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RESEARCH ARTICLE

Screening the Antibacterial and Antifungal Properties of *Holarrhena antidysenterica* (L.) and *Caesalpinia crista* (L.)

Joshi DM*, Verma RJ

Department of Zoology, University School of Sciences, Gujarat University, Ahmedabad-380009, India.

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ABSTRACT

The present study is an attempt to explore the plant wealth as a substitute of standard antibiotics. In order to fulfil the objective, a total of 48 assays were carried out to examine the antimicrobial property seed extracts of *Holarrhena antidysenterica* (L.) and *Caesalpinia crista* (L.). The assays involved the testing of aqueous, methanol and petroleum ether extracts of both the plants against eight micro-organisms and their comparison with four standard antibiotics. The micro-organisms include four strains of bacteria and four strains of fungi. Both the plants showed antimicrobial property against the selected strains of micro-organisms but with varied intensity. The study revealed that among the eight micro-organisms selected, *Fusarium oxysporum* is the most sensitive micro-organism and *Salmonella paratyphi A* is the most resistant one. Among the plants, *H. antidysenterica* is more potent as well as it showed broad spectrum of effectiveness.

KEYWORDS

Antibiotics, Aqueous, Methanol, Petroleum Ether, Antimicrobial Property

INTRODUCTION

Nature has nurtured mankind providing various resources since centuries. Rich wealth of plants is one of such resources and a precious gift of nature to mankind. Plants are the primary source of food and energy on earth. Apart from the said usages, mankind had begun to use the plants for curing diseases as well as maintaining healthy state. India has gained worldwide recognition for its indigenous and extensive Ayurvedic treatments, which originated as early as 5000 BC in the Himalayan regions. Besides, Ayurveda many other systems also came in existence like Siddha, Unani, Tebetan and Folk.

***Address for Correspondence:**
Joshi Devanshi M
Gujarat Ecological Education and Research (GEER) Foundation,
Indroda Nture Park, P.O. Sector-7
Gandhinagar-382007, India.
E-Mail Id: devanshi.joshi86@gmail.com

According to the World Health Organization (WHO), healing herbs are used as primary medicines by nearly 4 billion people worldwide, i.e. two-thirds of the world's population[1]. Current research has demonstrated the effectiveness of medicinal herbs on various dermatological conditions[2] and even cancer[3]; therefore it is clear that medicinal plants play an important role in global healthcare. The antimicrobial and antioxidant activities are responsible for the substantial use of plants to treat chronic and infectious diseases as well as maintaining healthy state. The antimicrobial property of plants is ascribed to certain chemicals which are produced by the plants in order to defend the parasites or predators like microorganisms, insects and herbivores.

Diverse microorganisms like bacteria, fungi, viruses, plankton etc. play various advantageous to adverse roles in human life. Bacteria give rise to various common to infectious fatal diseases and epidemics. Fungi stand second after insects, causing plant diseases, which results in heavy loss of plant products. Pathogenic fungi alone cause nearly 20% reductions in the yield of major food and cash crops[4]. It is reported that every year plant diseases eradicate one-third of the agricultural production worldwide[5].

The method to control fungi is the implication of chemical compounds that are toxic to fungi. It produces development of resistance in fungi against such chemicals that leads to find out another alternative and another hazard is persistence of some fungicides for many years in the environment. It is also observed that fungicides are not readily biodegradable so their persistence leads to the detrimental effect on organisms other than the target fungi[6].

In case of bacterial control, although antibiotics have also been proven boon since long time but some of they produce side effects like dizziness, hearing loss, kidney damage etc. which again triggered scientists to find out another way to overcome the microbial infections. In recent decades, the scientists have realized that the effective life span of any antibiotic is limited[7].

Hence, present study focalizes on the control of bacteria and fungi with some medicinal indigenous plants having no side effects with an endeavour to discover the plant wealth as a substitute of standard antibiotics.

The plants selected for this purpose are *H. antidysenterica* (Apocynaceae) and *Caesalpinia crista* (Fabaceae). *H. antidysenterica*, commonly known as Kurchi or Indrajaw is a shrub growing wild on mountains and is indigenous to India. It is one of the most commonly used herbs in fever associated with diarrhoea and also helpful to augment the digestion and appetite. This plant is reported to have phytochemicals like alkaloids, flavonoids, sterols, saponins, tannins etc.[8]. *Caesalpinia crista*, locally known as Kanchaka is used internally as well as externally for various healing treatments. Externally, the massage with its seed-oil particularly helps skin diseases. The seed powder is anthelmintic, controls the diarrhoea, and used to treat colic, convulsions, leprosy, and palsy[9].

MATERIALS AND METHODS

Collection and Extraction of Plant Material

For screening the antimicrobial property, seeds of *Holarrhena antidysenterica* and *Caesalpinia crista* were selected as per the usage of the plant and discussion with experts and local people. Seed powders of the selected plants were collected and their different extracts were made as described below.

Aqueous Extract

5 gm of the plant powder was dissolved in 100ml of distilled water and were heated at 50°C till volume becomes half. This was cooled and filtered using Whatmann filter paper No 1 and dried. The dried extract was then stored in clean brown bottles until used.

Methanol and Petroleum Ether Extracts

5 gm of the plant powder was soaked in 100ml of methanol and petroleum ether separately and kept for 24 hrs. Then it was filtered using Whatmann filter paper No 1. The above mentioned procedure was repeated once again. Then after the filtrate was dried and stored until used for the experiment.

Test Organisms

The extracts of the selected plants were screened against a total of eight microbial strains. The test organisms were collected from the Microbiology Department of School of Science, Gujarat University, India.

The test organisms include four strains of bacteria i.e., *Staphylococcus aureus*, *Salmonella paratyphi* A, *E. coli*, *Pseudomonas aeruginosa* and four of the fungi *Macrophomina phaseolina*, *Fusarium oxysporum*, *Aspergillus niger*, *Aspergillus nidulans*.

Antimicrobial Assay

Antimicrobial activities of the prepared plant extracts were evaluated by the agar well diffusion method[10].

Preparation of Culture Medium and Inoculation

The present study involves preparation of two different medium i.e., nutrient agar medium for bacteria and Rose -Bengal agar medium for fungi. The constituents of the medium (Nutrient agar: *1 gm peptone, 0.5 gm NaCl, 0.3 gm yeast extract, 3 gm agar powder*; Rose -Bengal agar: *3 gm Rose -Bengal agar powder, 3 gm agar powder)* were mixed with 100 ml water and autoclaved for 20 min at the of pressure 15 psi and temperature of 121°C. Along with these medium test-tubes for pour plate methods were also autoclaved.

Inoculation of the micro-organism was carried out by pour plate method. Further treatment is similar for both the bacteria and fungi. Following autoclave, the 20 ml of medium was poured in each Petri dish in aseptic conditions. The test tubes for pour plate were allowed to achieve 45°C temperature and then the previously prepared microbial solution was added to the test-tube. The test-tubes were rotated vigorously and finally the medium was poured on the agar plate and the plates were kept at room temperature for few minutes to solidify the medium.

Extract Application and Incubation

In order to prepare two different concentrations of the pre-stored extracts i.e. 0.1 gm/ml and 0.2 gm/ml, the aqueous extracts were dissolved in sterile distilled water; methanolic and petroleum ether extracts were dissolved in dimethylsulphoxide (DMSO). After solidification of the agar, wells of 6 mm diameter were bored in the agar. The plant extract (25 µL) was dispensed in each well using a sterile micropipette.

The plates were kept in refrigerator for half an hour. After that the plates were incubated at 37°C in the incubator for 24 hours and the antimicrobial activity was determined by measuring the diameter of zone of inhibition (mm). Along with the extracts of different plants, four standard antibiotics i.e., tetracycline, streptomycin, ampicillin and gentamycin were also tested so as to have comparison between antibiotic and plant extracts.

Statistical Analysis

To carry out the statistical analysis of the data student's t- test was used. The difference between the effects (diameter length of inhibition zone) of plant extracts and standard antibiotic was calculated. The analysis was carried out in Microsoft Excel 10.

RESULTS AND DISCUSSION

Both the plants essayed here have shown different degrees of the antimicrobial property. The radial growth of micro-organisms was reduced to a varying degree by different plant extracts. As well as different extracts of the same plant have also recorded showing varied extent of effectiveness on the organisms.

Some have broad spectrum that is inhibiting growth of many different micro-organisms while the others could inhibit the growth of few micro-organisms. The antimicrobial property of each plant is given in detail.

Holarrhena Antidysenterica

As shown in Table 1, the results revealed that *H. antidysenterica* is screened out as more potent antimicrobial plant among the two plants assayed. All the three seed extracts of this plant have exhibited appreciable antimicrobial activity in both the ways i.e. in terms of broad spectrum as well as high intensity of effectiveness.

As well as, it has inhibited the growth of all the eight selected micro-organisms. Aqueous extract has shown antimicrobial activity against eight micro-organisms. However, the methanol extract has affected two fungi; it did not display antibacterial property against the selected bacteria. The petroleum ether extract has affected four micro-organisms, two of them were bacteria and two were fungi as shown in the Table 1.

Successive isolation of phytochemicals from plant material is subject to the type of solvent used for extraction. Hence, for this instance maximum antimicrobial substances have been

Table 1: Antimicrobial activity of different plant extracts and standard antibiotics

Microorganism	**Extract Concentration**	**Diameter of Inhibition Zone (cm)**									
		H. Antidysenterica			***C. Crista***			**Antibiotics**			
		AE	**ME**	**PE**	**AE**	**ME**	**PE**	**S**	**G**	**T**	**A**
S. aureus	**0.1**	0	0	0	0	0	0	7.5	17	10	0
	0.2	8	0	0	0	9	1				
P. aeruginosa	**0.1**	8	0	0	9	10	0	0	11	11	0
	0.2	9	0	10	**13***	12.5	0				
E. coli	**0.1**	7	0	1	0	0	0	9.5	16	16	0
	0.2	8	0	4.5	0	8	2				
S. paratyphi A	**0.1**	0	0	0	0	0	0	0	15	9	0
	0.2	8	0	0	0	0	0				
M. phaseolina	**0.1**	9	0	0	8	12.5	1	13	18	13.5	0
	0.2	12	0	0	9	14.5	2				
F. oxysporum	**0.1**	0	0	0	0	0	2	8	10	10	8.5
	0.2	8	11	0	10	**12***	3.5				
A. niger	**0.1**	8	0	0	0	0	2	0	0	0	0
	0.2	**9***	0	0	0	0	3				
A. nidulans	**0.1**	13	0	0	0	8	1	16	19	12	23
	0.2	**24***	10	0	0	9	1.5				

(AE- Aqueous extract, ME-Methanolic extract, PE - Petroleum ether extract, S- Streptomycin, G-Gentamycin, T-Tetracycline, A-Ampicillin; * impact higher that the standard antimicrobial agent)

extracted in aqueous extract of *H. antidyesnterica*. Furthermore, it has been proven more potent on fungi. *A. nidulans* has been proven the most sensitive of all the micro-organism in case of aqueous extract and considerably very large zone of inhibition (24 mm diameter) was recorded. The petroleum ether extract was also recorded giving conspicuous zones of inhibition against the growth of *P. aeruginosa*. The data revealed that aqueous extract has maximum potency among the three extracts; this may be ascribed to the water soluble nature of the antimicrobial substances of this plant.

A study concluded that alkaloids from the ethanolic extract of *H. antidysenterica* seeds exhibited antibacterial activity against clinical isolates of enteropathogenic Escherichia coli (EPEC) *in vitro*[11]. One more study expresses the antimicrobial property of the aqueous and the alcoholic extracts of the stem bark of *H. antidyesnterica* on some enteric pathogens, in which antimicrobial activity of *H. antidysenterica* was observed at concentrations of 200, 300 and 400 mg/ml[12].

Caesalpinia Crista

Table-1 shows that all the three seed extracts of the *Caesalpinia crista* are active antimicrobial agents. *Caesalpinia crista* has affected seven of the eight selected strains of bacteria and fungi i.e., three of the bacteria and four of the fungi. The aqueous extract of the seed has shown antimicrobial activity on three microbial strains, one bacterium and two fungi. The methanolic extract has inhibited the growth of six micro-organisms three of them are bacteria and three are fungi. The petroleum ether extract has also affected six miro-organisms which involves two of the bacteria and all the fungi.

Caesalpinia crista has shown maximum inhibitory effect on bacteria *P. aeruginosa* and fungi *F. oxysporum.* Its maximum inhibitory effects are recorded from the extracts methanolic and petroleum ether, so it can be assumed that both the extracts must have some antimicrobial substances which have wide range of effect compared to the remaining extract. A study revealed that the seed extracts of *Caesalpinia crista* showed inhibitory effects against methicillin resistant (MR) *Staphylococcus aureus* and ampicillin resistant (AR) *Pseudomonas aeruginosa*. In vivo, the seed extracts showed a significant bacterial clearance from the lungs of the rats infected by *Pseudomonas aeruginosa* [13].

Collectively both the plants have affected all the microbial strains but *H. antidyesnterica* has shown the wider spectrum between the two plants with higher effectiveness over the eight selected micro-organism. *C. crista* has also shown wide spectrum inhibiting the growth of seven strains. It has been observed that bacteria are more resistant than fungi.

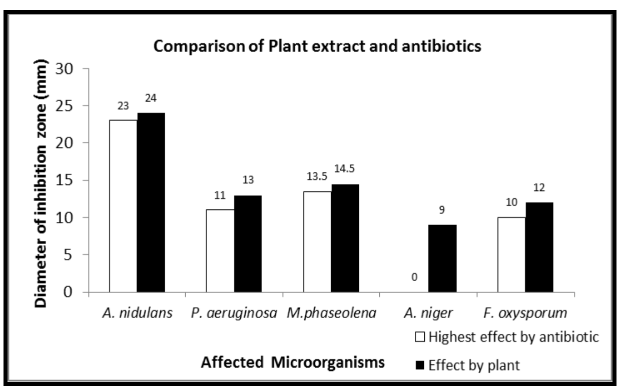

Figure 1: Comparison of Plant extract and Antibiotics

The standard antibiotics streptomycin, gentamycin, tetracycline and ampicillin were also demonstrated against the microbial strains, to compare them with the herbal sources. There are some microbes which exhibited more sensitivity against plant extracts than the antibiotics. The present study has revealed four plant extracts which have higher antimicrobial potential than the antibiotics on different microbes. Effectiveness of aqueous extract of *C. crista* is significantly ($p<0.001$) higher than the most effective antibiotic tetracyclin against the bacteria, *P aeruginosa*. The aqueous extract of the plant *H. antidysenterica* is significantly ($p<0.05$) more effective on *A. nidulans* in compare to ampicilin, which is the most effective among all the antibiotics tested. Tetracycline is the most effective antibiotic among all the antibiotics, against *F. oxysporum* but the effect of the methanolic extract of *C. crista* is even higher ($p<0.001$) than the antibiotic. Any of the antibiotic has not affected the growth of fungi *A niger* where as it is inhibited by both of the plants. Considering the extract type there is a generalized result, the *H. antidysenterica* showed broad spectrum when extracted with water; *C. crista showed* broad spectrum when extracted with methanol. (The effects of plant extracts and standard antibiotics were significantly different and the calculated *p* value is given.)

CONCLUSION

All the plant extracts have exhibited antibacterial as well as antifungal activity. This study has also derived that among the microbial strains *F. oxysporum* is the most sensitive one and *Salmonella paratyphi A* is the most resistant microbe. This study has also revealed the plants which are more effective than the standard antibiotics against a strain of bacteria and three strains of fungi, establishing a substitute for treating these pathogenic micro-organisms. These plants promise us to provide more effective and affordable antimicrobial substances without any side effects.

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