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IN VITRO SCREENING FOR ANTIBACTERIAL ACTIVITY OF VARIOUS EXTRACT OF *CENTRATHERUM ANTHELMINTICUM* SEEDS

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ABSTRACT

The antibacterial effect of various extracts of *Kalijiri (Centratherum Anthelminticum) seeds* was studied. *Kalijiri* seeds extracts were obtained by soxhlet extraction method solvent using solvents ethanol, petroleum ether, hexane, chloroform, diethyl ether. The extracts were assayed for antimicrobial activity and bacterial growth inhibition activity by agar well diffusion method. The results showed that ehtanolic extract has good antibacterial activity with Minimum Inhibitory Concentration (MIC) against *Pesudomonas aurigenousa* and *K.pneumoniae* of 2 mg/ml and 2.5 mg/ml of seed extract respectively with compare to standard antibiotic while other extracts did not show any antibacterial activity against all tested bacterial species.

Key words: Kalijiri, Antimicrobial Activity, Agar well diffusion method.

INTRODUCTION

Kaliziri is an Indian medicinal plant though it is vastly used in culinary purposes. The seeds of Kaliziri are generally popular for its usage in culinary practices and the entire plant is considered to be useful for treating diseases. Kaliziri is scientifically named as *Centratherum anthelminticum* Kuntze.

Centratherum anthelminticum (Kalajiri, Somraj) of the Asteraceae family is an erect, annual herb. It has been used for a number of medicinal purposes since ancient times as its seeds are anthelmintic and antiseptic. They are used to cure kidney troubles, asthma, hiccups, sores and white leprosy. Powdered seeds are also used externally to treat paralysis of legs. Roots are useful in curing diarrhoea, stomachache, ulcers and cough and the flowers are purgative [1].

Centratherum anthelminticum seeds contain 18% fixed oil and .02% volatile oil and also reported to contain flavonoids. The major constituents are, 7(Z)(28)-Stigmastadienol, 5-stigmasten-3β-ol, 7,22-stigmastadienol, 8,14(Z) - 24 (28) - stigmastatrienol acetate, verovan, p-hydroxybenzoyl vernovan, vernodalin, butein, 3,4,2‘,4‘,5‘-pentahydroxy-6‘-methoxy-2-methylchalcone, daucosterol, vernolic acid, acacetin-7-0-β-D-glucopyranosyl(1—4)-α-D-xylopyranoside.and others like stigmasterol, brassicasterol, 4α-methylvernosterol(Akihisa *et al*., 1992), vernosterol, avenosterol, (24αS)-stigmasta-5,22-dien-3β-ol, (24αS)-stigmasta-7,22-dien-3β-ol, (24αR)-stigmasta-7-en-3-one, (24αR)-stigmasta-7,9(11)-dien-3one: βamyrin; linoleic acid, oleic acid and sugars [2].

Ethanolic extract of seeds showed anthelmintic, hypotensive and laxative effects in rats. Ethanolic and ethanol: water (l: 1) extracts of seeds showed smooth muscle stimulant activity in guineapigs. Ethanol extract of seeds showed spermicidal activity in rats. Acetone, ethyl acetate and methanol extracts of seeds showed inhibition of spontaneous activity of whole worm as well as nerve muscle preparation of *Setaria cervi.* The alcoholic, ethereal and aqueous extract of seeds showed *In Vitro* anthelmintic activity against *Hymenolepis nana, Fasciolopsis buski* and *Ascaris lumhricoides* and *In Vivo* activity against *H. nana.* Further, the alcoholic extract also showed *In Vivo* anthelmintic activity against *F. buski* [3].

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The objective of the present work is to perform the *in vitro* screening for Antibacterial activity of various extracts of *Centratherum anthelminticum* seeds and to find minimum inhibitory concentration agaist tested microorganisms.

MATERIAL AND METHOD

Preparation of Extracts

Extraction of the *Centratherum anthelminticum* seeds were carried out by the percolation method using the Soxhlet apparatus. Kalijiri seeds were obtained from the local market and identified by botanist in Krushi Vighyan Kendra at Ganpat University. The dried fruits were then extracted using solvents ethanol, petroleum ether, hexane, chloroform, diethyl ether.

The soxhlet solvents extracts were obtained by soxhlet extraction of 25g of dried seeds in 100ml-200ml of solvents at 65° C using soxhlet apparatus. The extract was then concentrated to 10ml on a water bath and dried at room temperature. Percentage yield of various extracts were noted in each solvent.

Antibacterial activity

The antibacterial activity was determined by the diffusion method of Kirby Bauer described by Duguid *et al*., (1989). This method determines the antibacterial activity of the extracts.

Preparation of the nutrient medium

Nutrient agar medium was prepared by dissolving 2.8g of nutrient agar in 100ml distilled water. The solution was sterilized in an autoclave at 121oC (1.1N pressure) for 15 min. The suspension was cooled and poured into sterile Petri dishes to solidify. The agar depth of the medium was 4.0mm.

Preparation of culture and Inoculation

Pure culture of *Klebsiella pneumonia* (MTCC no. 432), *Micrococcus luteus* (MTCC no. 106), *Staphylococcus aureus* (MTCC no. 096), *Proteus vulgaris* (MTCC no. 426), *Bacillus subtilis* (MTCC no. 441), *Bacillus cereus* (MTCC no. 430), *Bacillus pumilus* (MTCC no. 1607), *Escherichia coli* (MTCC no. 443), *Vibrio cholera* (MTCC no.3906) and *Salmonella typhi* (MTCC no. 734) were obtain from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India, were separately used to inoculate the Petri dishes by streaking the surface of the plates in a zigzag manner until the entire surface was then covered. The inoculated plates were then incubated into the incubator at temperature specified by the MTCC for each strain.

Method for Antibacterial Assay

The extracts were dissolved in the appropriate solvent to obtain the concentration of 100µg/ml and 1000µg/ml. 4 wells were punched into the inoculated Petri plate using the 6 mm cork borer. 100ul of prepared extract were filled into the well using the micropipette using the sterile tip. The plates were incubated at the appropriate temperature. Streptomycin used as a standard drug taken as positive control [4-6.

The plates were examined for clear zones of inhibition. Presence of zones of inhibition indicated activity. The zones were measured and finally minimum inhibitory concentration (MIC) was found out.

RESULTS & DISCUSSION

Chloroform extract, diethyl ether extract, hexane and petroleum ether extract were tested on a panel of Gram positive and negative bacteria. The antibacterial activities of extracts and zones of inhibition exhibited by standard antibiotic (streptomycin) are also depicted in table-1.

Among all different extracts discussed above, ethanol extract produced positive anti bacterial activity while Chloroform extract, diethyl ether extract, hexane extract, petroleum ether as well as DMSO produced negative anti bacterial activity.

From above table, it was shown that ethanol extract was effective against all bacterial strain except *v.cholerae*. Among all bacterial strain, *p.aeruginosa* and *K.pneaumoniae* was considered as a highly sensitive against ethanol extract as minimum inhibitory concentration (MIC) of ethanol extract for *P.aeruginosa* and *K.pneaumoniae* were found to be 2.5 (mg/ml) and that of which was lowest MIC among remaining strain. DMSO was used as solvent which did not produce any activity on bacterial strain.

All the Ethanolic extract was found to be active at a concentration of 10mg/ml against all organisms except *V.Cholerae* (Table-2). Hence the further aunthenticate them antibacterial activity, MIC determination was carried out using cup plate method. In this method according to IP1996, in each well.1ml solution of different concentration was poured. The concentrations, which show minimum inhibition, were considered as MIC. From above study, the MIC of ethanol extract of Centratherum anthelminticum was found to be 2 mg/ml against *Pseudomonas aeruginosa*, 5mg/ml against *Proteus vulgaris* and *K.pneumoniae 2.5 mg/ml and Bacillus cereus 10 mg/ml.*

Table 1. Result for Antimicrobial assay of various extract of *Centratherum anthelminticum* using Agar well diffusion method

Sr. no.	Name of Strain	Crude Extract										Std Drug	Solvent
		Ethanol (mg/ml)		Petroleum ether (mg/ml)		Hexane (mg/ml)		Chloro-form (mg/ml)		Diethyl-ether (mg/ml)		Streptomycin (mg/ml)	DMSO
		10	40	10	40	10	40	10	40	10	40	10	99%
1	*P.aeruginosa*	++	+	-	-	-	-	-	-	-	-	++	-
2	*P. vulgaris*	+	+	-	-	-	-	-	-	-	-	++	-
3	*K.pneaumoniae*	++	+	-	-	-	-	-	-	-	-	++	-
4	*B.cereus*	+	+	-	-	-	-	-	-	-	-	++	-
5	*B.pumilus*	+	+	-	-	-	-	-	-	-	-	+++	-
6	*E.coli*	+	+	-	-	-	-	-	-	-	-	+++	-
7	*M.luteus*	+	+	-	-	-	-	-	-	-	-	++	-
8	*S.typhi*	-	+	-	-	-	-	-	-	-	-	++	-
9	*V.cholerae*	-	-	-	-	-	-	-	-	-	-	++	-

+ Zone of inhibition (1-9 mm), ++ Zone of Inhibition (10-15 mm), +++ Zone of inhibition (16 mm and above),
- No activity

Table 2. MIC of Ethanolic extracts of *Centratherum anthelminticum seed* aginst Different strain.

Sr. No.	Bacterial strain	MIC of ethanolic extract (mg/ml)
1.	*P.aeruginosa*	2
2.	*P.vulgaris*	5
3.	*K.pneaumoniae*	2.5
4.	*B.cereus*	10
5.	*B.pumilus*	10
6.	*E.coli*	10
7.	*M.luteus*	10
8.	*S.typhi*	40

Figure: 1 Seeds of *Kalijiri*

CONCLUSION

From the results, we can concluded that ethanolic extract of *Centratherum anthelminticum* seed was most effective against *P.aeruginosa* and *K.pneaumoniae* bacterial strain as the MIC of the extract for *P.aeruginosa* and *K.pneaumoniae* were 2mg/ml and 2.5 mg/ml respectively with compared to standard streptomycin (10 mg/ml). While in other extract the activity was not remarkable due to absence of activity responsible component. So the further investigation can be done using more purified extract by another sensitive method like Microtitre plate based Antimicrobial assay technique.

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