REPORT

In vitro evaluation of antimicrobial activity of *Kutajghan vati* – An Ayurvedic formulation

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Abstract: The present investigation focuses to determine the antimicrobial potential of an Ayurvedic formulation Kutajghan vati. In this study the activity of this formulation was compared with the standard antibiotics like Amikacin and Norfloxacin. Ethanol, methanol and acetone extract of *Kutajghan vati* demonstrated good antimicrobial activity and thus can form the basis for the development of a novel antibacterial formulation.

Keywords: *Kutajghan vati*, antimicrobial activity, Ayurvedic formulation.

INTRODUCTION

The search and use of drugs and dietary supplements of plant origin have accelerated in recent years. Ethno botanists, pharmacologists, microbiologists and natural-product chemists are combing the earth for phytochemicals which could be developed for treatment of infectious diseases. Out of 25 to 50% of current pharmaceuticals derived from plants, very few are used as antimicrobials. Since time immemorial, the traditional physicians have been using plants for prevention or cure of infectious conditions. Western medicine is trying to duplicate their success (Bansal *et al*., 2010). Today 80% of the world's population in African, Asian, Latin American and Middle Eastern countries is using plants as traditional health remedies due to minimal side effects (Bibitha *et al*., 2002; Maghrani *et al*., 2005; Doughari, 2006). In present scenario, pharmaceutical companies are investing significant amount of time and money for development of therapeutics based upon natural products obtained from plants (Ben Sassi *et al*., 2007; Coruh *et al*., 2007).

It is well known that use of most of the modern antimicrobials is fraught with adverse effects. The problem gets complicated since these antimicrobials are used for an extended period of time. Therefore, there is need to explore antibacterial activity of certain herbs and to create evidences for their efficacy. *Kutajghan vati* is a traditional Ayurvedic anti-dysenteric preparation which contains bark of Kurchi (*Holarrhena antidysenterica* L.) and roots of Aconite (*Aconitum napellus* L.) or whole plant of Atish (*Aconitum heterophyllum* Wall.). It is known to retrieve normal tone of intestine by dropping inflammation of small and large intestine. It is also known to enhance healing in colonic ulcers and restoring of digestion (Lather *et al*., 2010). At present there is no evidence for the antimicrobial potential of *Kutjghan vati.* So, the present study was undertaken to determine the *in-vitro* antimicrobial potential of this preparation.

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MATERIALAND METHODS

Drugs and chemicals

The present study was conducted in the Department of Microbiology, Government Medical College, Faridkot. Kutajghan Vati was procured from the local market of Faridkot.

Microbial strains

Different microbial strains of *Staphylococcus aureus* (MTCC 3160), *Pseudomonas aeruginosa* (MTCC 424), *Klebsiella pneumoniae* (MTCC 3384) and *Escherichia coli* (MTCC 739) used in the present study were procured from Institute of Microbial Technology, Sec. 39-A, Chandigarh, India.

Extraction of drug material

Method of Parekh *et al*. (2005) was used for the extraction of drug material (after some modifications). The aqueous extract was prepared by adding 20g of herbal preparations in 200 ml distilled water, heated at 60°C for 2 h, filtered and the filtrate was evaporated on sand bath. The dry mass (3.6%w/w) was then stored at

4°C. The organic solvent extract was prepared by adding 20g herbal preparation (powder) in 200ml of organic solvent (acetone, ethanol and methanol) in screw-capped bottles and was put at 190-220rpm on a rotary shaker. After 24h of shaking, the extract was filtered, evaporated in vacuum and dried by rotary evaporator at 60°C (Parekh *et al*., 2005, Tambekar *et al*., 2009). Dried extracts (2.9%, 3.1%, 3.7% w/w respectively) were stored in labeled sterile screw capped bottles at 4°C and later used for the *in vitro* study.

Formulation of extract

A known amount of powder was suspended in corresponding solvent to get desired concentration of suspension for the study of anti-microbial activity on the day of experimentation (Sharma *et al*., 2010).

Antimicrobial activity

Antibacterial activity was determined by using Disc Diffusion Method. The impregnated filter paper discs were employed to determine the antibacterial activity of both aqueous and organic solvent extracts of herbal preparation (NCCLS, 2002). For antibacterial properties, 0.1 ml bacterial suspension of 10^5CFU ml^{-1} was swabbed on Nutrient Agar plate to form lawn culture. The aqueous, acetone, ethanol and methanol extracts were prepared in their respective solvents. The filter paper discs (6mm in diameter) were separately impregnated with different concentrations of extract and then placed on the agar plates which had previously been inoculated with the test microorganisms. Discs were soaked in various organic solvents, dried and were placed on lawns as negative control. After incubation of 24h at 37°C, zone of inhibition of growth was measured in mm. The % inhibitory concentrations of the different extracts were measured and compared with the antibiotics (Kumar *et al*., 2009, Sharma *et al*., 2010) like Amikacin (30 µg) and Norfloxacin (10 µg) as standard.

RESULTS

From table it is evident that Ethanolic extracts of formulation showed more activity against *P. aeruginosa* as compared to other extracts of the formulation, Methanol extract showed significant activity against *Staphylococcus aureus* which is comparable with standard antibiotics while ethanol extract showed significant activity against *Staphylococcus aureus* which is

Table 1: Antimicrobial activity of different extract of *Kutajghan vati*

Sample	Microbial strains	Type of Extract	Zone of inhibition at different concentration (%) of extracts (mm)							
			100	80	60	40	20	C	Standard Antibiotics	
									Amikacin (30 µg)	Norfloxacin (10 µg)
A	*Pseudomonas aeruginosa* (MTCC 424)	Methanol	13	10	9	-	-	8	33	34
		Ethanol	**17**	13	12	7	-	7		
		Acetone	15	10	-	-	-	8		
		Water	-	-	-	-	-	-		
	Staphylococcus aureus (MTCC 3160)	Methanol	**20**	15	13	7	-	7	23	17
		Ethanol	17	12	10	8	-	7		
		Acetone	13	9	8	7	-	6		
		Water	-	-	-	-	-	-		
	Klebsiella pneumoniae (MTCC 3384)	Methanol	15	11	8	7	-	6	27	19
		Ethanol	**18**	13	10	-	-	8		
		Acetone	10	7	-	-	-	7		
		Water	-	-	-	-	-	-		
	E. coli (MTCC 739)	Methanol	**18**	13	11	9	-	8	17	17
		Ethanol	**17**	12	10	8	-	7		
		Acetone	**18**	11	9	8	-	7		
		Water	-	-	-	-	-	-		

comparable with norfloxacin (table). *Klebsiella pneumoniae* showed sensitivity against ethanol extract which is comparable to norfloxacin while *E. coli* was found sensitive to methanol, ethanol and acetone extracts of formulation which is comparable with standard antibiotics.

DISCUSSION

As mentioned above the *Kutajghan vati* is a compound formulation of Kurchi (*Holarrhena antidysenterica* L.) and roots of Aconite (*Aconitum napellus* L.) or whole plant of Atish (*Aconitum heterophyllum* Wall.). The reported antimicrobial activity in the present study seems to be the out come of antimicrobial action of its active components like norditerpenoids. The mechanism of action of terpenes is not fully understood but is speculated to involve membrane disruption by the lipophilic compounds (Bansal *et al*., 2010). In previous studies broad spectrum activity of aqueous and alcoholic extracts of stem bark of *H. antidysenterica* has been observed at concentrations of 200, 300 and 400 mg/ml against a number of enteric pathogens, but the inhibitory effect was best demonstrated at a concentration of 200 mg/ml of agar (Ballal *et al.*, 2001). In Ayurveda, kurchi has been reported to be used as antihelminthic, for diarrhoea and skin diseases. Portuguese have reported use of its hot decoction in bowel infection (Brown, 1992; Ahmad *et al.*, 1998). Two new aconitine-type norditerpenoid alkaloids 6-dehydroacetylsepaconitine and 13-hydroxylappa-conitine, alongwith three known norditerpenoid alkaloids lycoctonine, delphatine and lappaconitine have been reported from the roots of the *Aconitum heterophyllum* Wall. These compounds exhibited significant antibacterial activity (Ahmad *et al.*, 2008).

CONCLUSION

Our findings suggest that the Ayurvedic herbal preparation *Kutajghan vati* extracts have antimicrobial properties and they can be used in the treatment of infectious diseases. On comparing the zone of inhibition of *Kutajghan vati* extract to that of standard antibiotics (Amikacin and Norfloxacin) it is concluded that, the formulation in question shows comparable activity against *E. coli.* The most active extract can be further evaluated pharmacologically as well as for its chemically active components in the formulation.

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