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(RESEARCH ARTICLE)

Biochemical profiling of different extracts of *Centratherium anthelminticum* seeds and its synergistic, antimicrobial activity on clinical, drug resistant and standard strains of some common microorganisms

Thara KM *

Department of Biotechnology, University of Calicut, Thenjippalam, Malappuram District, Kerala, India, Pin-673635.

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Abstract

Extracts of seeds of *Centratherium anthelminticum* was prepared by Bioassay guided fractionation using different solvents. Different extracts from the plant *Centratherium anthelminticum*was biochemically analyzed for different bioactive components and properties. It shows an inhibitory action on different clinical strains within a range of 100 - 500µg/ ml.

Keywords: Antimicrobial activity; MRSA; HPLC; *Centratherumanthelminticum*; Biochemical profiling

1. Introduction

Centratherumanthelminticum (L) Kuntze., (Purple flebane) is a common ingredient in different ayurvedic preparations. The biological activities are related to the structure of the compound to an extent. Most important classes of biologically active compound belong to phenolic, flavonoids, alkaloids, terpenoids etc. *Centratherum anthelminticum (L)* Kuntze., (Purple flebane).

This plant belongs to the family *Astearace* and other common names are 'Kattujeerakam' 'Vanajeera', 'Aranyageera' and Kalijiri'. It is seen as herb in hilly areas and sub-tropical regions. Medicinal properties are vested in its seeds, roots, leaves etc. It is effective in treating thread worm infections and gastric troubles. Dry seeds were used in the present study [1].

2. Material and methods

Plant material: The seeds of *Centratherum anthelminticum* were collected from the plants Wayanad district, Kerala. It was identified by the Department of Botany, University of Calicut. Voucher no.7214 and was deposited in the herbarium.

Plant material and seeds were collected, washed air died and aqueous extract was prepared. The extract was prepared as by the method described by Thara et al., 2013[4].Different biochemical analysis were also performed for testing the presence of phenolics, flavanoids,terpenoids, saponins, glycosides and proteins[4],[5],[6],[7].HPLC profiling was also conducted as described earlier[4],[8].

* Corresponding author
E-mail address: tara_menon2003@yahoo.co.in

2.1. Test organisms

2.1.1. Standard strains

Escherichia coli MTCC 41, *Proteus vulgaris* MTCC 426, *Pseudomonas aeruginosa* MTCC 424, *Staphylococcus aureus* MTCC 87, *Candida albicans* MTCC 183. These cultures were purchased from Institute of Microbial Technology (IMTECH), Chandigargh.*Klebsilla pneumonia* and *Aspergillus nige*r were collected from the Department of Life Sciences, University of Calicut.

2.1.2. Clinical strains

The following clinical strains were also tested. The strains were collected from Department of Microbiology, Govt. Medical College, Calicut.*Acenetobacter, Enterobacter, Klebsillia, Proteus mirabilis, Proteus vulgaris, Salmonella typii,Shigella* and *Staphylococcus aureus.*

2.1.3. MDR strains

MRSA (ATCC 43300) –Collected from Jubilee mission medical college, Thrissur.

2.2. Antibacterial activity

2.2.1. Determination of minimum inhibitory concentration (MIC)

MICs for each extract against each microbial strains were determined using nutrient broth. 10 ml of the medium was taken in a 50 ml conical flask and sterilized. The extract (stock diluted to 10mg/ ml in DMSO) was then added to 10 ml of medium in a series of conical flasks to get a concentration of 10000, 1000, 100, 10, 0 µg/ ml initially (10 times dilution). Then double dilution was used to determine the range of inhibitory activity (MIC value) of each extract. A loop-full of fresh culture at log phase (1 x 10^8 CFU/ml, turbidity equal to that of 0.5 MacFarland solution) was added to each flask so as to have concentration of 5 x 10^5 CFU/ ml. It was then incubated at 36^0 for 18 to 24 hrs. The OD at 600 nm was monitored at different concentrations of the drug in the culture media. The least concentration at which the growth of the organism was completely inhibited was recorded as MIC of that extract, comparing the OD with that of the blank. It was then confirmed by inoculating to fresh agar plate [9],[10],[11]. The experiment was done in triplicate and average value of MIC was then taken. It was then statistically analyzed and $p< 0.05$ was considered as significant. The experiment was repeated for different strains and extracts. The values were compared for gram positive and negative bacteria. The difference in inhibitory effect of the extract on the growth of clinical and standard strains was compared.

2.2.2. Synergistic effect

The method was done using a 96-well micro-titer plate. In this 200 µl of the sterile nutrient broth was added to each well. In the first well of series 1, ie A1, antibiotic at a concentration of 100 µg/ ml was added. In the well B1 sample AS1 at a concentration of 500 µg/ml was added. In the well C1 both antibiotic and AS1 were added. The concentration of the antibiotic and AS1 were the respective MICs or above the MIC values. All the wells were then serially diluted to next well.ie 100 µl from first well has transferred to second and 100 µl from second to third and so on so that a double dilution had obtained. All wells were then inoculated with culture at log phase, mixed well, covered and incubated at 36^0C for 18 hrs. The growth was observed and MICs were noted and Fractional inhibitory concentrations (FICs) were calculated [12], [13].
Fractional inhibitory concentration (FIC) was calculated using the formula
FIC= MIC of drug in combination/ MIC of drug alone + MIC of extract in combination /MIC of extract alone.

2.3. Statistical analysis

All data were analyzed using SPSS version 16.0.

3. Results and discussion

The yield of the aqueous extract was yield was 3.5%. Biochemical analysis shows the presence of alkaloids, saponins and glycosides in aqueous extract (Table 2, 3). HPLC profiling shows two major peaks at R_T 1.6 and 7.6 [Fig. 1]. Results of the *in vitro* anti-bacterial activity of the aqueous and methanol extract were summarized in Table 4 and 5.

Table 1 Percentage yield of the extracts

Name of the plant	Part used	Solvent	Yield	% yield	Designated as
Centratheriumanthelminticum	Seeds	Methanol	2.5	5.0	CA1
		Ethanol	1.0	2.5	CA2
		Water	1.1	2.8	CA3
		Chloroform	0.1	0.20	CA4

Table 2 Various metabolites present in the extracts

Name of the plant	Extract tested	Phenolic acids	Flavonoids	Alkaloids	Saponins	Glycosides	Terpenoids	Protein
Centratheriumanthelminticum	HCI	++	+	+	-	-	-	-
	HC3	-	-	+	+	+		++

+++ Present high level, ++ medium level, + low level,- absent

Table 3 Total phenolics and flavonoids in the extracts

Name of the plant	Extract used for the estimation	Total phenolics as Gallic acid equivalents (GE)	Total flavanoids as Quercetin equivalents (QE)
Centratheriumanthelminticum	HCI	8 + 3	0.8 + 0.2
	HC3	2.5	ND

Table 4 Antimicrobial activity of extracts against standard strains: Micro dilution test.

Name of the plant	**Extract used for the estimation**	***Escherichia coli* (µg/ ml)**	***Pseudomonas aeruginosa* (µg/ ml)**	***K.pneumonia* (µg/ml)**	***Proteus mirabilis* (µg/ml)**	***Staphylococcus aureus* (µg/ml)**	***Candida albicans* (µg/ml)**	***Aspergillus niger* (µg/ml)**
Centratheriumant helminticum	HCI HC3	250 220	440 200	250 150	220 150	260 160	450 250	ND ND
Gentamicin	GEN	30	30					
Streptomycin	SRT	50	40					
Penicillin	PEN	10u	10 IU					
Nystatin	NYS	40u	40					
DMSO	DMSO	-	-					
Water (sterile)		-	-					
Ampicillin		10 mcg						

ND-Not Done, NDT-Not detected. Mean ± SD of three values are calculated. P<0.05 is considered as significant comparing the MIC without extract.

Table 5 Antimicrobial activity of extracts against clinical strains

Name of the plant	**Extract used**	**Shigellaflexineri (µg/)**	**Escherichia coli (µg/ ml)**	**Klebsiella pneumonia (µg/ ml)**	**Proteus vulgaris (µg/ ml)**	**Staphylococcus aureus (µg/m l)**	**Enterobacter (µg/ ml)**	**Acinetobacter (µg/ml)**	**Proteous mirabilis** **Salmonela (µg/ml) typhi**	
Hemigraphiscolorata, (Blume) Hallier f.	HCI HC3	150 145.6	200 180	150 140	212 200	250 220	175 200	300 150	150 150 175 150	625

Table 6 MICs of extracts on MRSA (ATCC 43300)-Tube dilution test.

Name of the plant	Extract used for the estimation	MRSA (µg/ml)
Centratherium anthelminticum	HCI	200
Centratherium anthelminticum	HC3	100

Table 7 FIC of different extracts in combination with antibiotics against Staphylococcus aureus and Escherichia coli.

Microorganism tested	Extract used in combination	Antibiotic used	MIC of antibiotic alone	MIC of antibiotic in combination	MIC of extract alone	MIC of extract in combination	FIC INDEX
Staphylococcus aureus	HC3	Penicillin	20	7.0	250	150	0.95
E.coli	HC3	Gentamicin	15	6.0	250	200	1.0

FIC value < 0.5, synergy; 0.5-.75 partial synergy; 0.76 -1.0 additive; 1-4 indifference; and >4 antagonism

Aqueous extract showed significantly higher activity against microbes test compared with methanol extract. It showed maximum activity against *S. aureus* and *K. pneumonia*. The activity against clinical strains was also significant. It showed significant activity against *C.albicans* also. The extract showed good activity against drug resistant organism like MRSA [Table 6]. It showed synergistic effect against the microbes when tested along with antibiotics. When treated along with pencillin the concentration of pencillin was significantly reduced to less than half-fold [Table 7]. Plants are alternative source of medicines [14, 15, 16]. Present study shows *C. anthelminticum* is a potential source against microbial diseases.

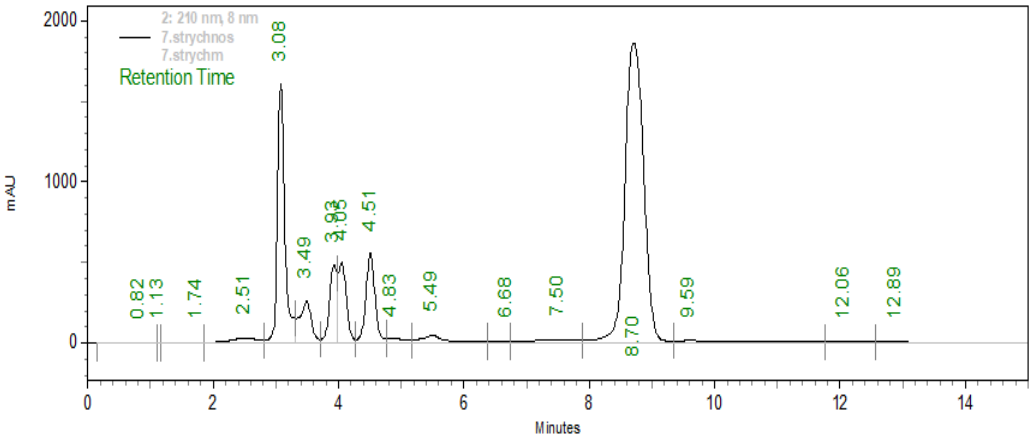

Figure 1 HPLC fingerprint of methanol extract of *Centratherium anthelminticum*

2: 210 nm, 8 nm							
Pk #	**Retention Time**	**Area**	**Area %**	**Height**	**Height %**	**Start Time**	**Stop Time**
1	0.82	13231	0.02	300	0.01	0.14	1.11
2	1.13	634	0.00	214	0.00	1.11	1.16
3	1.74	56952	0.08	2419	0.04	1.16	1.85
4	2.51	904046	1.19	31504	0.58	1.85	2.81
5	3.08	13500610	17.84	1604062	29.61	2.81	3.32
6	3.49	3452380	4.56	259187	4.78	3.32	3.71
7	3.93	4097439	5.42	482020	8.90	3.71	3.99
8	4.05	4417337	5.84	497379	9.18	3.99	4.28
9	4.51	5728857	7.57	556158	10.27	4.28	4.77
10	4.83	535907	0.71	26074	0.48	4.77	5.17
11	5.49	1345632	1.78	46849	0.86	5.17	6.37
12	6.68	202812	0.27	9504	0.18	6.37	6.74
13	7.50	1027991	1.36	22079	0.41	6.74	7.88
14	8.70	38969965	51.50	1859639	34.33	7.88	9.34
15	9.59	981843	1.30	13427	0.25	9.34	11.77
16	12.06	151680	0.20	3264	0.06	11.77	12.57
17	12.89	279929	0.37	3169	0.06	12.57	15.53
Totals		**75667245**	**100.00**	**5417248**	**100.00**		

HPLC chromatogram at 210 nm shows the presence of three major compounds with retention time 3.08 min, 4.51 min and 8.7 min and area of 13500610 mAu 5728857 mAu and 38969965 mAu respectively.

4. Conclusion

We can conclude that *Centratherium anthelminticum*has the potential source to develop into an antimicrobial agent. Both methanol and aqueous and methanol extract showed significant effect against standard and clinical strains respectively. It has synergistic effect with certain antibiotics also against *E.coli* as well as *S.aureus*. This has to be further studied as a combating strategy against drug resistance.

Compliance with ethical standards

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