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Research Article

COMPARATIVE ANTI-MICROBIAL EFFICACY EVALUATION OF HYDROALCOHOLIC EXTRACT OF MEDICINAL PLANTS IN VULVOVAGINITIS: AN IN-VITRO STUDY

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ABSTRACT

Vulvovaginitis is infection of vulva and vagina where bacteria are the most common etiological agent. Treatment of vaginal infection should aim not only to eradicate pathogenic organisms, but also at supporting the normal vaginal microflora. Most of the antibiotics used for its treatment are associated with development of resistant micro-organisms and drug-induced side-effects. So, an attempt has been made to investigate in-vitro antimicrobial susceptibility testing of medicinal plant extracts for better therapeutic outcome. **Aim and objective:** To screen out antimicrobial activity of hydroalcoholic extract of *Azadirachta indica, Symplocos racemosa, Curcuma longa* and *Pongamia pinnata* with an objective to treat vaginal infections. **Material and method:** This in-vitro study evaluates antimicrobial activity by measuring zone of inhibition against standard ATCC bacterial strains responsible for vulvovaginitis using spot test assay. **Result and Discussion:** C. longa and S. racemose exhibited strongest antimicrobial activity for gram-negative bacteria and gram-positive bacteria respectively. Among different extracts, 70% ethanolic extract of A. indica and P. pinnata, ethanolic extract of *S. racemosa* and 50% ethanolic extract of C. longa exhibited the highest antimicrobial activity. **Conclusion:** Hence, these plants can be used to treat vulvovaginitis that may serve as lead in the development of new potent herbal antibiotics to which pathogen strains are not resistant.

INTRODUCTION

Vulvovaginitis is a very common gynaecological problem in women of all age groups which is caused by single or mixed vaginal infections. [1] The most common causes of vulvovaginitis are bacterial vaginosis, vulvovaginal candidiasis, and trichomoniasis. Among these, bacterial vaginosis is implicated in 40% to 50% of cases.[2] The bacteriological agents associated with vulvovaginitis include Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa[3], Citrobacter freundii, Staphylococcus aureus [4] and Enterococcus faecalis [5].

According to Ayurveda, local route of approach (*Sthanika chikitsa*) to the local vaginal pathology is the most preferred route.[6] It has some additional advantages over oral delivery, such as avoiding hepatic first-pass metabolism, reducing, or eliminating the incidence and severity of side effects and gastrointestinal incompatibility.[7] For its treatment, plant extracts and biological active compounds are receiving more attention due to multiple drug resistance against many antibiotics. The continuous evolution of bacterial resistance to available antibiotics has necessitated the search for novel and effective antimicrobial Ayurvedic drugs. [8]

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Based on the clinical features of Vulvovaginitis, vaginal douching with decoction of *Aragwadhadi* group of drugs containing *Azadirachta indica* (*Neem*) and *Pongamia pinnata* (*Karanja*) for foul smell[9,10]; fumigation with paste of *Curcuma longa* (*Haridra*) rhizome for vaginal itching [11] and vaginal douching with decoction of stembark of *Symplocos racemosa* (*Lodhra*) for excessive abnormal vaginal discharge[12] are mentioned in *Ayurveda*. Based on these references, these four drugs are selected for in-vitro evaluation of its anti-microbial activity.

MATERIAL AND METHODS

This is a prospective laboratory trial study which was undertaken to evaluate and compare the possible antimicrobial action of the aqueous, hydroethanolic (50% and 70% ethanol) and ethanolic extracts of leaf of A. indica, stem bark of *S. racemose*, rhizome of *C. longa* and leaf of *P. pinnata.*

Plant Material

A. indica leaves and P. pinnata leaves were collected from BHU campus, C. longa rhizome and S. racemose stem bark were purchased from local drug dealer from Varanasi and identified in Lab of Morphogenesis, Department of Botany, BHU, Varanasi, India.

Preparation of sample extract

All the above-mentioned plant parts were shade dried and grounded coarsely. 5gm of each of the four drugs was added separately in 100ml of distilled water, 50% ethanol, 70% ethanol and 95% ethanol [13] to prepare aqueous, hydroethanolic (50% and 70% ethanol) and ethanolic extract by cold extraction method with occasional shaking. The extracts were then concentrated in a rotary evaporator. [14]

Screening of antibacterial activity

Test micro-organism

A total of 6 bacterial strains viz. Escherichia coli ATCC 25922, Klebsiella pneumoniae ATCC 700603, Pseudomonas aeruginosa ATCC 27853, Citrobacter freundii ATCC 13316, Enterococcus faecalis ATCC 29212 and Staphylococcus aureus ATCC 25923 were used for the antimicrobial assay. All cultures were obtained from American Type Culture Collection (ATCC), preserved at Department of Microbiology, Institute of Medical Sciences, BHU, Varanasi, India. The cultures were maintained by subculturing periodically and preserved at 4°C.

Media used

Muller-Hilton agar (MHA) media (Hi-media, Mumbai, India) was used for culturing bacterial strains. MHA plates were prepared by pouring 15ml of molten media into sterile petri plates. [15] The plates were allowed to dry for 5 min.

Preparation of microbial inoculum

1.0 ml of an 18 h old culture of bacteria was adjusted to 0.5 MacFarland standards in sterile saline to achieve a concentration of 10^7 CFU/mL. Lawn culture of the bacterial suspension was made over the surface of MHA agar plates.[16]

Inoculation of test plates: Optimally within 15 minutes after adjusting the turbidity of the inoculum, a sterile cotton swab was dipped into the adjusted suspension. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inoculum. To obtain a uniform and confluent growth, the dried surface of MHA plate was inoculated by streaking the swab over the entire sterile agar surface three times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar was swabbed. The lid was left ajar for 3 to 5 minutes, but no more than 15 min, to allow for any excess surface moisture to be absorbed before dropping the stock solution.

Determination of Zone of inhibition

To determine zone of inhibition, first 50mg of all the dried extracts were separately dissolved in 1ml of Dimethyl sulphoxide (DMSO) to get the concentration of 50mg/ml. Further, spot test assay was performed by dropping 10µl of each extract separately into sixteen quadrants; four for each drug for four different extracts.

Reading plates and interpreting result: Plates were incubated at 37°C for 24 hours, and then the sensitivities of the microorganism species to the plant extracts were determined by measuring the diameter of inhibitory zones The zones were measured to the nearest whole mm using a zone size measuring scale, which is held on the back of the inverted petri plate.

All the tests were performed in triplicate and mean or average values of the diameter of inhibition zones were calculated.

RESULT

Table 1: Mean ± Standard Deviation of Diameter of Inhibition Zone (in mm) by Different Extracts on Different Bacterial Strain

Bacteria / Plant Extract		E. coli	K. pneumoniae	P. aeruginosa	C. freundii	E. faecalis	S.aureus
A. indica	NA	-	-	-	-	7±1.41	-
	NE_{50}	8	6	7±1.41	7.5±2.12	9±1.73	-
	NE_{70}	9.5±0.70	9.33±1.15	7	8.5±0.70	9.5±2.12	9.33±2.30
	NE	9±1.41	8.66±0.57	6	9±0.00	8.5±2.12	8.66±1.15
S. racemose	LA	-	-	6.5±0.70	10	7.66±1.52	7.66±1.52
	LE_{50}	-	6	8.33±2.08	9±4.24	12.66±1.15	12±2.00
	LE_{70}	-	-	9±1.41	8±2.82	11.66±0.57	9.66±2.51
	LE	-	-	9	8.5±3.53	12.66±1.15	14±2.00
C. longa	HA	-	-	-	-	-	-
	HE_{50}	9±1.00	9±1.73	9.33±1.15	10.33±0.57	10±2.00	9.66±0.57
	HE_{70}	8.33±1.52	7±2.00	7.66±2.08	10±1.00	9.33±1.15	11.33±1.15
	HE	9.33±1.52	7.66±0.57	7.66±1.52	9.33±2.51	9±1.41	10.33±0.57
P. pinnata	KA	-	5	-	-	-	-
	KE_{50}	6	5	8	9.5±0.70	9±1.00	-
	KE_{70}	6.5±2.12	7.66±1.52	8.5±0.70	9±2.82	10	-
	KE	8±1.41	8.66±1.52	7±1.73	9±2.64	8	6

Where, *N- Neem, L- Lodhra, H- Haridra, K- Karanja,* A- Aqueous extract, E_{50}- 50% ethanolic extract, E_{70}- 70% ethanolic extract, E- 95% ethanolic extract

The hydroethanolic extract of all the four drugs exhibited higher zones of inhibition than aqueous extract for all antimicrobial categories.

Among gram-negative bacteria, E. coli, P. aeruginosa and C. freundii were found most sensitive to C. longa and K. pneumoniae was most sensitive to A. indica whereas among gram-positive bacteria E. faecalis was found more sensitive than S. aureus to S. racemose. Hence, C. longa and S. racemose exhibited strongest antimicrobial activity for gram-negative bacteria and gram-positive bacteria respectively.

Range of zone of inhibition for gram-positive bacteria was found to be 7-14mm and 5-10.33mm for gram-negative bacteria which shows that gram-positive bacteria were more susceptible towards plants extracts as compared to Gram-negative bacteria. These differences may be attributed to fact that the cell wall in Gram positive bacteria is of a single layer, whereas the Gram-negative cell wall is multilayered structure due to presence of lipopolysaccharides. [17]

To compare the overall antimicrobial activity of different extracts, SPSS Windows version 19 (SPSS Inc., USA) was used for statistical analyses by applying mean values of zone of inhibition using analysis of variance (ANOVA).

Table 2: Overall Antimicrobial Activity of Different Extracts

Extract	Mean ± Standard deviation of diameter of inhibition zone (mm)			
	A. indica	S. racemose	C. longa	P. pinnata
Aqueous	7.00	8.00±1.63	-	5.00
50% Ethanol	7.50±1.04	8.83±3.18	9.57±0.53	7.50±1.87
70% Ethanol	8.71±1.11	9.00±2.23	8.85±1.34	8.16±1.32
Ethanol	8.28±1.11	10.40±2.88	8.85±0.69	7.66±1.03

Among all, 70% ethanolic extract of leaves of A. indica and P. pinnata, 50% ethanolic extract of rhizome of C. longa and ethanolic extract of bark of S. racemose exhibited highest antimicrobial activity.

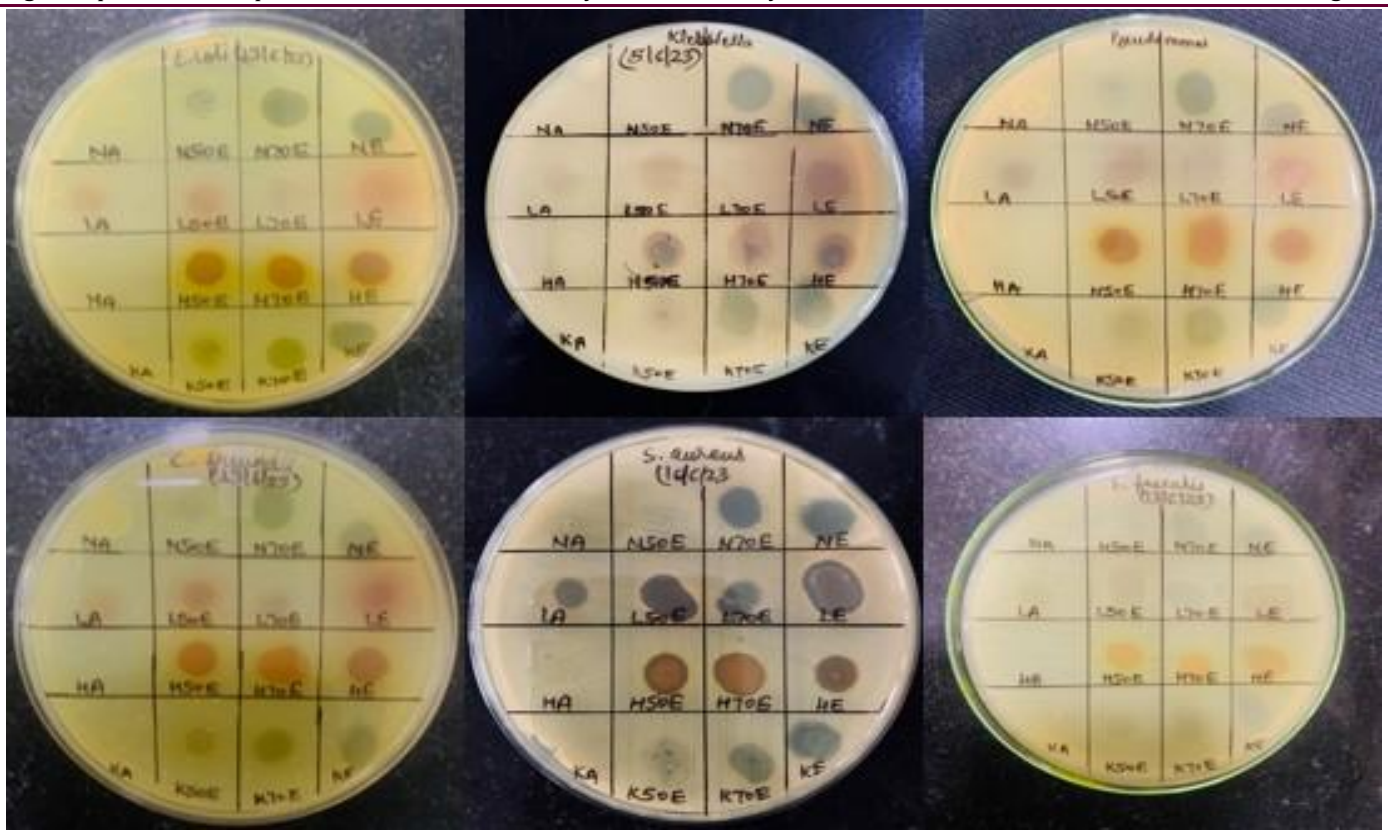

Fig. 1: Antibacterial activity

DISCUSSION

The inhibitory effects of plants on microorganisms may be therefore, due to the presence of certain phytochemicals such as alkaloids, flavonoids, saponins and tannins [18] which make the drugs effective enough to cure the disease.

The mechanism of action of alkaloids as an antimicrobial is by inhibiting the synthesis of nucleic acids by inhibiting the enzymes dihydrofolate reductase and topoisomerase I. Alkaloids can disrupt the constituent components of peptidoglycan on bacterial cells so that the cell wall layers are not formed intact and cause cell death. Flavonoids provide bacteriolytic effects, inhibit protein synthesis, DNA synthesis, RNA synthesis and damage cell membrane permeability. The mechanism of action of saponin as an antibacterial is by lysis of the bacterial cell wall and leakage cytoplasm along with AKP (Alkaline Phosphate) resulting in cell death. Tannin is a water-soluble polyphenol which prevent the development of microorganisms by precipitating microbial proteins and making nutrient proteins unavailable to bacteria. [19]

CONCLUSION

The antimicrobial activity of these drugs was due to the presence of various secondary metabolites. Hence, these plants can be used to treat vulvovaginitis which may serve as leads in the development of new potent herbal antibiotics.

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