Research Article

Available online through
www.ijrap.net

IJRAP
ISSN 2229-3566

IN VITRO ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *ERIGERON FLORIBUNDUS*

Joshi R. K.[1]*, Mujawar M. H. K.[2], Badakar V. M.[1], Khatib N. A.[3]

[1]Department of Phytochemistry, Regional Medical Research Center (ICMR), Belgaum, Karnataka 590010, India
[2]Department of Genomics, National Institute of Biomedical Genomics (NIBMG), Netaji Subhas Sanatorium (T.B. Hospital), Kalyani, West Bengal 741251, India
[3]Department of Pharmacology, KLE College of Pharmacy, J. N. Medical College, K. L. E. University, Belgaum, Karnataka 590010, India

Received on: 03/01/2011 Revised on: 22/01/2011 Accepted on: 09/02/2011

ABSTRACT

The essential oil of *Erigeron floribundus* was screened against ten human pathogenic bacteria and fungi. The oil was found active against *Staphylococcus aureus, Escherichia coli, Candida albicans*, *Aspergillus niger*, *Saccharomyces cerevisiae* and *Penicillium chrysogenum* with minimum inhibitory concentration (MIC) of 0.41±0.18, 0.72±0.47, 0.36±0.23, 0.45±0.28, 0.57±0.59 and 0.88±0.63 mg/ml, respectively. The essential oil of *E. floribundus* was found more active against the tested fungal strains.

KEYWORDS: Essential oil, *Erigeron floribundus,* antimicrobial activity

***Corresponding author**

Dr. R. K. Joshi, Scientist & Head, Department of Phytochemistry, Regional Medical Research Center (ICMR), Belgaum, Karnataka-590010, India E-mail: joshirk_natprod@yahoo.com

INTRODUCTION

There is an increasing interest in the medicinal plants as a natural alternative to synthetic drugs[1]. The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases[2]. Over the years, essential oils and plant extracts have evoked interest as sources of natural products. Essential oils produced by plants is believed to be predominantly a defense mechanism against pathogens and pests[3]. Essential oils and their compositions are gaining increasing interest beacuse of their relatively safe status, wide acceptance by consumers and their exploitation for potential multi-purpose functional use[4].

Erigeron floribundus (Kunth) Sch. Beep. Syn. *Conyza sumatrensis* (Retz.) (Asteraceae) known as a reputed medicinal plant, traditionally used for the treatment of skin disorders by the rural populace as well as those from the urban areas in South Africa[5]. This species is widely distributed in temperate regions of India[6]. The extracts of *E. floribundus* have been reported antifungal[7], antipyretic and anti-inflammatory activities[8]. However, volatile constituents and antifungal activity of the essential oil of *E. floribundus* has been previously investigated[9]. The aim of present study was to evaluate the antimicrobial efficacy of the essential oil extracted from *E. floribundus* collected from Western Ghats region of North Western Karnataka, which has not previously investigated.

MATERIALS AND METHODS

Plant material

The aerial parts of *E. floribundus* were collected from the campus of Regional Medical Research Centre (RMRC), (ICMR), Belgaum, Karnataka at a height of 800 m. The plant was identified and authenticated in RMRC, Belgaum, where a voucher specimen (No. 421) is deposited.

Isolation of essential oil

The aerial parts (1 kg) of *E. floribundus* were steam distilled using copper still fitted with spiral glass condensers for 3 h and water distillate was extracted with n-hexane and dichloromethane. The organic phase was dried over anhydrous sodium sulphate and the solvent was distilled off using thin film rotary vacuum evaporator at temperature range 25°-30° C. The oil yield was 0.01% (v/w).

Microbial strains

The microbial strains namely *Bacillus subtilis* (NCIM 2063), *Micrococcus flavus* (NCIM 2379), *Staphylococcus aureus* (NCIM 2079) (Gram-positive

bacteria), *Escherichia coli* (NCIM 2574), *Klebsiella pneumoniae* (NCIM 2957), *Salmonella typhimurium* (NCIM 2501) (Gram-negative bacteria), *Aspergillus niger* (NCIM 620), *Candida albicans* (NCIM 3471), *Penicillium chrysogenum* (NCIM 733) and *Saccharomyces cerevisiae* (NCIM 3090) (Fungi) were obtained from the National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune.

Screening of antimicrobial activity

The antimicrobial activity was tested using disk diffusion method. The inocula of microbial strains were prepared from 18 hours old culture and suspensions were adjusted to 0.5 McFarland standard turbidity (~10^4 CFU (colony forming unit)/ml for bacteria and ~10^3 CFU/ml for fungi[10]. Petri plates were prepared by pouring 20 ml of nutrient agar (HI MEDIA, India) for bacterial strains and YPG agar (HI MEDIA, India) for fungal strains and allowed to solidify for half an hour. The inoculum (0.05 ml) was spread on the top of the solidified media and allowed to dry for 10 min. The essential oil was dissolved in 10% dimethyl sulfoxide (DMSO) which is reported to be non toxic to microorganisms at this percentage[11] with Tween 80 (0.1% v/v for easy diffusion) to a concentration range 5.0 to 0.009 mg/ml. Negative controls were prepared using discs impregnated with 10% aqueous DMSO with Tween 80 (0.1% v/v) as solvent control. Streptomycin (Nicholas Piramal India Limited, Gujarat, India) and fluconazole (Shwarde Pharmaceuticals (I) Pvt. Ltd., Goa, India) (20 µl of 1 mg/ml) were used as positive reference standards for bacterial and fungal strains, respectively. After deliver the essential oil and reference drugs, the plates were left for 30 min at room temperature to allow the diffusion of essential oil. The plates were than incubated for 24 h and 48 h at 37 ^{0}C for bacteria and fungi, respectively. The diameters of inhibition zones were measured in milliliters[12]. Assay was carried out in triplicate and mean values are reported (Tables 1).

Determination of the minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was determined by using the tube dilution method[13]. Bacteria and fungi were grown in peptone water. The inocula of microbial strains were prepared from 18 hours old culture and suspensions were adjusted to 0.5 McFarland standard turbidity (~10^4 for bacteria and ~10^3 for fungi colony forming unit (CFU) per milliliter[10]. After this, a different concentration of the essential oil of *E. floribundus* was prepared at a concentration range of 5.0-0.009 mg/mL. The reference antibiotics 1 mg/ml of streptozotocin for bacteria and fluconazole for fungi were used at a concentration range 1.02-0.001 mg/ml. Serial two fold dilution was prepared for different concentrations of the essential oil and reference antibiotics. The tubes were incubated for 24 and 48 hours at 37 ºC, for bacteria and fungi, respectively. The lowest concentration of test drugs that inhibits the visible microbial growth considered as MIC[14]. All determinations were performed in triplicate and mean ± SEM is reported (Table 1).

RESULTS AND DISCUSSION

The essential oil of *E. floribundus* showed qualitative (diameter of inhibition zone) and quantitative (minimum inhibitory concentration) *in vitro* antimicrobial property against tested pathogens summarized in Table 1. The qualitative antimicrobial activity evaluated by using disk diffusion method. The diameters of inhibitory zones (IZ) recorded in millimeter. It observed that the oil was effective against all tested microbial strains. The essential oil of *E. floribundus* was found active against the tested microorganisms, with average IZ of 18.7 mm. The maximum IZ of the oil showed against *C. albicans* followed by *A. niger, E. coli* and *S. cerevisiae* with IZ of 26.6, 20.3, 21.0 and 18.0 mm, respectively. All tested pathogens were effective at a concentration of 1.25 mg/ml except *P. chrysogenum*. Moreover, the oil was effective at a lower concentration of 0.62 mg/ml against *A. niger* and *C. albicans* (Table 1).

The quantitative *in vitro* antimicrobial activity of the essential oil of *E. floribundus* is reported as minimum inhibitory concentration (MIC) in mg/ml (Table 1). The oil was found more susceptible against the fungi *C. albicans* with MIC value of 0.36±0.23 however, the oil showed slightly less effective against *A. niger* and *S. cerevisiae* with MIC values of 0.45±0.28 and 0.57±0.59, respectively. The oil was less effective against the *P. chrysogenum* with MIC value of 0.88±0.63. The oil was found more susceptible against Gram-positive bacteria like *S. aureus* with MIC value of 0.41±0.18, while very less effective against *M. flavus* and *B. subtilis* with MIC value of 1.66±0.72 and 2.91±1.09, respectively. The oil was also moderately susceptible against *E. coli* with MIC value of 0.72±0.47, which is higher than *S. aureus*. Other microorganisms *K. pneumoniae* and *S. typhimurium* were found less effective with MIC value of 0.83±0.36 and 1.35±1.09, respectively. The essential oil of *E. floribundus* possessed lesser antimicrobial activity than that of standard reference antibiotics. The finding of antimicrobial activity of the essential oil of *E. floribundus* attributed the traditional use as antifungal infections but this plant can be use for external application as antibacterial contagion also, however, is less potent towards bacteria.

REFERENCES

1. Fabio AC, Cermelli C, Fabio G, Nicoletti P, Quaglio P. Screening of the antibacterial effects of a variety of essential oils on microorganisms responsible for respiratory infections. Phytother Res 2007; 21: 374-377.
2. Prabuseenivasan S, Jayakumar M, Ignacimuthu S. *In vitro* antimicrobial activity of some plant essential oil. BMC Compl Alter Med 2006; 6: 1-8.
3. Feng W, Zheng X. Essential oils to control *Alternaria alternata in vitro* and *in vivo*. Food Cont 2007; 18: 1126-1130.
4. Ashafa AOT, Grierson DS, Afolayan AJ. Composition and antibacterial activity of essential oil from *Felicia muricata* Thunb. leaves. J Biol Sci 2008; 8: 784-788.
5. Tra Bi FH. Utilisations des plantes, par lhomme, dans les forets classees du Haut-Sassandra et de Scio, en Cote dIvoire, These 3eme cycle, Universite dAbidjan-Cocody, Abidjan, 1997.
6. Hajra PK, Rao RR, Singh DK, Uniyal BP. Flora of India. Botanical survey of India, Calcutta, 1995, pp 109-110.
7. Tra Bi FH, Kone MW, Kouame NF. Antifungal activity of *Erigeron floribundus* (Asteraceae) from Cote dIvoire, West Africa. Trop J Pharm Res 2008; 7: 975-979.
8. Asongalen EA, Foyet HS, Ngogang J, Folefoc GN, Dimo T, Kantchouing P. Analgesic and antiinflammatory activities of *Erigeron floribundus*. J Ethnopharmacol 2004; 91: 301-308.
9. Kuiate JR, Tsona AA, Foko J, Bessiere JM, Menut C, Zollo PHA. Chemical composition and *in vitro* antifungal properties of essential oils from leaves and flowers of *Erigeron floribundus* (H.B. et K.) Sch. Bip. from Cameroon. J Essent Oil Res 2005; 17: 261-264.
10. McFarland J. Standardization of bacterial culture for the disc diffusion assay. J Am Med Assoc 1987; 49: 1176–1178.
11. Pujol V, Seux V, Villard J. Recherche de substances antifongiques secretes par les champignons superieurs en culture. Ann Pharm Fr 1990; 48: 17–22.
12. Joshi RK, Pande C, Mujawar MHK, Kholkute SD. Chemical composition and antimicrobial activity of the essential oil of *Anaphalis nubigena* var. *monocephala*. Nat Prod Commun 2009; 4: 993-996.
13. Joshi RK, Mujawar MHK, Kholkute SD. Antimicrobial activity of the extracts of *Craniotome furcata* (Lamiaceae). J Ethnopharmacol 2010; 128: 703–704.
14. Murthy MM, Subramanyam M, Giridhar KV, Jetty A. Antimicrobial activities of bharangin from *Premna herbaceae* Roxb. and bharangin monoacetate. J Ethnopharmacol 2006; 104: 290–292.

Table 1: Zone of inhibition (mm) and MIC (mg/ml) of the essential oil of *Erigeron floribundus*

Sl.no.	Microbial strains	Zone of inhibition (mm)						MIC	RA
		5 mg	2.5 mg	1.25 mg	0.62 mg	oil	RA		
Gram-positive bacteria									
1	*Bacillus subtilis*	10.3	8.3	7.0	-	15.6	20.6	2.91±1.09	0.003±0.001
2	*Micrococcus flavus*	10.6	9.3	9.0	-	19.3	21.0	1.66±0.72	0.002±0.001
3	*Staphylococcus aureus*	11.0	9.6	8.3	-	19.0	23.0	0.41±0.18	0.003±0.001
Gram-negative bacteria									
4	*Escherichia coli*	13	11	9.6	-	15.6	24.0	0.72±0.47	0.002±0.001
5	*Klebsiella pneumoniae*	13	11.6	9.3	-	21.0	21.6	0.83±0.36	0.003±0.001
6	*Salmonella typhimurium*	10	9.3	9.0	-	15.3	17.6	1.35±1.09	0.003±0.001
Fungi									
7	*Aspergillus niger*	12.6	12	11.3	11	26.6	15.0	0.45±0.28	0.007±0.001
8	*Candida albicans*	12	11	11	10	20.3	17.6	0.36±0.23	0.001±0.006
9	*Saccharomyces cerevisiae*	10.3	9.6	9.3	-	18.0	13.3	0.57±0.59	0.001±0.002
10	*Penicillium chrysogenum*	9.8	9.1	-	-	15.0	14.6	0.88±0.63	0.006±0.002

RA, reference antibiotics (streptomycin for bacteria, fluconazole for fungi).
Values are mean±standard deviation of six experiments in replicate.
-, no inhibitioz

Source of support: Nil, Conflict of interest: None Declared