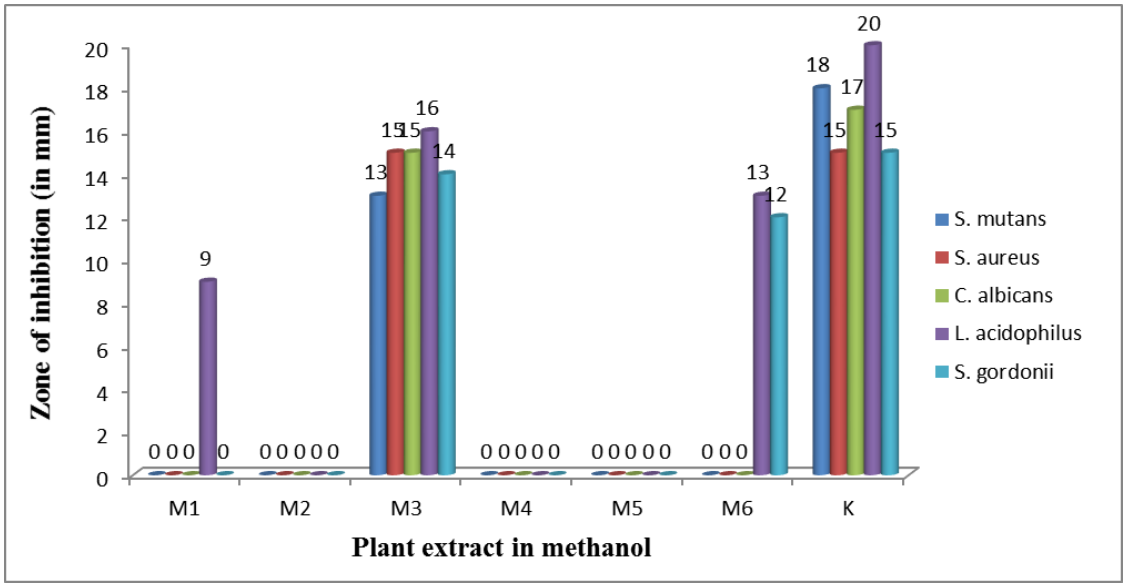


Graph 1: Zone of inhibition: Aqueous extract of plants

Table 5: Antimicrobial activity of Methanolic extract (in mm)

Sample	*S. mutans*	*S.aureus*	*C. albicans*	*L. acidophilus*	*S. gordonii*
M1		-	-	9	-
M2	-	-	-	-	-
M3	13	15	15	16	14
M4	-	-	-	-	-
M5	-	-	-	-	-
M6	-	-	-	13	12
K	18	15	17	20	15

M3 has had the most significant zone of inhibition against *L. acidophilus* and considerably equal zone of inhibition against other strains also which varied from each other just by 1 or 2 mm. M1 displayed the activity only against *L. acidophilus* and M6 showed activity against *L. acidophilus* and *Streptococcus gordonii* . M2, M4 and M5 exhibited no antimicrobial activity against any of the bacterial strain.

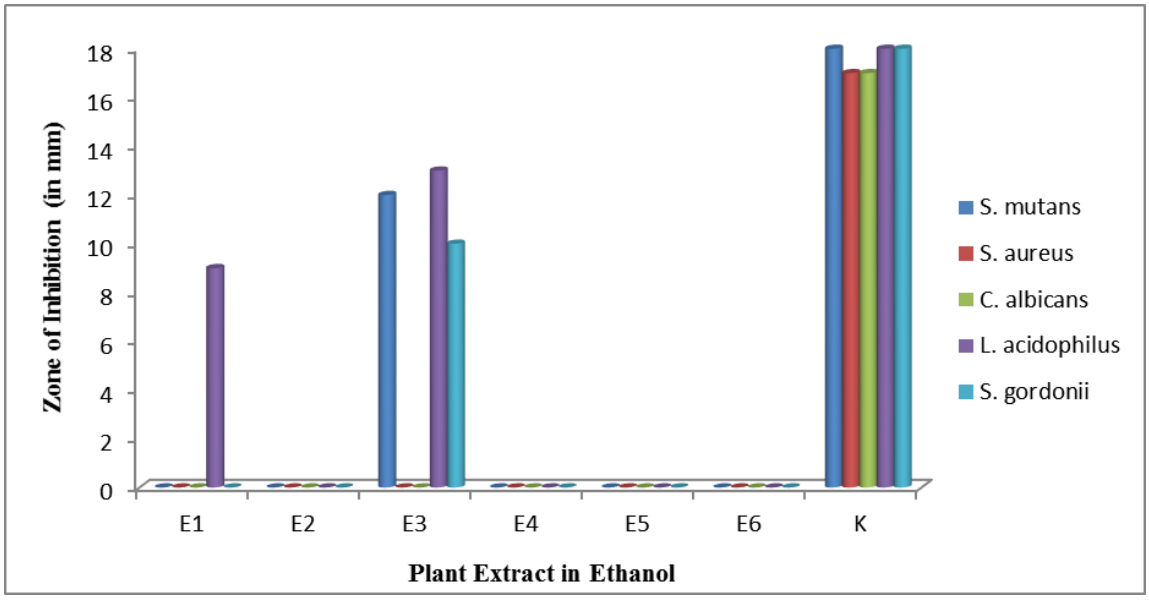


Graph 2: Zone of inhibition: Methanolic extract of plants

This graph has illustrated the antimicrobial activity of methanolic extracts of plants. The extract of *Dhalia* (M3) showed antimicrobial activity against all the five cariogenic bacterial strains. The biggest zone of inhibition measured 16mm is against *L. acidophilus*. M1 and M6 displayed activity against *L. acidophilus*. M6 inhibited the growth of *S. gordonii*.

Table 5: Antimicrobial activity of Ethanolic extract (in mm)

Sample	*S. mutans*	*S. aureus*	*C. albicans*	*L. acidophilus*	*S. gordonii*
E1	-	-	-	9	-
E2	-	-	-	-	-
E3	12	-	-	13	10
E4	-	-	-	-	-
E5	-	-	-	-	-
E6	-	-	-	-	-
K	18	17	18	18	17



Graph 3: Zone of inhibition: Ethanolic extract of plants

E3 has had the most significant zone of inhibition against *L. acidophilus.* It also inhibited the growth of *S. mutans* and *S. gordonii.* E1 showed the activity only against *L.acidophilus*. E2, E4, E5 and E6 exhibited no antimicrobial activity against any of the bacterial strains.

The results of antimicrobial potency of ethanol, methanol and aqueous extracts of various medicinal plants, the positive control Kanamycin and the zone of inhibition of these extracts against the test pathogens are presented in the study. Methanolic extract of plant *Dahlia* showed antibacterial activity against all five bacteria l strains chosen. Aqueous extract of plant *Hibiscus rosa-sinensis* showed the widest zone of inhibition, which was against *C. albicans*.

Images of antimicrobial activity against different oral pathogens

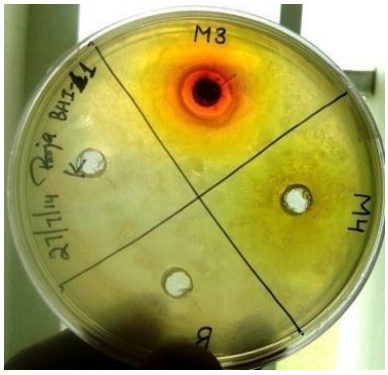


Fig 1: Activity of M3 against *S. mutans*

Fig 2: Activity of E3 against *S. mutans*

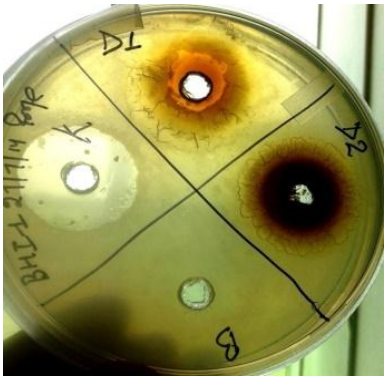


Fig 3: Activity of D1 against *S. mutans*

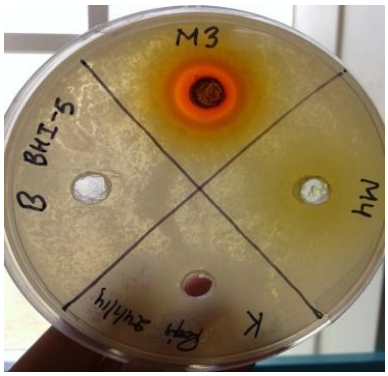


Fig 4: Activity of M3 against *S. gordonii*

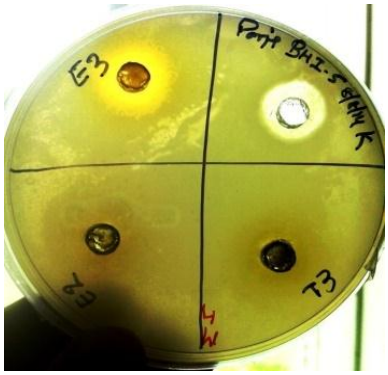


Fig 5: Activity of E3 against *S. gordonii*

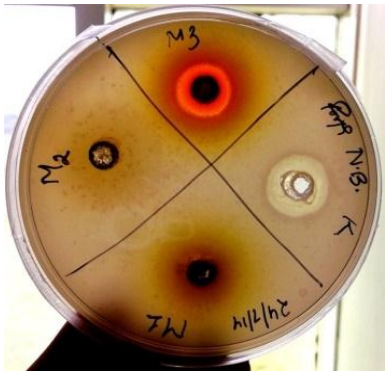


Fig 6: Activity of M3 against *S. aureus*

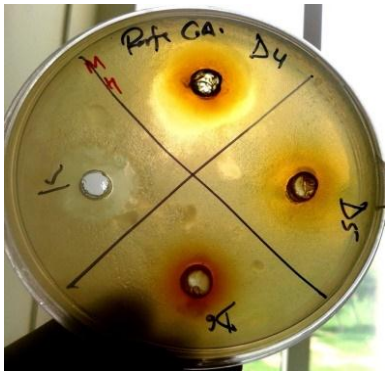


Fig 7: Activity of D4 against *C. albicans*

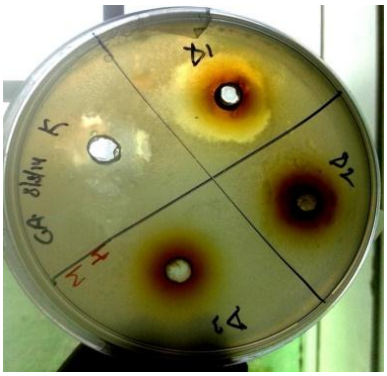


Fig 8: Activity of D1 against *C. albicans*

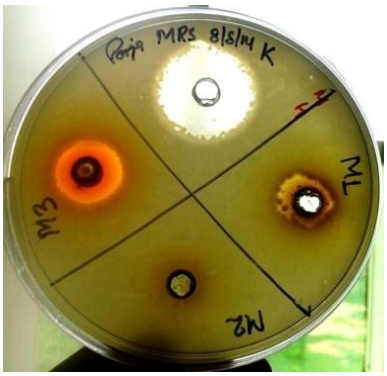


Fig 9: Activity of M3 and M1 against *L. acidophilus*

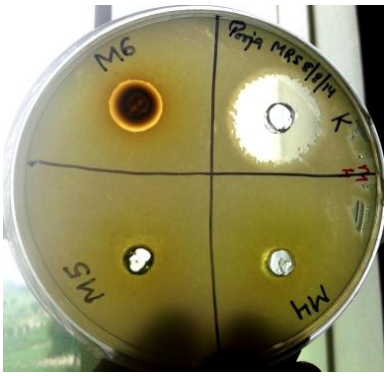


Fig 10: Activity of M6 against *L. acidophilus*

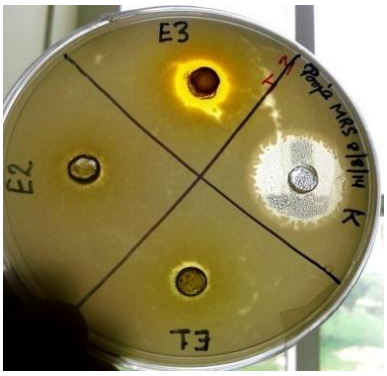


Fig 11: Activity of E3 and E1 against *L. acidophilus*

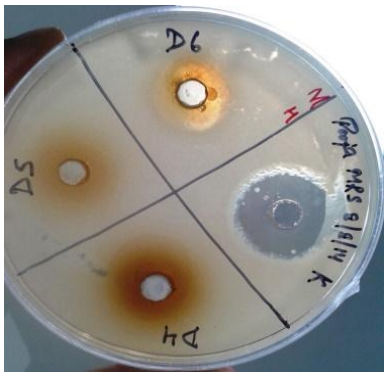


Fig 12: Activity of D6 against *L. acidophilus*

Conclusion

As pathogenic microbes readily becoming resistant to traditional antibiotics, so there is need to discover new antibiotics. The above study explored the antimicrobial activity of methanol, ethanol and distilled water extracts of six Indian medicinal plant species growing in different kind of habitats. These plant species showed different range of antimicrobial activity against test microorganism (*S. mutans, S. aureus, C. albicans, L. acidophilus* and *S. gordonii*). Out of these six plant species *Dahlia* could serve as broad spectrum antibiotic whereas plants such as *Bombax ceiba* and *Cordia* could serve as narrow spectrum antibiotics. D1 and D4 are the plant extracts which are found to have the best result of antimicrobial activity against *C. albicans*. The largest zone of inhibition was observed in the activity of *H. rosa-sinensis* against *C. albicans* that was recorded as 20mm. D1, D4 and D6 inhibit the growth of *S. mutans*, *S. gordonii*, *L. acidophilus* respectively. D2, D3 and D5 did not show activity against any of the bacteria.

Best activity against different bacterial strains was shown by

- *S. mutans* – methanol extract of *Dahlia* (flowers)
- *S .aureus* – methanol extract of *Dahlia* (flowers)
- *C. albicans* – aqueous extract of *Hibiscus rosa-sinensis* (leaves)
- *L. acidophilus* – methanol extract of *Dahlia* (flowers)
- *S. gordonii* – methanol extract of *Dahlia* (flowers)

Future Prospectus

An elaborate research is needed to identify compounds with specific activity. Plants may be used as an antibiotic source for pharmaceutical industries. It requires isolation and purification of various plant strains from various regions around the world and analyzes their antimicrobial activity against various pathogens for potent anti- pathogenic activity.

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