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Research Article

STUDY OF ANTIMICROBIAL, ANTIFUNGAL, AND ANTHELMINTIC ACTIVITY OF THE ESSENTIAL OIL EXTRACTED FROM *BLUMEA ERIANTHA*

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ABSTRACT

Microbial and parasitic diseases are one of the most prevalent diseases in developing and developed countries. Their adverse effect on health and social-economic society are more visible and has been considered with tremendous public health importance. The universal role of plants in the treatment of diseases is established by their extensive usage in all important systems of medicine such as Ayurveda and Siddha. Medicinal plants are rich sources of secondary metabolites such as essential oils, which are potential sources of useful drugs. Blumea genus consists of varied species spread in tropical and sub tropical Asia and Africa. This genus includes various species which are used in preparation of traditional and endemic medicines. Blumea eriantha is known to show anti-diarrheal, anti-acne and anti-diabetic activity. The extraction of essential oil from Blumea eriantha leaves was done by hydrodistillation using Clevenger type apparatus. The anti-fungal and anti-microbial activity was appraised by Couplet method. The microbes used for anti-microbial activity were Salmonella typhi, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa and Staphylococcus aureus. For anti-fungal activity, fungi Candida albicans was used. The study of anthelmentic activity was done by using Pheritima posthuma (Indian adult earthworm). Further the susceptibility testing and anthelmentic activity results showed enormous therapeutic potential.

Keywords: Blumea eriantha, Anti-microbial, Anti-fungal, Anti-helmentic

INTRODUCTION

Emergence of new diseases and resistant bacterial strains are major health problems leading to increased mortality around the globe, so there is need for better and efficient anti-microbial agents (Ventola, 2015). Since ancient era, herbs have gained a major role in treating various diseases & disorders. Medicinal plant produces various primary and secondary metabolites which contributes to the pharmacological actions. The essential oils are one such secondary metabolite which are lipophilic; causing membrane expansion, increased permeability and alteration in transport mechanisms resulting in bacterial cell death, and hence can be used as antibacterial agent and anthelmentic agent (Domenico *et al*., 2005).

Herbal drugs produce comparatively less side effects than synthesized drugs so considered safer by population (Sharma *et al.*,2008). So herbal products are preferred by patients. Blumea genus consists of varied species spreading tropical and sub tropical Asia and Africa. This genus includes various species which are used in preparation of traditional and endemic medicines. *Blumea eriantha* is perennial herb is commonly known as Nimurdi & Kukronda. It is widely distributed in Karnataka, Maharashtra, Uttarpradesh, Madhyapradesh, Bihar and Orissa. The

plant has been reported to have carminative property, anti-diarrhoeal, antiacne and antidiabetic activity; while the essential oil extracted from its stem and leaves posses antimicrobial and antioxidant activity (Singh and Parthasarathy, 2012). The present study was carried out to determine anti-bacterial activity of essential oil extracted from *Blumea eriantha* against *Salmonella typhi*, *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* , anti-fungal activity against *Candida albicans* and anthelminthic activity against *Pheritima posthuma*.

MATERIALS AND METHODS

Plant collection & extraction procedure:

The medicinal plant was collected from Central Park, Kharghar in month of November and was authenticated at St. Xavier's Institute, Churchgate. The fresh plant material with its aerial part, stem and leaves were chopped into small pieces. 750g of fresh plant material was used and subjected to hydro distillation using Clevenger type apparatus of capacity 5 Litres. To this 3 litres of water was added. The mixture was heated on heating mantle at 85^0C. The distillation was continued for three hours. The essential oil obtained was then dried over anhydrous sodium sulphate and stored at room temperature in sealed vials until analysis.

Bacterial strains collection:

The microbes that were used for the anti-bacterial activity were *Salmonella typhi* (ATCC 23654), *Escherichia coli* (ATCC 8739), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC9027), and *Staphylococcus aureus* (ATCC 6538) . The fungi that was used for assessment of anti-fungal activity was *Candida albicans* (ATCC 2091). All the strains were procured from National Collection of Industrial Micro-organisms (NCIM), National Chemical Laboratory, Pune, India. Bacterial strains were maintained on Nutrient Agar slants and fungal strain was maintained on Sabouraud Dextrose Agar slants (Hi Media Laboratories Pvt. Ltd.) at 4ºC.

Earthworm collection:

The study of anthelmintic activity was done by using (*Pheritima posthuma*) Indian adult Earthworm. *Pheritima posthuma* was collected from Bio Genik system, Malviya road.

Controls and standards:

Streptomycin was used as standard for anti-bacterial activity while Tioconazole was used as standard for anti-fungal activity. Antimicrobial standards were procured from Abbot Healthcare Pvt. Ltd., Mumbai.

Antimicrobial activity test:

Antimicrobial activity of essential oil from *Blumea eriantha* was determined using agar well diffusion method. 25 ppm and 50 ppm solutions of extracted essential oil were prepared in DMSO. Inoculums were prepared in 0.9% saline solution and were adjusted to produce approximately 10^8 CFU/ml using 0.5 McFarland's standard. Agar plates were prepared. 100 μl of inoculums were spread on respective agar plates and then wells were made using sterile cork borer of 6 mm in diameter. 100 μl of each concentration of extract was then carefully poured into respective wells. Streptomycin and Tioconazole were taken as positive control. Negative control agar plates contain agar, inoculums and solvent used for dilutions (Collins *et al.*, 1998; Gaud and Gupta, 2006). This assay was done in triplicate and the anti-microbial activity was expressed as mean of inhibition diameters (mm) ± standard deviation.

Determination of minimum inhibitory, minimum bactericidal concentrations and minimum fungicidal concentrations:

The minimum inhibitory concentrations (MIC) for the micro-organisms were determined as using tube dilution assay. The cultures were diluted in nutrient broth and the density was adjusted using McFarland's standard, 0.5 ml of bacterial suspension was then added to 4.5 ml of test broth to which diluted extract solution was added. Positive controls were containing broth and suspension only. The negative control tubes were containing broth, inoculums and solvents used for dilution of the extracts. Minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined by applying the test dilutions on to fresh agar medium and followed by incubation for 24 and 48 hr respectively. The highest dilution producing no bacterial growth on agar medium was taken as MBC where as the highest dilution producing no fungal growth on agar medium was taken as MFC (I.P., 2010 ; Sahoo *et al*., 2013).

Anthelmintic activity test:

The evaluation of anthelmintic activity of essential oil was done by using *Pheritima posthuma* (Indian earthworm). Worms were divided into 4 groups with 3 worms in each group. Group I were treated with 1ml of 25 ppm solution of extracted essential oil in DMSO, Group II were treated with 1ml of 50 ppm solution of extracted oil in DMSO, Group III were treated with standard Albendazole (20mg/ml) and Group IV served as negative control (DMSO). The worms were placed in petridishes of respective groups. These petridishes were then placed at room temperature and the time taken for paralysis and death of worms was noted. Paralysis was said to occur when the worms do not receive any sense even in presence of normal saline. Death was concluded when the worms lost their motility and their body color faded when dipped in warm water (Patil *et al.,* 2015).

RESULTS AND DISCUSSION

The antimicrobial activity of essential oil obtained from *Blumea eriantha* was determined using agar well diffusion method using microbial cultures of *Salmonella typhi* (ATCC 23654), *Escherichia coli* (ATCC 8739), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC9027), and *Staphylococcus aureus* (ATCC 6538) and *Candida albicans* (ATCC 2091). The results (Table no. 1, Fig. 1) show that the extracted oil was capable of inhibiting the growth of above mentioned micro-organisms at both doses reflecting antimicrobial activity. The inhibitory diameters of test were compared with inhibitory diameters of standard antibiotics (Streptomycin and Tioconazole). It was found that with increased doses of extracted oil area of inhibition of microbial growth was increased. MIC, MBC and MFC of extract were determined (Table no. 2). MIC value for *Bacillus subtilis* and *Staphylococcus aureus* was recorded as 5.25mg/ml whereas for *Escherichia coli* and *Candida albicans* MIC value was 2.12mg/ml while for *Salmonella typhi* the MIC value was 4.12mg/ml. MBC values for *Bacillus subtilis*, *Staphylococcus aureus* were recorded as 10.5mg/ml and 11.5mg/ml respectively whereas, for *Escherichia coli* and *Salmonella typhi* MBC value was recorded as 3.5mg/ml. MFC value for *Candida albicans* was recorded as 3.5mg/ml. Lowest MIC value i.e. 2.12mg/ml was recorded for *Escherichia coli* and *Candida albicans*. Lowest MBC value i.e. 3.5mg/ml was recorded for *Escherichia coli*, *Salmonella typhi* and *Candida albicans*. Anthelmintic activity of essential oil was determined using *Pheritima postuma* (Indian earthworm) the time required for death of earthworms was noted and compared with DMSO control and standard drug Albendazole. The results (Table no. 3, Fig. 3) showed that the extracted oil has anthelmintic activity.

TABLE NO 1: ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL OBTAINED FROM *BLUMEA ERIANTHA*

Dose a -100 µl of 25ppm solution of extracted oil.

Group (n=3)	Zone of Inhibition (mm)					
	Salmonella typhi (ATCC 23654)	*Escherichia coli* (ATCC 8739)	*Bacillus subtilis* (ATCC 6633)	*Pseudomonas aeruginosa* (ATCC9027)	*Staphylococcus aureus* (ATCC 6538)	*Candida albicans* (ATCC2091)
Dose a (25ppm)	11.00 ± 0.9	11.98 ± 0.7	12.17 ± 0.7	13.80 ± 1.2	11.84 ± 0.9	12.34 ± 0.9
Dose b (50ppm)	13.60 ± 0.9	13.72 ± 0.8	13.60 ± 0.7	13.40 ± 0.6	14.20 ± 0.2	13.50 ± 0.5
Streptomycin (10µg/ml)	19.50 ±3.9	16.30 ± 1.8	15.10 ± 0.2	13.80 ± 1.2	NA	NA
Tioconazole (10µg/ml)	NA	NA	NA	NA	NA	28.10 ± 3.5

Dose b-100 µl of 50 ppm solution of extracted oil.
Values are expressed as mean of inhibition diameters (mm) ± standard deviation.
'-' indicates no zone of inhibition.

TABLE NO 2: ANTIMICROBIAL SUSCEPTIBILITY OF ESSENTIAL OIL

Microorganism	MIC (mg/ml)	MBC (mg/ml)	MFC (mg/ml)
B. subtilis	5.25	10.5	NA
S. aureus	5.25	11.5	NA
E. coli	2.12	3.5	NA
S. typhi	4.12	3.5	NA
C. albicans	2.12	NA	3.5

NA-Not applicable
N=3 indicates assay was done in triplicates.
MIC-Minimum inhibitory concentration
MBC-Minimum bactericidal concentration
MFC-Minimum fungicidal concentration
NA-Not applicable

TABLE NO 3: ANTHELMENTIC ACTIVITY OF ESSENTIAL OIL OBTAINED FROM *BLUMEA ERIANTHA*

Groups	Concentration	paralysis attack(min)			
		P_1	P_2	P_3	D
Group I Dose a	25ppm	13±0.3	22 ±0.2	26±0.9	75±0.5
Group II Dose b	50ppm	10.2±0.59	20± 0.9	23±0.3	68±0.9
Group III Albendazole	20mg/ml	23±0.3	26±0.6	30±0.75	58±0.54
Group IV Control(DMSO)	-	-	-	-	-

Dose a -1mlof 25 ppm solution of extracted oil.
Dose b-1ml of 50 ppm solution of extracted oil.
Values are expressed as time in mins ± standard deviation.
Where, P_1, P_2, P_3 = paralytic attack, D = death

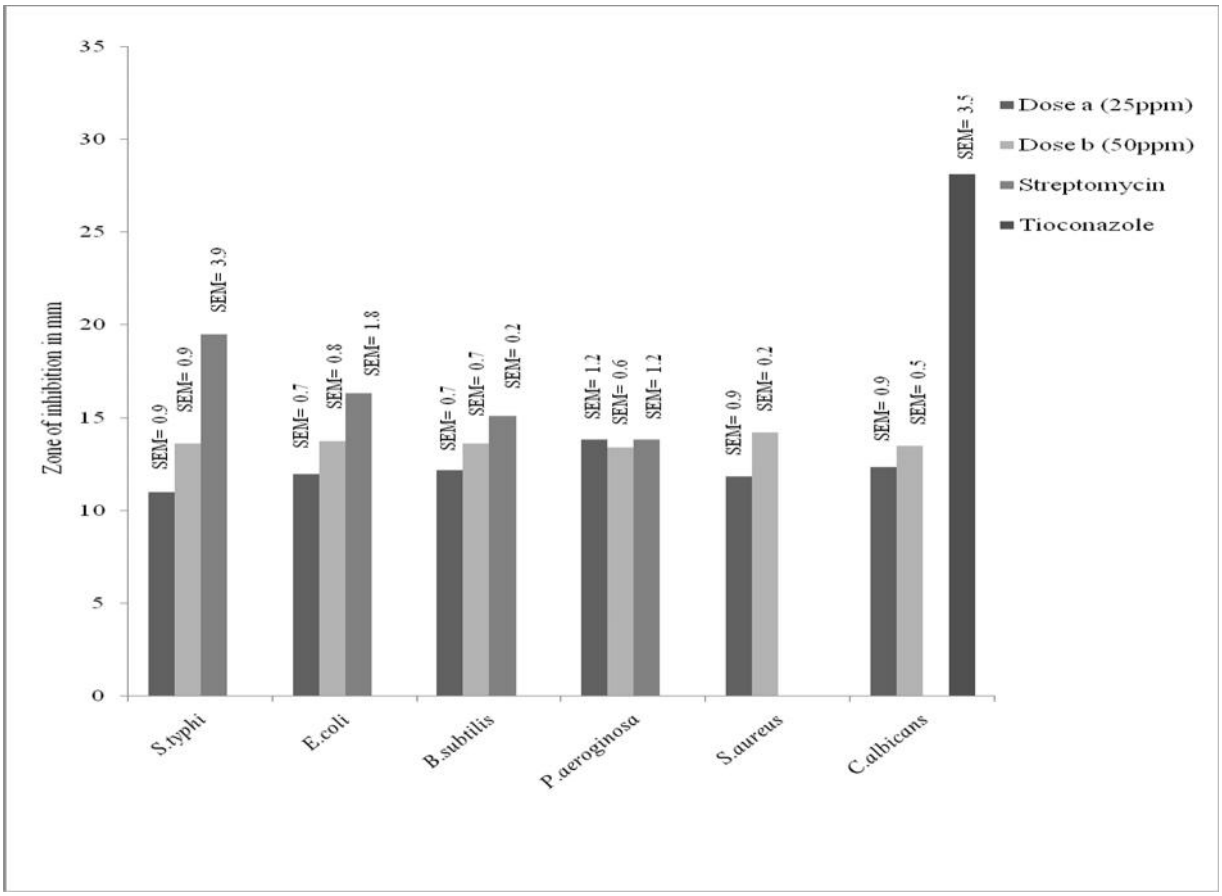

Fig 1: ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL OBTAINED FROM *BLUMEA ERIANTHA*

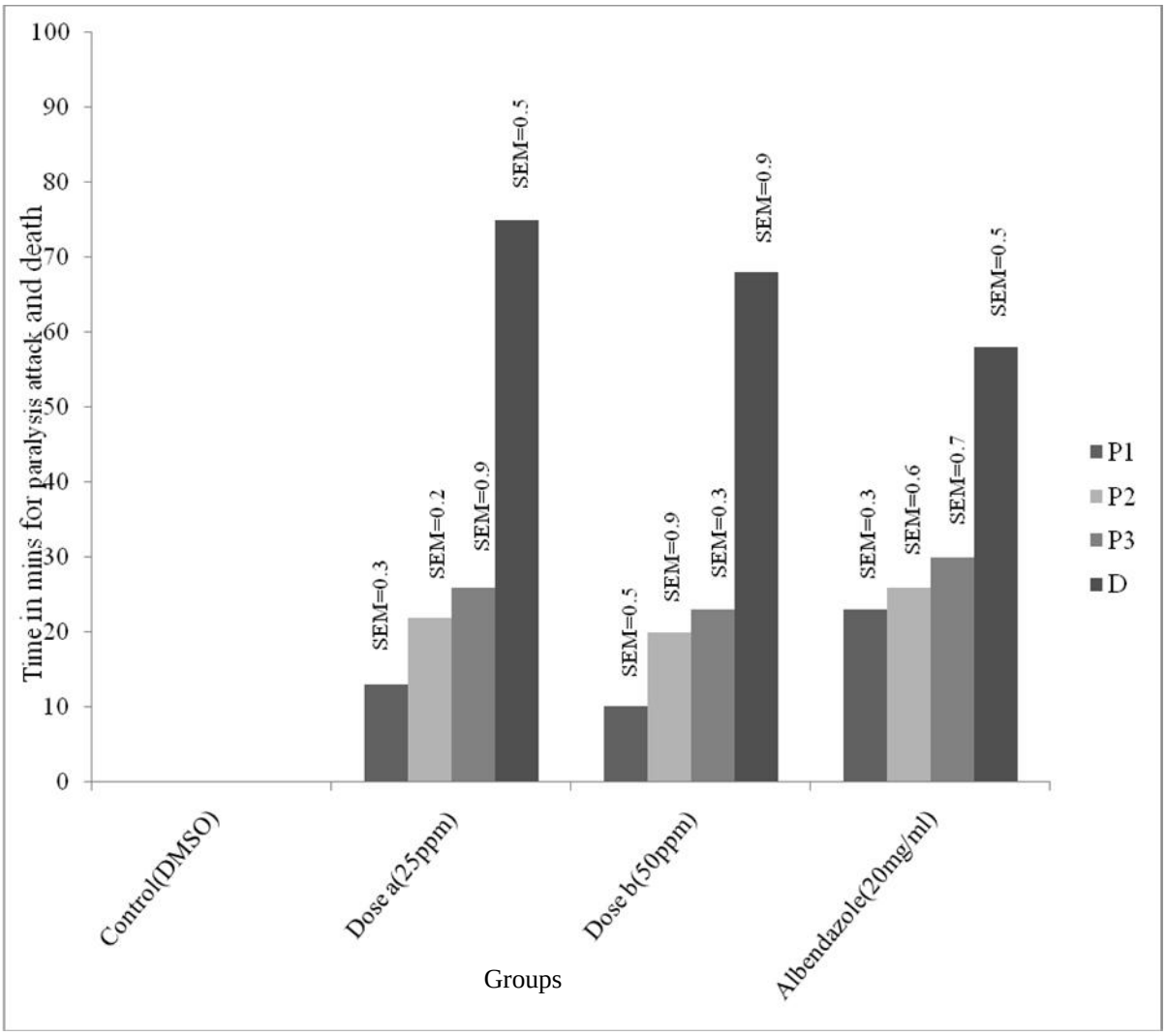

FIG 2: ANTHELMENTIC ACTIVITY OF ESSENTIAL OIL OBTAINED FROM *BLUMEA ERIANTHA*

CONCLUSION

Thus, from present study it can be concluded that the essential oils extracted from *Blumea eriantha* possess good potential antimicrobial, antifungal and anthelmentic activity. Further there is need to isolate and identify the actives of essential oils of *Blumea eriantha* followed by analytical confrmation of the moieties.

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