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In-vitro Antimicrobial Activities of different Hibiscus Leave Extract

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ABSTRACT

Solvent preparations of the different Hibiscus leaf have been mostly used in folk medicine for various purposes. In present study in this paper we have studied the antimicrobial activities of the leaf extract of *Hibiscus rosa sinersis, H. aculeatus, H. sabdariffa, H. mutabillis* and *H. ficulness*. We have evaluated the antimicrobial activity of the leaf extracts of all five Hibiscus sp. by agar well diffusion method. In the first screening all of the bacterial and fungal showed varying degrees of sensitivity to the Hibiscus leaf extract. Minimum antimicrobial activity was found by the petroleum either extracts. In among all four different solvent extract shows Ethyl acetate extract was found to be most favorable and suitable solvent for extraction and give maximum results in antimicrobial activities.

Keywords - Hibiscus, Antimicrobial, Leaf, solvent, extract.

I. INTRODUCTION

One of the well-known plants of *Hibiscus* is an evergreen woody glabrous showy shrub with about 1.5-2.5 m height. Leaves are coarsely toothed above and entire below ovate bright green and 3 nerved base. The flowers are axillary solitary companulate (Kaushik *et al.*, 1999). Plants of the genus *Hibiscus* (family: Malvaceae) are widely distributed throughout the world. *Hibiscus,* (genus *Hibiscus)*, genus of numerous species of herbs, shrubs, and trees in the family Malvaceae are native to temperate and tropical regions of the world. Several species are cultivated as ornamentals for their showy flowers and a number are useful as fibre plants. Hibiscus is widely growth as an ornamental plant throughout the tropical as well as sub tropical region of the world.

Natural products have been a source of drugs for centuries. Ayurveda, Siddha, Unani and Folk (tribal) medicines are the major organizations of indigenous medicines in India. Among these systems, Ayurveda is most developed and widely practised in India. Plants, especially used in Ayurveda can provide biologically active molecules and lead structures for the development of modified derivatives with enhanced activity and /or reduced toxicity.

An antimicrobial is substance that kills or inhibits the growth of microbes such as bacteria, fungi and viruses. Antimicrobial drugs either kill microbes or prevent the growth of microbes. (Patel *et.al.*, 2012) Medicinal plants are used in traditional

treatments to cure variety of syndromes. In the last few decades there has been an exponential growth in the field of herbal medicine. Medicinal plants have been used by human being since ages in traditional medicine due to their therapeutic potential and the search on medicinal plants have led the discovery of novel drug candidates used against diverse diseases. According to the World Health Organization (WHO), more than 80% of the world's populace relies on traditional medicine for their primary healthcare needs (Elhoussine *et al*., 2010). Various *Hibiscus* species are studied by various researchers, and the plant has been described traditionally for the various medicinal and general purpose uses. However, the links between the traditional knowledge and the current scientific perspective are missing. The aim of present study to investigate the antimicrobial acclivity of different Hibiscus species.

II. Material and Method

Collection of plant sample-

Various Hibiscus species like *H. rosa sinersis, H. aculeatus, H. sabdariffa, H.mutabillis and H. ficulness* plant leaves were collected in different plant nurseries, Rani Durgavati University, Jabalpur. Jawahar Lal Nehru Agriculture college, Jabalpur and collected all Hibiscus species were identified by State forest Research Institute, Jabalpur. All samples were carried to the laboratory for further use.

Organisms used for evaluation of antimicrobial activity

In-vitro antimicrobial susceptibility test were performed using a set of microbes such as Gram negative, Gram positive bacteria and fungi. All bacterial and fungal strains were obtained from ATCC and National collection for industrial microorganism (NCIM) India.

Extraction of bioactive compound from various Hibiscus species

10 gram of dried Hibiscus leaf powder was subjected to soxhlet extraction apparatus for 10-12 hours using 150 ml of following solvent's viz. (Methanol, Ethyl acetate, Petroleum ether and Distilled water) Cycles were done 8-10 time and extract was recovered by filtration. (Avnish *et.al.,* 2020)

Agar well diffusion Method

Nutrient agar media were used for bacteria and potato dextrose agar media were used for fungi. Once the agar was solidified, 50 µl of the different bacterial cultures were spread onto the plates using a sterile spreader. The plates were punch with six millimeter diameter wells and filled with 25 µl of the Hibiscus plant extract. The tests were carried out in triplicates. The experimental plates were incubated at incubator. The diameter of the zone of inhibition was measured in millimeters. (Perez *et.al.,* 1990 and bauer *et.al.,* 1966)

III. Results and Discussion

All the five Hibiscus sp. Taken for the study were screened for antimicrobial activity using methanol, ethyl acetate, petroleum ether and distilled water extract of Hibiscus sp.

Antimicrobial activity of *Hibiscus rosa sinensis*. –

Hibiscus rosa sinensis tested against above mentioned microbial cultures for the antimicrobial activity. Aqueous extract showed maximum activity against *staphylococcus aureus* with 23 mm zone of inhibition followed by *salmondla abony* 22 mm. minimum antimicrobial activities were showed by *E-coli* and *streptococcus pneumonia* but *Aspergillus niger* and *candida albicans* showed no activity against aqueous extract of *Hibiscus rosa-sinensis*. The ethyl acetate and petroleum ether extract showed not good activity against selected all micro organisms. (Table - 1) similar results have been reported (Cheruiyot *et.al.,* 2009, Dubey *et.al.,*2021)

Table 1 : Antimicrobial activity of leaves of *Hibiscus rosa sinensis* against bacteria and fungi

S. No.	Bacteria & fungi	Extracts of *Hibiscus rosa sinensis*				
		Aqueous	Methanol	Ethyl acetate	Petroleum ether	Control
1	*Escherichia coli* NCIM 2256	12.83 ± 0.76	10.17± 0.29	00.00	00.00	19.0 ± 0.57
2	*Staphylococcus aureus* NCIM 2079	22.33 ± 0.76	20.67± 0.76	17.3 ± 1.04	12.17 ± 0.29	16.5 ± 0.48
3	*Salmonella abony* NCIM 2257	21.83 ± 0.29	17.67± 1.04	11.5 ± 0.50	00.00	14.0 ± 0.63
4	*Streptococcus pneumoniae* ATCC 49619	14.00 ± 0.57	11.50± 0.50	00.00	00.00	17.5 ± 0.66
5	*Klebsiella pneumoniae* NCIM 2957	21.5 ± 0.87	16.67± 0.76	00.00	00.00	19.5 ± 0.49
6	*Aspergillus niger* MTCC	00.00	00.00	00.00	00.00	12.5 ± 0.73
7	*Candida albicans* ATCC 10231	00.00	00.00	00.00	00.00	14.0 ± 0.55

Antimicrobial activity of *Hibiscus aculeatus*

Aqueous, Methanol, ethyl acetate and petroleum ether extracts of *Hibiscus aculeatus* did not show any zone of inhibition against all selected microorganism.

Table 2 : Antimicrobial activity of leaves of *Hibiscus aculeatus* against bacteria and fungi

S. No.	Bacteria & fungi	Extracts of *Hibiscus aculeatus*				
		Aqueous	Methanol	Ethyl acetate	Petroleum ether	Control
1	*Escherichia coli* NCIM 2256	00.00	00.00	00.00	00.00	19.0 ± 0.57
2	*Staphylococcus aureus* NCIM 2079	00.00	00.00	00.00	00.00	16.5 ± 0.48
3	*Salmonella abony* NCIM 2257	00.00	00.00	00.00	00.00	14.0 ± 0.63
4	*Streptococcus pneumoniae* ATCC 49619	00.00	00.00	00.00	00.00	17.5 ± 0.66
5	*Klebsiella pneumoniae* NCIM 2957	00.00	00.00	00.00	00.00	19.5 ± 0.49
6	*Aspergillus niger* MTCC	00.00	00.00	00.00	00.00	12.5 ± 0.73
7	*Candida albicans* ATCC 10231	00.00	00.00	00.00	00.00	14.0 ± 0.55

Antimicrobial activity of *Hibiscus sabdariffa* –

Only Ethyl acetate extract showed maximum activity against *staphylococcus aureus* with 32 mm followed by *streptococcus pneumonia, klebsiella pneumonia* and minimum activity were showed by *Salmonella abovy*, *E. coli* and *Aspergillus niger* but no zone of inhibition showed by *Candida albicans*. Aqueous, Methanol and Petroleum ether extract of *Hibiscus sabdariffa* did not show any zone of inhibition against all selected microorganism. (Table-3) Similar results have been also obtained by masoodi *et.al.,* 2008.

Table 3 : Antimicrobial activity of leaves of *Hibiscus sabdariffa* against bacteria and fungi

S. No.	Bacteria & fungi	Extracts of *Hibiscus sabdariffa*				
		Aqueous	Methanol	Ethyl acetate	Petroleum ether	Control
1	*Escherichia coli* NCIM 2256	00.00	00.00	28.00 ± 0.5	00.00	19.0 ± 0.57
2	*Staphylococcus aureus* NCIM 2079	00.00	00.00	31.67 ± 0.29	00.00	16.5 ± 0.48
3	*Salmonella abony* NCIM 2257	00.00	00.00	26.83 ± 0.76	00.00	14.0 ± 0.63
4	*Streptococcus pneumoniae* ATCC 49619	00.00	00.00	29.33 ± 0.5	00.00	17.5 ± 0.66
5	*Klebsiella pneumoniae* NCIM 2957	00.00	00.00	29.33 ± 0.76	00.00	19.5 ± 0.49
6	*Aspergillus niger* MTCC	00.00	00.00	19.67 ± 0.29	00.00	12.5 ± 0.73
7	*Candida albicans* ATCC 10231	00.00	00.00	00.00	00.00	14.0 ± 0.55

Antimicrobial activity of *H. mutabilis*

Ethyl acetate extract showed maximum activity against. *Salmonella abony* with 12 mm zone of inhibition followed by *klebsiella pneumonias*. Minimum activities were showed by *E. coli* and *staphylococcus aureus* but no zone of inhibition showed by *Aspergillus niger* and *candida albicans*. Aqueous, Methanol and petroleum ether expect of *H mutabilis* did not show any zone of inhibition against all selected Micro organism. (Table-4) similar results have been also obtained by Cheruiyot *et.al.,* 2009

Table 4 : Antimicrobial activity of leaves of *Hibiscus mutabillis* against bacteria and fungi

S. No.	Bacteria & fungi	Extracts of *Hibiscus mutabillis*				
		Aqueous	Methanol	Ethyl acetate	Petroleum ether	Control
1	*Escherichia coli* NCIM 2256	00.00	00.00	8.33 ± 0.29	00.00	19.0 ± 0.57
2	*Staphylococcus aureus* NCIM 2079	00.00	00.00	8.17 ± 0.50	00.00	16.5 ± 0.48
3	*Salmonella abony* NCIM 2257	00.00	00.00	12.17 ± 0.76	00.00	14.0 ± 0.63
4	*Streptococcus pneumoniae* ATCC 49619	00.00	00.00	00.00	00.00	17.5 ± 0.66
5	*Klebsiella pneumoniae* NCIM 2957	00.00	00.00	10.5 ± 0.29	00.00	19.5 ± 0.49
6	*Aspergillus niger* MTCC	00.00	00.00	00.00	00.00	12.5 ± 0.73
7	*Candida albicans* ATCC 10231	00.00	00.00	00.00	00.00	14.0 ± 0.55

Ethyl acetate extract showed maximum activity against *Streptococcus pneumoniae* with 38.33 mm zone of inhibition followed by *Salmonella abony* and *Staphylococcus aureus* . Minimum activities were showed by *Candida albicans* and *Escherichia coli.* While aqueous extract shows zone of inhibition against only *Salmonella abony* and *Klebsiella pneumonia.* Methanol and petroleum ether expect of *Hibiscus ficulneus* did not show any zone of inhibition against all selected Micro organism. (Table-5)

Table 5 : Zone size (in millimeter) of antimicrobial activity of aqueous, methanol, ethyl acetate and petroleum ether extracts of leaves of *Hibiscus ficulneus* against standard bacteria and fungi.

S. No.	Bacteria & fungi	Extracts of *Hibiscus ficulneus*				
		Aqueous	Methanol	Ethyl acetate	Petroleum ether	Control
1	*Escherichia coli* NCIM 2256	00.00	00.00	14.33 ± 0.58	00.00	19.0 ± 0.57
2	*Staphylococcus aureus* NCIM 2079	00.00	00.00	20.67 ± 0.58	00.00	16.5 ± 0.48
3	*Salmonella abony* NCIM 2257	15.33 ± 0.87	00.00	25.33 ± 1.15	00.00	14.0 ± 0.63
4	*Streptococcus pneumoniae* ATCC 49619	00.00	00.00	38.33 ± 1.53	00.00	17.5 ± 0.66
5	*Klebsiella pneumoniae* NCIM 2957	11.7 ± 1.04	00.00	17.67 ± 0.58	00.00	19.5 ± 0.49
6	*Aspergillus niger* MTCC	00.00	00.00	14.67 ± 0.58	00.00	12.5 ± 0.73
7	*Candida albicans* ATCC 10231	00.00	00.00	10.67 ± 1.15	00.00	14.0 ± 0.55

IV.CONCLUSION

The aim of the study was to investigate whether the folk used of the herbal preparation derived from the plant Hibiscus in some disease caused by microbes. In this paper study shows that Hibiscus plant sp. have great potential for antimicrobial activity. The study focused on the solvent extracts of *H. rosa sinersis, H. aculeatus, H. sabdariffa, H.mutabillis* and *H. ficulness* solvent extract was proven to be most suitable solvent for extraction and antimicrobial activity.

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