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Research Article

ANTIMICROBIAL ACTIVITY OF *FICUS RACEMOSA L.* AND *CISSAMPELOS PAREIRA L.* VAR. *HIRSUTA* (DC) FORMAN., AGAINST *E. COLI*, *P. AEROGINOSA*, *S. AUREUS* AND *B. SUBTILIS*

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ABSTRACT

The present work was designed to assess the antimicrobial activity of the methanolic extracts of the bark of *Ficus racemosa L.* and root of *Cisampelos pareira L.* var. hirsuta (DC) Forman. The antimicrobial activity of the extracts was assessed by disc diffusion method using four microbial strains. Many plants of the family Moraceae are used in the treatment of infectious diseases. *Ficus racemosa*, belongs to this family, is used traditionally against dyspepsia, dermal infections, abdominal pains and diarrhoea. Similarly, *Cissampelos pareira*, a plant belongs to family Minispermaceae found to have analgesic, anti-inflammatory and febrifuge properties. In the present study, both the plant extracts exhibited the antimicrobial effect against four strains of Escherichia coli, Pseudomonas aeroginosa, Staphylococcus aureus and Bacillus subtilis. The effects were compared with that of the standard antibiotic loaded discs, Ofloxacin 5 µg/disc. Finally, the petridishes were incubated at 37^0C for 24 hours. After the incubation period the zone of inhibition produced by samples and standard were measured. Comparatively, the activity of *Cissampelos pareira* was found better than that of *Ficus racemosa*. But, in case of P. aeuroginosa, it showed less activity than *Ficus racemosa*. *Cissampelos pareira* showed better activity against B. subtilis which is comparable with that of the standard drug Ofloxacin. It was clearly observed that methanolic extracts of both the herbals exhibit impact antimicrobial activity against B. subtilis.

Key words: *Ficus racemosa, Cissampelos pareira*, Disc diffusion Method, Medicinal plant (Herbal)

INTRODUCTION

Medicinal plants have been inducted as a common source of alternative remedy for treating human diseases because they contain numerous bio-active constituents of therapeutic values[1]. Infectious diseases can be the result of colonisation of the various microbes in human systems and the pathogen is a micro-organism that has the potentiality to cause diseases. An infection is the invasion and multiplication of pathogenic microbes in healthy human systems. To cause a microbial infection, microbes enter into the body through four sites namely, (a) respiratory tract (including oral cavity), (b) gastrointestinal tract, c) urogenital tract and (d) dermal regions. Virulent strains of *E. coli* cause gastroenteritis, urinary tract infections and neonatal meningitis. In rare cases virulent strains are also responsible for haemolytic-uremic syndrome, peritonitis, mastitis, septicaemia and Gram-negative pneumonia[2]. *Pseudomonas aeuroginosa* frequently causes nosocomial infections such as pneumonia, urinary tract infections (UTI)[3]. *Staphylococcus aureus* infects tissues when the skin or mucosal barriers have been breached. This can lead to different types of infections including furuncles and carbuncles[4]. *Bacillus subtilis* has been known to cause disease in severely immune compromised patients rarely causes food poisoning[5]. A scientific study has been made possible by extracting the phyto-active chemical principles through a pharmacognostic approach keeping pace with their systemic evaluations[6].

Phyto-constituents like tannins act as a barrier for microorganisms for the protection of the plant. Flavonoids exhibit inhibitory activity against bacteria due to their β-rings which are more active against microorganisms and the more in the hydroxylation, is the greater antimicrobial activities[7]. Thus, in the present study methanolic extracts of bark of Ficus racemosa and root extract of *Cissampelos pareira* have been investigated against various microbes and interesting results have been obtained.

PHYTOGRAPHY ANALYSIS OF THE HERBALS

1. *Ficus racemosa* Linn.

Morphological profile:

Ficus racemosa Linn., locally known as 'Dimiri' (Odia) belongs to family *Moraceae*. It is a tree, highly cosmopolitan in occurrence, grows all over India especially in habitats like forests and hills. The tree is of medium height up to 10-16 meters; bark reddish grey, often cracked at outer surface with easily removable translucent flakes,greyish to rusty brown; uniformly hard and non-brittle. Many ancient scriptures of Ayurveda like Susruta Samhita described the properties of its bark as astringent, promotes healingprocess of fractured wounds by formation of callus (bhagna sandhaniya), alleviates hematemesis (Rakta pitta), burning sensation, obesity and useful in vaginal disorders.

Phytochemical profile

Bark of *Ficus racemosa* contain chemicals like new tetracyclic triterpene-glaunol, Sitosterol, unidentified long chain ketone, Ceryl behenate, lupeol and its acetate, amyrin acetate, stigmasterol sitosterol, glucoside and friedelin as per report outstanding[8].

Phytotherapeutic profile

The plant is used both, internally and externally as well, to meet many therapeutic remedies as:

External use: The latex is applied externally on chronic infected wounds to alleviate oedema and pain, it has been found to be effective in promoting the healing process. The decoction of its bark is used as an effective gargle against stomatitis and sore throat. Application of latex alleviates the oedema in adenitis, parotitis, orchitis, traumatic swelling and toothache[8].

Internal use: It incorporates vast range of maladies. The decoction of bark is useful in diarrhoea, dysentery and ulcerative colitis in gastrointestinal tract. In children, the latex is given along with sugar to combat diarrhoea and dysentery. In diabetes, the ripe fruits or decoction from bark is useful as it works well as anti-diuretic. In uterine bleeding

due to abortion, leucorrhoea and vaginitis, the decoction of its bark is given orally or in form of pessaries/suppository (basti) as well. The latex admixed with sugar removes sexual debility in males. The powdered bark works well as an anorexient[9].

2. *Cissampelos pareira* L. var. *hirsuta* (DC) Forman

Morphological profile

Cissampelos pareira locally known as 'Akanabindi'- Oriya; belongs to family Minispermaceae. Herbal of softly tomentose, herbaceous climbers; petiole to 2.5 cm long; lamina ovate to orbicular; inflorescence dioecious, subtended with many conspicuous bracts imbricate arranged; pistillate inflorescence longer than staminate ones; flowers greenish white.

Phytochemical profile

Cissampelos pareira contains a group of phytochemicals called isoquinoline alkaloids[10]. Out of thirty-eight alkaloids so far discovered; one, called tetrandrine is the most well documented[7]. Protoberberine alkaloids have been found in the roots.

Phytotherapeutic profile

Clinical research over the years has found tetrandrine to have pain-relieving, anti-inflammatory, and fever-reducing properties. Used in menstrual problems (pain, cramps, excessive bleeding, fibroids, and endometriosis) as a female tonic (hormonal balancing, menopausal libido loss, hormonal acne, premenstrual syndrome, childbirth) for heart problems (irregular heartbeat, high blood pressure, heart tonic) as a general antispasmodic and muscle-relaxer (asthma, stomach cramps, muscle pain/strains, irritable bowel syndrome [IBS], diverticulitis) for kidney support (kidney stones, kidney/urinary infections and pain)

MATERIAL AND METHODS

Ficus racemosa (barks) and *Cissampelos pareira* (roots) were collected from Tanginiguda of Malkangiri district in Odisha, the herbaria, so prepared from both the herbal species were identified, confirmed and duly authenticated by S. K. Dash, Head, P.G. Department of Biosciences, CPS. Mohuda, Berhampur (Odisha) and were preserved in the institutional museum of College of Pharmacy (Poly), Pandharpur of Solapur district, Maharashtra for future reference. The plant parts were washed, shade dried and extracted with methanol (90% v/v). The extracts so collected air dried at 50^0 to 60^0C; proceeded further for preliminary phytochemical analysis. The antimicrobial activities of the extracts were assessed following disc diffusion method[11,20.] Nutrient agar medium was prepared and sterilized in autoclave. Under aseptic condition, the media were poured into petri-dishes to uniform depth of 4 mm and allowed to be solidified at room temperature. The test microbes Escherichia coli (MTCC 1683), Pseudomonas aeuroginosa (MTCC 4673), Staphylococcus aureus (MTCC 7443) and Bacillus subtilis (MTCC 6942) were spread over the media with the help of a sterile swab soaked in respective bacterial cultures and thus used for antibacterial study. Both the extracts were dissolved separately in dimethyl sulfoxide (DMSO) to produce two concentrations of 200 and 400µg/disc and used for the study. Ofloxacin 5 µg/disc was used as the standard in these experiments. The sterile filter paper discs (6 mm) were immersed in definite concentration of plant extracts and placed over the solidified agar in such a way that there is no overlapping of the zone of inhibition. Plates were kept at room temperature for half an hour for the diffusion of the sample into the agar media. The organism inoculated petri-dishes were incubated at 37ºC for 24 hours. After the incubation period is over, the zone of inhibition produced by the samples and standard were measured. All tests were performed in triplicates.

Table 1: Comparative Phytochemical screening of the methanolic herbal extracts

Plants Constituents	*F. racemosa*	*C. pareira*
Carbohydrates	(--)	(--)
Alkaloids	(--)	(+)
Tannins	(+)	(+)
Flavonoids	(+)	(+)
Saponins	(+)	(--)
Proteins	(--)	(--)
Fats	(--)	(--)
Steroids	(+)	(+)
Triterpenoids	(--)	(+)

(+) – Presence, (--) - Absence

Table 2: Comparative antimicrobial activity of *Ficus racemosa* and *Cissampelos pareira*

Drug/ Extract	Concentration (µg/disc)	Mean diameter of growth inhibition zones (mm)			
		E. coli	S. aureus	P. aeuroginosa	B. subtilis
Ofloxacin	5	24	22	22	26
C. pareira	200	12	9	8	21
	400	18	12	12	18
F. racemosa	200	8	7	9	11
	400	10	8	10	13

Values are expressed in mean for zone of inhibition (n=3)

RESULTS

Phytochemical screening

Test solutions were prepared by dissolving the extracts in specific menstrum. In the present study, the methanolic extract of *Ficus racemosa* and that of *Cissampelos pareira* were found positive ascertaining the presence of tannins, flavonoids and steroids as in Table 1.

Antimicrobial activity

An effective antimicrobial activity was noticed from the herbal extracts in their both dilutions (200 & 400 µg/disc) comparing to that of a standard drug (Ofloxacin 5µg/disc) as evident from the data in Table 2. However, the activity of C. pareira was found better than that of *F. racemosa*. Also, *C. pareira* exhibited better activity against Bacillus subtilis comparatively with that of the standard drug used.

DISCUSSION

Antimicrobial drug resistance in human bacterial pathogens is a continuing worldwide issue and as a consequence, effective treatment and control of such organisms remains as yet an important challenge. Bacterial resistance has appeared for every major class of antibiotic. Since their introduction, the emergence of resistance to antibiotics has become increasingly evident, particularly for important pathogens such as Escherichia coli (E. coli), Salmonella sp., Campylobacter sp., Enterococcus sp. and Staphylococcus sp.[12, 13].

Literature says that medicinal plants are the backbone of traditional medicine[14] and the antibacterial activity of plant extracts are due to different bioactive chemical agents which were classified as active antimicrobial compounds[15]. Tannins are usually found in large quantities in the bark of trees where they act as a barrier for micro-organisms like bacteria and fungi and protect the tree. Tannins restraints the entries of any unwanted pathogenic substances, hence, are important from the microbial stand point.

Similarly, flavonoids (specifically catechins) are "the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants"[16, 17]. The flavonoids in significant quantities are incorporated into the human systems through the regular diet. Preliminary research indicates that flavonoids may modify allergens, viruses, and carcinogens, and so may be biological "response modifiers". *In-vitro* studies show that flavonoids also have anti-microbial activity[16,17,21]. Hence it is a need based to explore investigations in this regard. The present study facilitates a step accordingly to establish more in it. Many phytochemicals too encompass phytosterols. The ability of phytosterols to reduce cholesterol levels was first demonstrated in humans in 1953[18]. The present study attests a step further to establish the antimicrobial activityof the extracts containing phytosterols of *Ficus racemosa* and *Cissampelos pareira*.

From the above study it reveals that the presence of compounds like sterols, tannins and flavonoids[19], found in these two plants supposed to play an active role to guard the integrated live systems against the microbes.

CONCLUSION

The use of plants and plant preparations has been in existent since prehistory. The World Health Organization (WHO) reported that about 80% of the world's population depend mainly on traditional medicine and the traditional treatment involve mainly the use of plant extracts (WHO, 1993). In the present study, the root extract of *Cissampelos pareira* showed promising anti-microbial activities against Escherichia coli, Pseudomonas aeroginosa, Staphylococcus aureus and Bacillus subtilis comparative to that of the standard drug Ofloxacin. These findings suggest new pathway in elucidating antimicrobial potential from the two test species such as *Ficus racemosa* and Cissampelos pareirera.

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REFERENCES

1. Nostro, A., M.P. Germano, V. D'Angelo, A. Marino and M.A. Cannatelli. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett. Applied Microbial, 2000; 30: 379-384.
2. Todar, K. "Pathogenic E. coli". Online Textbook of Bacteriology. University of Wisconsin–Madison Department of Bacteriology, 2007; 11-30.
3. Pollack M. Pseudomonas Aeuroginosa. In: Mandell GL, Bennett JE, Dolin R, eds. *Principles and Practice of Infectious Diseases*. 5th ed. New York, NY: Churchill Livingstone, 2000; 2310-27.
4. Kluytmans J, Van Belkum A, Verbrugh H. "Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks". Clin. Microbiol. Rev, 1997; 10 (3): 505–20. PMC 172932. PMID 9227864.
5. Ryan K.J., Ray C.G. Congenital cytomegalovirus infection; Sherris Medical Microbiology; 4th edn. McGraw Hill, 2004; 566–9; ISBN 0-8385-8529-9.
6. Gordon, D. M., Riley, M. A. and Pinou, T. Temporal changes in the frequency of colicinogeny in Escherichia coli from house mice. Microbiology, 1998; 144: 2233-2240.
7. Sato M., S. Fujiwara, H. Tsuchiya, T. Fujii and M. Iinuma. Flavones with antibacterial activity against cariogenic bacteria. J. Ethnopharmacol, 1996; 54: 171-176.
8. Padmma M. Paarakh. "*Ficus racemosa* Linn An-Overview", Natural Product Radiance, 2009; 8(1): 84-90.
9. A. Poongothai,K. P. Sreena, K. Sreejith, M. Uthiralingam and S. Annapoorani. Preliminary phytochemicals screening of *Ficus racemosa* linn. Bark. Int. J. Pharma Bio Scs, 2011; 2(2): 431-434.
10. Samanta Jhuma, Bhattacharya S. *Cissampelos pareira* a promising anti-fertility agent, IJRAP, 2011; 2 (2): 439-442.
11. Sahoo, S., Kar, D.M., Mohapatra, S., Rout, S.P., Dash, S.K. Antibacterial activity of Hybanthus enneaspermus against selected UTI pathogens. Indian Journal of Pharmaceutical Sciences, 2006; 68(5): 653-655.
12. Adesiyun A., Offiah N., Seepersadsingh N., Rodrigo S., Lashley V., Musai L. Antimicrobial resistance of Salmonella spp. and Escherichia coli isolated from table eggs. Food Cont, 2007; 18: 306-311.
13. Rodrigo S, Adesiyun A, Asgarali Z, Swanston W. Antimicrobial resistance of Campylobacter spp. isolated from broilers in small poultry processing operations in Trinidad. Food Cont, 2007; 18: 321-325.
14. R. M. Coopoosamy. Traditional Information and antibacterial activity of four bulbine species (Wolf). African journal of Biotechnology; 10(2): 220-224.
15. Rojas A., Hernandez L., Pereda–Miranda R. and Mata R., 1992. Screening for antimicrobial activity of crude drug extracts and pure natural products from Mexican medicinal plants., J. Ethonopharmacol, 2011; 35:275-283
16. Cushnie T.P.T., Lamb A.J. "Recent advances in understanding the antibacterial properties of flavonoids". International Journal of Antimicrobial Agents, 2011; 38 (2): 99–107. doi:10.1016/j.ijantimicag.2011.02.014. PMID 21514796.
17. Cushnie T.P.T., Lamb A.J. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents, 2005; 26(5): 343-356. doi:10.1016/j.ijantimicag.2005.09.002. PMID 16323269.
18. Pollak, O.J. Reduction of blood cholesterol in man. Circulation, 1953; 7 (5): 702-6. doi:10.1161/01.CIR.7.5.702. PMID 13042924.
19. 19. Sanchez Medina, A. Evaluation of biological activity of crude extracts from plants used in Yucatecan traditional medicine part l. Antioxidant, antimicrobial and beta-glucosidase inhibition activities. Phytomedicine, 2001; 8(2):144-51.
20. Prabhat Kumar Jena, Subas Chandra Dinda and P. Ellaiah. Physical Evaluation and Antibacterial Activity of Various Leafy Extracts of Smilax zeylanica Linn. Journal of Pharmacy Research, 2012; 5(9): 4723-4725.
21. Surya Narayan Das, Varanasi Jagannath Patro and Subas Chandra Dinda. Anti-microbial activity of Leucas-clarkei, Bangladesh J Pharmacol, 2012; 7: 135-139.

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