ANTIMICROBIAL AND ANTHELMINTIC EFFECT OF *CYPERUS ESCULENTUS* LINN. AND *CYPERUS ROTUNDUS* LINN. ESSENTIAL OILS

S.S.Biradar*, V. P. Rasal, S.S.Biradar, Megha Thakur

Sandeep S. Biradar*

Vijaykumar P Rasal, Professor

Shridharkumar S. Biradar, Lecturer

Megha Thakur

Department of Pharmacology,

K.L.E.S' College of Pharmacy, Belgaum, Karnataka, India

Correspondence to: S.S.Biradar*

E-mail: sandeepbiradar84@gmail.com

Summary

The present paper was undertaken: 1: To screen activity of *Cyperus esculentus* Linn. and *Cyperus rotundus* Linn Essential oils on antimicrobial activity. 2: To screen activity of *Cyperus esculentus* Linn. and *Cyperus rotundus* Linn Essential oils on anthelmintic activity.The essential oil of Cyperus esculentus (C.e) and Cyperus rotoundus (C.r) has multiple pharmacological activities. In the present study the essential oil of Cyperus esculentus and Cyperus rotoundus was used in concentration of 250mg-0.5mg/ml for antimicrobial activity and 100-300 mg/ml for anthelmintic activity. The antimicrobial activity was performed by serial dilution method on gram positive, gram negative and fungi viz: *Staphylococcus aureus, Staphylococcus albus,Pseudomonas aeruginosa, E.coli, Candida albicans, Aspergillus.* Anthelmintic activity was performed using indian earthworm viz: *Pheretima posthuma, Ascardia galli* respectively.The essential oil of both the plants were active against all the microorganisms at higher doses (250mg/ml). Essential oil showed significant paralysis of earthworm and altered uptake of glucose at higher dose. In conclusion the essential oils (C.e and C.r) showed antimicrobial activity. The anthelmintic action of essential oils (C.e and C.r) on *A.galli* may be related to (a) reduction in glucose uptake resulting in energy crisis (b) Paralysis of worm as a result of inhibitory motor action.

Key words: Essential oil, antimicrobial, anthelmintic, *Cyperus esculentus*, *Cyperus rotundus*, Serial dilution.

Introduction

Essential oils are used extensively in aromatherapy and various traditional medicinal systems. Antimicrobial drugs are designed to inhibit/kill the infecting organism and to have no/minimal effect on the recipient. Anthelmintic are that drugs that either kill or expel infesting helminthes, since the essential oils are noncorrosive and safe to take internally, the oil has been chosen. The *Cyperus rotoundus* (C.r) showed multiple pharmacological activities like antidirrhoeal[1], hepatoprotective[2], antimutagenic and radical scavengers[3]. Traditionally these plants are also reported for its anthelmintic activity[4]. Hence the present study is to investigate the antimicrobial and anthelmintic activity of C.e and C.r essential oils.

Materials and Methods

Drugs, reagents and chemicals

1. **Serial dilution method**:- As prescribed by the bioassay methods, one ml of the original sample was added it to 9 ml of sterile water, it will give 1: 10 or 10^{-1} dilution of original sample, i.e. the original sample had been diluted to 1/10th. Similarly we prepared 1: 100 ($10^{-}2$), 1: 1,000 ($10^{-}3$), 1: 10,000 ($10^{-}4$) and so on, dilutions of the original sample. Finally one ml aliquot of any dilution was added to a sterile eppindroff tubes to which are added 9 ml of sterile, cool, molten agar medium with the specified bacteria and the sensitivity and the resistance was checked.[5]

2. **Motility study**:- Samples of the anthelmintic study was prepared by dissolving the essential oil in 25ml of 1% gum acacia solution prepared in normal saline to obtain a stock solution. Five groups of approximate equal size Indian earthworm consisting of six earthworms consisting of six earthworms in each group was used for one of the following: vehicle (1% gum acacia normal saline and different concentration of the essential oils. Observation was made for the time taken to paralyze and/or death of individual worms. Paralysis was said to occur when the worm do not revive even in normal saline. Death was concluded when the worms lost their motility, followed with fading away of their body colour.[6]

3. **Glucose uptake study**:- *Ascardia galli* worm were incubated in different concentration of the essential oils *Cyperus esculentus* Linn. and *Cyperus rotundus* Linn and drug free tyrode medium (control) for 20 hours. The glucose estimation was done by using glucose oxidase (GOD)/peroxidase (POD) methods.[7]

Results:

1. Antimicrobial study (By the serial dilution method):- Sensitive to *Staphylococcus albus, Candida albicans, Aspergillus* were sensitive to both the essentialoils and resistant to *E.coli* at all concentration. *Pseudomonas aeruginosa* showed sensitivity at 16.6 mg/ml and 31.250 mg/ml for Of both the essential oils *Cyperus esculentus* Linn. and *Cyperus rotundus* Linn. respectively. *Staphylococcus aureus* showed sentivity at 125mg/ml and 31.250mg/ml for *Cyperus esculentus* Linn. and *Cyperus rotundus* Linn. Respectively. (Table no.01)

Antimicrobial study (By the serial dilution method):- Table no.1

Concentration	**250mg/ml**	**125 mg/ml**	**62.5 mg/ml**	**31.250 mg/ml**	**16.6 mg/ml**	**8.3 mg/ml**	**4.015 mg/ml**	**2 mg/ml**	**1 mg/ml**	**0.5 mg/ml**
E.coli										
C.e	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
C.r	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
Pseudomonas aeruginosa										
C.e	**S**	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**R**	**R**
C.r	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**R**	**R**	**R**
Staphylococcus aureus										
C.e	**S**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
C.r	**S**	**S**	**S**	**S**	**R**	**R**	**R**	**R**	**R**	**R**
Staphylococcus albus										
C.e	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
C.r	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
Candida albicans										
C.e	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
C.r	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
Aspergillus										
C.e	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
C.r	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**

C.r: *Cyperus rotundus*, C.e: *Cyperus esculentus*

2. Motility study: The mass motility with both the essential oil was significantly decreased at higher concentration (300mg/ml), but at higher concentration were as there was no significant decrease in the motility with (100 mg/ml) concentration. (Table no.02)

Motility study: Table no.2

Group	Conc. (mg/ml)	Pyrethema Postuma	
		PARALYSIS	DEATH
I control		-----	--
I C.r	100	25.83 ± 0 .60**	34.17 ± 0.70**
II C.r	200	22.5 ± 0 .67**	27.83 ± 0.70**
II C.r	300	17.67 ± 0 .76 **	23.5 ± 0.76**
VII C.e	100	27.11 ± 0.90**	33.50 ± 0.84**
VI C.e	200	25.0 ± 0.73 **	34.50 ± 0.34**
VII C.e	300	21.33 ± 0.49**	21.67 ± 0.49 **
Piperazine citrate	15	20.33± 0.45**	24.33 ± 0.47**

Mean ± SEM, when compared with control **P< 0.01.
C.r: Cyperus rotundus, C.e: Cyperus esculentus

3. Glucose uptake studies: Incubation of *Ascardia galli* with *Cyperus esculentus* Linn. and *Cyperus rotundus* Linn. essential oil in tyrode solution resulted in significant inhibition of glucose uptake.(Table no.03)

Glucose uptake studies: Table no.3

Extract	Glucose Uptake	**Glycogen content in glucose containing medium (mg %)**	**Glycogen content in glucose free medium (mg %)**
Control	3.94 ± 0.10	30.31 ± 0.15	0.91 ± 0.018
C.r 50	3.44 ± 0.15^{ns}	2.85 ± 0.10*	1.12 ± 0.011*
C.r 100	2.94 ± 0. 05**	1.13 ± 0.12**	1.82 ± 0.083**
C.e 50	3.34± 0.12*	2.83± 0.11*	1.33 ± 0.015*
C.e 100	2.80 ± 0.05**	1.014 ± 0.06**	1.80 ± 0.087 **
Mebendazole	1.50± 0.15**	1.31 ± 0.006**	1.20 ± 0.010**

Mean ± SEM .when compared with control,*P< 0.05, **P<0.01, P>0.05^{NS}
C.r: *Cyperus rotundus*, C.e: *Cyperus esculentus*

Discussion

The essential oils showed variable activities against tested bacteria (gram +ve and gram -ve) and fungi. It has been already shown that the antimicrobial activity of volatile compounds results from a combined effect of direct vapour on microorganism and indirect effect through the medium that absorbed the vapour[8]. The results showed that tested oils are resistance to gram –ve bacteria (*E.coli*) at all concentrations. *Pseudomonas aeruginosa* (Gram-ve) bacteria is susceptible to C.e from conc. 16.6 -250 mg/ml but C.r from conc. Of 125-250 mg/ml. *S.aureus* showed susceptibility to C.e from conc. 125-250 mg/ml but C.r from conc. 31.25-250 mg/ml. *S.albus* (Gram +ve), Candida albicans, Aspergillus (Fungi) showed susceptibility at conc. of both the oils .

The outer layer of the earthworm is a mucilaginous layer and composed of complex polysaccharides. This layer being slimy, enables the earthworm to move freely. Any damage to the mucopolysaccharide membrane will expose the outer layer and this restricts its movement and can cause paralysis. This action may lead to the death of worm by causing damage to the mucopolysaccharide layer. This causes irritation leading to paralysis[9]. The essential oils (C.e and C.r) at higher conc. (300mg/ml) significantly decrease the motility of earthworm and hence lead to paralysis and death.

On the basis of molar concentration, MBZ is relatively more potent than essential oils (C.e and C.r) in inhibiting the glucose uptake in *A.galli.* The results suggest that essential oil (C.e and C.r) induces energy crisis consequent to the blockade of glucose uptake. However, parasites can still mobilize their glycogen source for the production of ATP21. In view of this possibility, the effect of essential oils (C.e and C.r) has been studied on tissue glycogen level of *A.galli.* In the presence of glucose in the incubation medium essential oils (C.e and C.r) 100µg/ml and MBZ 100 µg/ml causes a significant depletion of tissue glycogen. Thus, the mechanism of action of essential oils (C.e and C.r) appears to be similar to that of MBZ. Because of the presence free of glucose in the medium, it is difficult to assess the effect of the drug directly on the glucose metabolism as glycogen depletion might result consequent to the inhibition of glucose uptake and maintain the normal physiological functions of the Parasite. In order to rule out such a possibility, the effect of MBZ and essential oils (C.e and C.r) on the tissue glycogen level were studied in glucose-free medium. Interestingly, MBZ and essential oils (C.e and C.r) failed to alter the glycogen level of *A.galli* in the absence of glucose suggesting that MBZ and essential oils-induced glycogen depletion was due to marked inhibition of glucose uptake[7].

Conclusion

In conclusion the essential oils (C.e and C.r) showed antimicrobial activity. The anthelmintic action of essential oils (C.e and C.r) on *A.galli* and *Pheretima posthuma* may be related to (a) reduction in glucose uptake resulting in energy crisis (b) Paralysis of worm as a result of inhibitory motor action, respectively.

References

1) Uddin SJ et al. antidiarrhoeal activity of cyperus rotundus. Fitoterapia2006;77(2):134-36.
2) SVS Kumar, HS Mishra. Hepatoprotective activity of rhizomes of cyperus rotundus linn against carban tetrachloride induced hepatotoxicity. Ind J pharm. Sci.2005;67(1):84-88.
3) Kilani S et.al. investigation of extracts from(Tunisian) cyperus rotundus as anti mutagens and radical scavengers. Environ. Toxicol. pharmacol.2005;20:478-84.
4) Indian medicinal plants a compendium of 500 species, vol.2: 1997;293-296.
5) Indian pharmacopoeia 2007:2.2.10.
6) Mounnissamy VM. Et.al. anthelmintic activity of cansjera rheedii J. gmelin(opiliaceae).J Bio Sci.2008;8(4):831-33.
7) D. Kumar, s.k. Mishra, s.k. Tandan, h.c. Tripathi. Possible mechanism of anthelmintic action of palasonin on *Ascaridia galli.* Ind J pharmacol 1995; 27: 161-66.
8) Matasyoh LG,et al. Chemical composition and antimicrobial activity of the essential oil of ocimum gratissimum L. growing in Eastern Kenya, African J biotech 2007;6 (6): 760-65.
9) Chandrashekar CH, Latha KP, Vagdevi HM, Vadia VP. Anthelmintic activity of the crude extraxts of Ficus racemosa. Intern J green Pharm 2008; 100-3.