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Research Article

IN VITRO ANTIBACTERIAL ACTIVITY OF CHITRAKADI VATI - A HERBO-MINERAL AYURVEDIC FORMULATION AGAINST *E. coli*

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Abstract

Present study was carried out with an objective to investigate the antibacterial potentials of Chitrakadi vati (a herbo-mineral Ayurvedic formulation). The aim of the study was to assess the antibacterial activity and to determine the zone of inhibition of methanol extracts of it. For this, its methanol extracts were evaluated against medically important bacterial strain using agar cup diffusion method. The antibacterial activities of extracts of Chitrakadi vati in concentrations (5, 25, 50, 100, 250 µg/ml) were tested against the Gram negative - *Escherichia coli,* human pathogenic bacteria, occurring commonly in intestines. Zone of inhibition of extracts were compared with that of standards of different generations like ampicillin, chloramphenicol, ciprofloxacin and norfloxacin for antibacterial activity. The results showed that the remarkable inhibition of the bacterial growth was shown by Chitrakadi vati against the tested organism. The antibacterial potential of the extracts were found to be dose dependent. The total microbial count of the drug was also found within permissible limits. The phytochemical analyses of the drug were carried out. The antibacterial activity of the Chitrakadi vati was due to the presence of various secondary metabolites. The study justifies the traditional use of Chitrakadi vati in most of the Gastro-intestinal disorders.

Key words: Chitrakadi Vati, Antibacterial; *E. coli*; In vitro.

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INTRODUCTION

Man is closely influenced by the activities of microorganisms. Microorganisms are a part of our lives in more ways than most of us understand. They have shaped our present environment and their activities will greatly influence our future also. Microorganisms should not be considered separate from human beings, but profound beneficial influence as a part of our life. Despite the established useful functions and potentially valuable activities of microorganisms, these microscopic norms of life may be best known as agents of food spoilage and causal agents of human beings. Animals and plants have also been known to be victims of microbial pathogens. Control of microbial population is necessary to prevent transmission of disease, infection, decomposition, contamination and spoilage caused by them. Bacterial infections are most common amongst these.

Antibiotics are one of our most important weapons in fighting these bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades, these health benefits are in danger as many commonly used antibiotics have become less effective against certain diseases, not only because of their adverse reactions, but also due to emergence of drug-resistant bacterial strains. Hence it is the need of present era to explore new antibiotics with lesser resistance.

Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. In many developing countries, traditional medicine is one of the primary healthcare systems.[1][2] Herbs are widely exploited in the traditional medicine and their curative potentials are well documented.[3] About 61% of new drugs, developed between 1981 and 2002 were based on natural products and they have been very successful, especially in the areas of infectious disease and cancer.[4] Recent trends, however, show that the discovery rate of active novel chemical entities is declining.[5] Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action.[6][7] Plants are rich in a wide variety of secondary metabolites such as tannins, steroids, alkaloids, flavonoids, glycosides, etc., which have been found *in vitro* to have antimicrobial properties.[8][9] Herbal medicines have been known to man for centuries. Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine.[10] Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population.[11]

In an effort to expand the spectrum of antibacterial agents from natural resources, Chitrakadi vati, a herbo-mineral Ayurvedic formulation has been selected here for evaluation of its antibacterial action. The very first description of its therapeutic use has been documented in Charaka samhita for the treatment of Grahani, a gastro-intestinal disorder; manifested as a result of weak digestive capacity.[12] This formulation exhibits Deepana (appetizing) and Pachana (digestive) activities by virtue of its different herbal and mineral contents. It has been also recommended in Grahani chikitsa by different Ayurvedic Acharyas.[13,14] Its main content is Chitraka (*Plumbago zeylanica*) which is considered to be good appetizer.[15] This formulation specifically works in various types of gastric disorders including anorexia, indigestion, nausea, diarrhea etc.

Escherichia coli (commonly abbreviated *E. coli*) is a Gram negative, facultative anaerobic, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms. Most *E. coli* strains are harmless, but some serotypes can cause serious food

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poisoning in humans. They are incriminated as pathogens because in certain instances some strains have been found to produce gastroenteritis, urinary tract infections, meningitis, septicemia, inflammations of liver and gall bladder, pneumonia etc. [16][17]

Hence present study has been carried out with an objective to investigate the antibacterial potentials of Chitrakadi vati against medically important Gram-negative bacteria strain E. coli.

MATERIAL AND METHODS

Procurement and preparation of test drug

The crude drugs mentioned in Charakasamhita in Grahani dosha chikitsa adhyaya[12] for the preparation of Chitrakadi vati, were procured from the Pharmacy of Gujarat Ayurved University, Jamnagar, after proper authentication by the Pharmacognosy laboratory of the Institute. The crude drugs used in Chitrakadi vati, with their botanical identities, parts used and proportions are given in Table 1.

Preparation of tablet

All the ingredients were taken in same proportion and vati (tablet) were prepared as per the standards mentioned in the Ayurvedic Pharmacopoeia of India.[18] These vatis were crushed, dried and passed through a no. 20 sieve. The formulation was then compressed in a single – punch tablet press with a target weight of 500 mg.

Preparation of extract

The extraction of Chitrakadi vati was carried out using standard procedures as per the Ayurvedic Pharmacopoeia of India.[19] Initially 5 g of coarse powder of the drug was extracted which yielded a yellowish to light brown solid residue weighing 0.612 g (30.59 % w/w). More yields of extracts were collected by the same method. The extracts were then kept in sterile bottles, under refrigerated conditions, until further use. The extracts were preserved at 2 to 4°C. These methanol extracts were used for further investigations.

Preliminary Phytochemical Screening

The extract was subjected to preliminary phytochemical testing to detect the presence of different chemical groups of compounds like saponins, tannins, alkaloids, flavonoids, steroids, glycosides, carbohydrates, and amino acids (protein) as described in literatures.[20-22]

Total microbial count (Total plate count)

For estimation of Microbial load,[23] bacterial and fungal growth study was carried out.

Antibacterial Activity - Test Micro-organisms and Growth Media

The microorganism *Escherichia coli* (MTCC 443) was chosen based on its clinical and pharmacological importance.[24] The bacterial strain procured from Institute of Microbial Technology, Chandigarh, was used for evaluating antimicrobial activity. The bacterial stock cultures were incubated for 24 hours at 37°C on nutrient agar and potato dextrose agar (PDA) medium (Microcare laboratory, Surat, India), following refrigeration storage at 4°C. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C (the bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C).

Antimicrobial agents

Ampicillin, Chloramphenicol, Ciprofloxacin, Norfloxacin for antibacterial activity. Dimethyl sulfoxide (DMSO) was used as diluents / vehicle to get desired concentration of drugs to test upon Standard bacterial strains.

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Table 1: Crude Drugs of Chitrakadi vati

Sr. No.	Drug Name	Botanical name	Family	Part Used	Propo-rtion
1	Chitraka	*Plumbago zeylanica*	Plumbaginaceae	Root	1 part
2	Pippalimula	*Piper longum* Linn.	Piperaceae	Root	1 part
3	Shunthi	*Zingiber officinale*	Zingiberaceae	Rhizome	1 part
4	Maricha	*Piper nigrum* Linn.	Piperaceae	Fruit	1 part
5	Pippali	*Piper longum* Linn.	Piperaceae	Fruit	1 part
6	Ajamoda	*Apium graveolus*	Umbelliferae	Fruit	1 part
7	Chavya	*Piper chaba*	Piperaceae	Root	1 part
8	Hingu	*Ferula foetida*		Exudate	1 part
9	Yavakshara	*Hordeum vulgare*	-	-	1 part
10	Sarjikshara	Crude alkaline earth	-	-	1 part
11	Sauvarchala Lavana	Black salt	-	-	1 part
12	Saindhava lavana	Rock salt	-	-	1 part
13	Vida lavana	Black salt	-	-	1 part
14	Samudra lavana	Sea salt	-	-	1 part
15	Audbhida lavana	Rock salt*	-	-	1 part
16	Matulunga	*Citrus aurantifolia*	Rutaceae	Fruit juice	Q.S.

*Official substitute

Determination of Zone of Inhibition Method

In vitro antibacterial activity of methanol extracts was carried out by using the Agar cup method.[25][26] It is one of the non automated in vitro bacterial susceptibility tests. This classic method yields a zone of inhibition in mm result for the amount of antibacterial agents that is needed to inhibit growth of specific microorganisms. It was carried out in petri plates. Each purified extracts were dissolved in DMSO, sterilized by filtration using sintered glass filter and stored at 4°C. For the determination of zone of inhibition (ZOI), gram negative bacterial strain was taken and as a standard antibiotic for comparison of the results. The extract was screened for its antibacterial activity against the E. coli. The sets of five dilutions (5, 25, 50, 100, and 250 µg/mL) of Chitrakadi vati extract and standard drugs were prepared in double distilled water using nutrient agar tubes. Muller Hinton sterile agar plates were seeded with indicator bacterial strains (10^8cfu) and allowed to stay at 37°C for 3 hours. The zones of growth inhibition around the disks were measured after 18 to 24 hours of incubation at 37°C for bacteria.

The sensitivity of the microorganism species to drug extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks, and values < 8 mm were considered as not active against microorganisms.

RESULTS

Preliminary phytochemical screening

It was found that methanol extract of Chitrakadi vati contains tannins, steroids, flavonoids, volatile oils, glycosides and alkaloids. (Table 2)

Table 2: Phyto-chemical screening of Methanol extract of Chitrakadi vati

Sr. No	Phytochemical Group	Results
1	Tannins	+
2	Saponins	–
3	Steroids	+
4	Flavonoids	+
5	Volatile Oils	+
6	Glycosides	+
7	Alkaloids	+
8	Carbohydrates	–
9	Amino acids	–

(+) Present and (–) Absent

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Table 3: Total Microbial Count and Pathogens (Total Plate Count) of Chitrakadi vati

Sr. No.	Test Parameters	Result	Limit
1	**Total Microbial Count**	30 CFU per gm	100 CFU per gm
	Total Bacterial Count	30 CFU per gm	
	Total Fungal Count	00 CFU per gm	
2	**Pathogens**		Should be Absent per 10 gm
	E. coli	Absent	
	Salmonella spp.	Absent	
	Streptococci spp.	Absent	
	Pseudomonas aeruginosa	Absent	
	S. aureus	Absent	

Table 4: Antibacterial activity (Zone of Inhibition in mm) of Chitrakadi Vati & Standard Drugs against *E. Coli*

Sr. No.	Gram negative bacteria		*E. Coli* (MTCC 443)				
	Drug Concentration (µg/ml)		5	25	50	100	250
1	Test Drug	*Chitrakadi Vati*	11	13	16	17	21
2	Standard Drugs	Ampicillin	14	15	16	19	20
		Chloramphenicol	14	17	23	23	23
		Ciprofloxacin	20	23	28	28	28
		Norfloxacin	22	25	26	27	29

Total microbial count (Total plate count)

Findings of the analysis of microbial count and pathogens of Chitrakadi vati tablet were within normal limits as shown in Table 3.

Microbial activity

The antibacterial activity of methanol extract of Chitrakadi vati was studied in different concentrations (5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml, 250 µg/ml) against the pathogenic bacterial strain, gram negative *E. coli* (MTCC 443). Antibacterial potential of extracts were assessed in terms of zone of inhibition of bacterial growth. The results of the antibacterial activities are presented in Table 4. The results revealed that the test drug extracts showed remarkable inhibition zone which ranged from 11–21 mm on measurement for selected bacterial strain. The antibacterial activity of the extracts increased linearly with increase in concentration of extracts (µg/ml).

The extracts of Chitrakadi vati were found to be effective against the selected microbe tested in all concentrations. (Table 4)

DISCUSSION

Medicinal plant matters normally carry bacteria and moulds often originating in soil in high numbers. In the present formulation, the microbial count was found within permissible limits.[27] which indicates that the proper hygiene norms were followed during the preparation of formulation and packing.

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies. In this work, the methanol extracts obtained from Chitrakadi vati showed remarkable activity against the tested bacterial strain. The results were compared with standard antibiotic drugs.

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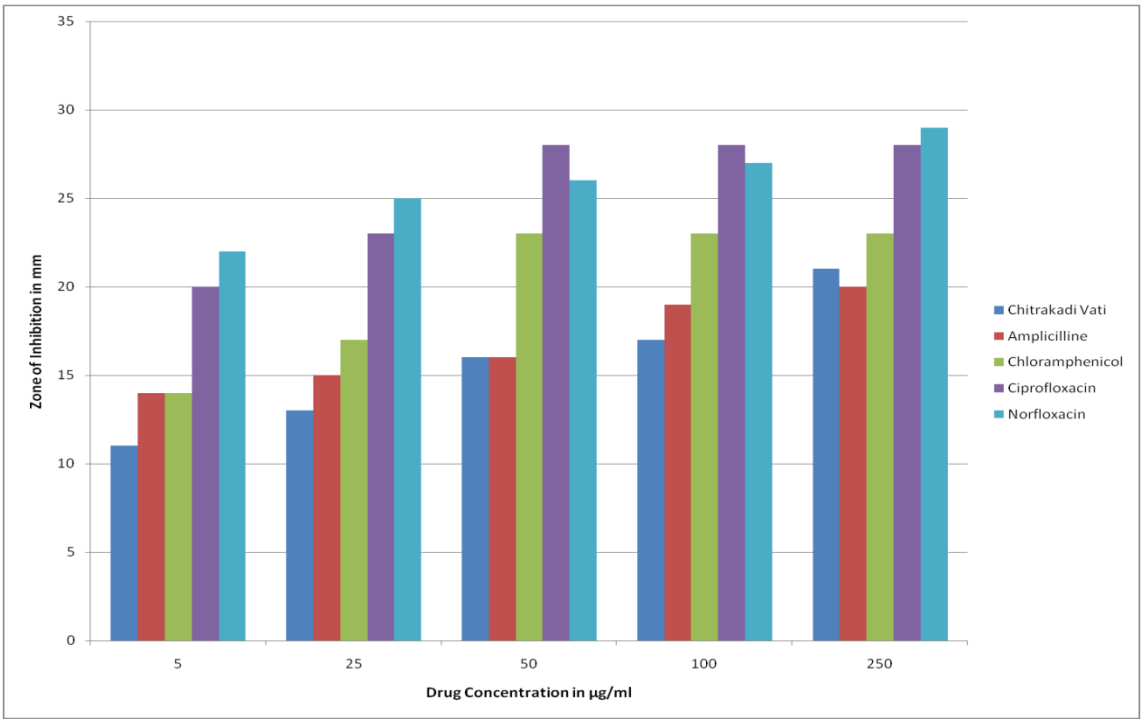
Figure 1: Antibacterial Activity against *E. coli* (MTCC 443)



The doses of the extracts of test drug used for screening were less than its therapeutic dose (2 g/day in divided doses). Still, no extracts of Chitrakadi vati were found to be inactive against the organism in this study. The growth of bacteria was effectively inhibited by all the extracts of Chitrakadi vati. This study showed the presence of different phytochemicals with the biological activity that can be of the valuable therapeutic index. The result of phytochemicals in the present investigation showed that this formulation contains tannins, steroids, flavonoids, volatile oils, glycosides and alkaloids. From the above results, the extracts of Chitrakadi vati showed significant antibacterial activity.

CONCLUSION

Antimicrobial resistance is a global problem. Emergence of multidrug resistance has limited the therapeutic options. Hence, this study was aimed to focus the antimicrobial properties of Chitrakadi vati on most common intestinal pathogenic organism *E.coli*.

In the current investigation, the methanol extract of Chitrakadi vati was found to be active on Gram negative bacteria *E. coli* in the dose which was lesser than its therapeutic dose in comparison to standard antibacterial drugs of different generations and the total microbial load of the drug was also found within normal limits. The antibacterial activity may be found more in therapeutic dose of its basic form. This study has justified the traditional use of Chitrakadi vati in most of the Gastro-intestinal disorders.

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