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Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

COMPARATIVE ANTHELMINTIC ACTIVITY OF *CITRUS LIMON* L OSBECK AND *CITRUS LIMON* L BURMF FROM NORTH EASTERN INDIA

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ABSTRACT

Citrus limon L Osbeck and *citrus limon* L Burmf belong to family Rutaceae. Citrus food and derive product have beneficial impacts on human health. More than just a vitamin C rich fruits, lemon is a powerful medicinal plant with numerous benefits that have been enjoyed for over 3000 years. Helminthiasis or worm infection is a common infection in human, affecting a large population of the world. Considering this, comparative study of anthelmintic activity of *Citrus limon* L Osbeck and *Citrus limon* L Burmf leaves and peel was carried out in this experiment. Ethyl acetate, ethanol and methanol extracts of leaves and peel of both *Citrus limon* have been evaluated for anthelmintic activity against Indian Earthworm species *Eicinia foetida*. The results reveal a dose dependent increase in activity of the extracts at 5, 10 and 20 mg/ml concentrations. All the extracts exhibited better activity than standard compound Albendazole. C*itrus limon* L Osbeck leaves and peel extracts are found to be more active than respective *citrus limon* L Burmf extracts.

Keywords: *Citrus limon*, Anthelmintic activity, *Eicinia foetida*, Albendazole.

1. INTRODUCTION

The infection due to worm is a great threat to human health. It contributes to malnutrition, anemia, pneumonia and other health issues [1, 2]. Despite this prevalence of parasitic infections, the research on anthelmintic drug is limited [3]. Anthelmintics are drugs that may act locally to expel worms from the gastrointestinal tract (GIT) or systematically to eradicate adult helminths or development forms that invade organs and tissues [4]. Most of the existing anthelmintic produces nausea, vomiting, headache and diarrhoea. Chemotherapy is the only treatment and effective tool to cure and control helminths. Effective vaccines against helminths have not been developed so far [4]. Indiscriminate use of synthetic anthelmintic can lead to resistance of parasites [5]. Herbal drugs have been in use since ancient times for the treatment of parasitic diseases in human and could be of value in preventing the development of resistance [6].

Citrus limon, is a species of small evergreen tree in the flowering plant family Rutaceae. *Citrus limon* L Burmf also known as Assam Lemon is one of the most important varieties of lemon, found in north eastern region of India and valued for fragrance, acidic content and mainly used in green matured stage for culinary purposes. *Citrus limon* L Osbeck which is round in shape, another important variety of lemon largely available in Assam and other north eastern states. It is an excellent source of vitamin C and also contains some iron, fiber, copper, and calcium. Citrus fruits do not undergo rapid chemical or physical changes, after harvest lemons are the only citrus fruits held in prolonged storage [7]. Fruit of *Citrus limon* is widely used for culinary, beverages, industrial and medicinal purposes. The leaves of the lemon tree are used to make a tea and for preparing cooked meats and sea foods. The peel can be used in the manufacture of pectin, a polysaccharide used as a gelling agent and stabilizer in food and other products [8].The fruits are sour, rich in vitamin C which strengthens the immune system, acts as an antioxidant and protects cells from radical damage [9]. Infusion of the bark or peel of the fruits is given to relive colic. It is used as antiseptic, antibiotic and antiviral. Anthelmintic activity of *Citrus limon* fruit empty juice sacs was investigated [10]. Comparative study of antioxidants changes including total flavonoid, total phenol and total antioxidant capacity of *Citrus limon* fruits grown at the north of Iran was examined [11]. Antimicrobial activities of juice in ripen and unripe forms of *Citrus limon* were studied [12]. Phenol and flavinoid contents of *Citrus limon* L Burmf

leaves and peel was published in author's previous publications [13, 14]. Traditionally, *Citrus limon* claims as anthelmintic but scientifically scant of work is available [15]. Anthemintic activity of *citrus limon* L Burmf leaves was carried out and reported in author's previous publication [16]. Anthemintic activity of *Citrus limon* L Burmf peel extracts was reported by S. Upadhyaya et al. [17]. As per the available reports a comparative study of anthelmintic activity of *Citrus limon* L Burmf and *Citrus limon* L Osbeck is not available. Keeping this point in mind a comparative anthelmintic activity study of *Citrus limon* L Burmf and *Citrus limon* L Osbeck leaves and peel extracts was carried out in this experiment. As per the data available, this is the first comparative study of anthelmintic activity of ethyl acetate, ethanol and methanol extracts of *citrus limon* L Burmf and *Citrus limon* L Osbeck leaves and peel extracts collected from Biswanath district, Assam, India.

2. MATERIAL AND METHODS

The plant material was collected from Naduar area, Biswanath district, Assam, India. The plant was authenticated at Department of Life Sciences, Dibrugarh University, Assam, India.

2.1. Preparation of plant extract

Air shade dried and powdered leaves and peel material of *Citrus limon* L Buemf and *Citrus limon* L Osbeck (50 mg) were treated separately with ethyl acetate, ethanol and methanol (250ml) by refluxing for 18 hours. Solvents were recovered under reduced pressure to obtain the respective crude extracts of leaves and peel of both the plants.

2.2. Anthelmintic activity

2.2.1. Chemicals

Albendazole, normal saline were purchased from authorized pharmaceuticals. The solvents and other chemicals used during experimental protocol were of analytical grade.

2.2.2. Animal

Indian earthworm species *Eicinia foetida* was collected from Mahatma Phule Agriculture University, Pune. Maharashtra, India. All earthworms were of approxi-mately equal size (9-10). The anthelmintic assay was carried out as per the method reported by Ajaycoda *et al* with minor modification [18]. The assay was performed on adult Indian Earth species *Eicinia foetida* due to its anatomical and physiological resemblance with the intestinal round worm parasite of human. Albendazole in normal saline solution was used as standard compound for the assay and normal saline was served as control. The time taken for complete paralysis and death was recorded. External stimuli were applied as ascertain the paralysis time. The time taken by worm to become motionless was considered as paralysis time and lethal time was ascertained by death of motionless worm followed by fading of their body colour.

2.2.3. Administration of extract

The suspension of different solvent extract of leaves and peel of *Citrus limon* L Burmf and *Citrus limon* L Osbeck in different concentrations (5-20mg/ml) were prepared using saline and final volume was made to 10ml. Five groups of approximately equal size worms consisting of five earthworms individually in each group were released into 10ml of desired concentration of drug and extracts.

2.2.4. Administration of Albendazole

Albendazole (5, 10, 20mg/ml) was prepared by using 50mg, 100mg, and 200mg powder in 10ml saline as a suspending agent.

2.3. Anthelmintic potency of *citrus limon* L Osbeck and *Citrus linom* L Burmf in Indian earthworm (*Eicinia foetida*)

For evaluation of anthelmintic activity of *Citrus limon* (L Burmf and L Osbeck) leaves and peel, group I, II and III received standard Albendazole in normal saline while group IV (BL), V(BL), VI(BL) are different concentration of ethanol extract, VII(BL),VIII(BL), IX(BL), different concentration of methanol extract and X(BL), XI(BL), XII(BL), different concentration of ethyl acetate extract of *citrus limon* L Burmf leaves respectively. Similarly group IV(BP), V(BP), VI(BP) are different concentration of ethanol extract, VII(BP),VIII(BP), IX(BP), different concentration of methanol extract and X(BP), XI(BP), XII(BP), are different concentration of ethyl acetate extract of *citrus limon* L Burmf peel respectively.

IV (OL), V(OL), VI(OL) are the different concentration of ethanol extract, VII(OL),VIII(OL), IX(OL), different concentration of methanol extract and X(OL), XI(OL), XII(OL) different concentration of ethyl acetate extract of *citrus limon* L Osbeck leaves, respectively. Similarly group IV (OP), V(OP), VI(OP), different concentration of ethanol extract, VII(OP),VIII(OP), IX(OP), different concen-tration of methanol extract and X(OP), XI(OP), XII(OP), different concentration of ethyl acetate extract of *citrus limon* L Osbeck peel respectively.

2.4. Statistical Analysis

All the results were expressed as mean±SD of five animals in each group.

3. RESULTS AND DISCUSSION

The results of anthelmintic activity of *Citrus limon* L Osbeck and *Citrus limon* L Burmf leaves and peel extracts are presented in table 1. All values represent Mean±S.D; n=5 in each group. All values are significantly different from reference standard (Albendazole). This activity was concentration dependent. The potency was found to be inversely proportional to the time taken for paralysis and time of death of the worms.

Table 1: Anthelmintic activity of *Citrus limon* L Osbeck and *Citrus limon* L Burmf.

Treatmet	Group	Concentration (mg/ml)	Time of paralysis (min)	Time of death (min)
Albendazole	I	5	247 ± 4.06	178 ±1.78
	II	10	202 ± 2.34	168 ±1.58
	III	20	200 ± 1.64	155 ±1.81
***Citrus limon* L Burmf leaves**				
Ethanol	IV (BL)	5	180 ± 3.53	155 ±3.53
	V ((BL)	10	160 ± 3.80	132 ± 2.07
	VI(BL)	20	98 ± 3.89	101 ± 2.54
Methanol	VII(BL)	5	200 ±1.81	124 ±1.67
	VIII(BL)	10	178 ±1.30	100 ± 1.51
	IX(BL)	20	160 ±1.51	79 ± 1.64
Ethyl acetate	X(BL)	5	194 ±1.14	165 ± 1.30
	XI(BL)	10	181 ± 2.16	164 ± 1.94
	XII(BL)	20	174 ±1.92	145 ± 1.92
***Citrus limon* L Burmf peel**				
Ethanol	IV (BP)	5	188 ± 1	127 ± 0.83
	V (BP)	10	164 ± 0.70	87 ± 1.22
	VI(BP)	20	142 ± 0.81	64 ± 1.30
Methanol	VII (BP)	5	203 ± 1.51	132 ± 1.34
	VIII (BP)	10	186 ± 1.30	129 ± 0.70
	IX (BP)	20	158 ± 1.64	81 ± 1.30
Ethyl acetate	X (BP)	5	202 ± 1.34	166 ± 1.51
	XI (BP)	10	182 ± 1.09	137 ± 0.83
	XII (BP)	20	178 ± 0.83	127 ± 1.30
***Citrus limon* L Osbeck leaves**				
Ethanol	IV (OL)	5	106 ± 2.77	147 ± 3.31
	V (OL)	10	84 ± 3.16	125 ± 3.80
	VI (OL)	20	70 ± 1.58	105 ± 3.16
Methanol	VII (OL)	5	97 ± 3.67	120 ± 2.54
	VIII (OL)	10	80 ± 1.58	105 ± 1.87
	IX (OL)	20	60 ± 2.73	93 ± 2.30
Ethyl acetate	X (OL)	5	85 ± 2.30	122 ± 1.92
	XI (OL)	10	77 ± 2.28	115 ± 1.48
	XII (OL)	20	58 ± 2.54	95 ± 3.80
***Citrus limon* L Osbeck peel**				
Ethanol	IV (OP)	5	95 ± 2.23	100 ± 3.16
	V (OP)	10	74 ± 3.24	80 ± 2.54
	VI (OP)	20	61 ± 4.18	55 ± 4.30
Methanol	VII (OP)	5	105 ± 2.16	120 ± 2.23
	VIII (OP)	10	80 ± 1.58	100 ± 2.23
	IX (OP)	20	65 ± 1.48	70 ± 2.54
Ethyl acetate	X (OP)	5	72 ± 1.51	95 ± 3.80
	XI (OP)	10	63 ± 1.58	73 ± 3.20
	XII (OP)	20	55 ± 1.87	50 ± 2.54

From the above result, it is clear that all extracts of both plants exhibited better result than standard drug Albendazole. Ethanol extracts of *citrus limon* L Burmf leaves and peel have significant anthelmintic activity in dose dependent manner when compared with standard anthelmintic drug Albendazole as well as other extracts. In case of *Citrus limon* L Osbeck leaves, ethyl acetate extract showed more significant activity than methanol and ethanol extracts. Similarly ethyl acetate extract of *Citrus limon* L Osbeck peel has more significant activity than ethanol and methanol extracts.

From the experimental data, it is observed that ethyl acetate and methanol extract of leaves of *Citrus limon* L Osbeck are more active than respective ethyl acetate and methanol extract of *citrus limon* L Burmf leaves. Same trend is observed for ethanol extract for both the plants.

Considering the activity of *citrus limon* peel, all three extracts, ethyl acetate, ethanol and methanol of *citrus limon* L Osbeck showed more potent activity than respective extracts of *Citrus limon* L Burmf. It is clear that *Citrus limon* L Osbeck leaves and peel extracts are more active anthelmintic agent than respective *Citrus limon* L Burmf extracts.

The biochemical mechanism of anthelmintic action of the plant extracts may be due to interfering with metabolic processes, interfering with neuromuscular physiology of parasites. They may inhibit the glucose uptake and depleted the glycogen content in the presence of glucose and thereby affect the energy generating mechanism of the parasite. In general the possible mechanism of anthelmintic action of the plant extract may be related to either inhibition of energy metabolism or alternation in the motor activity of the parasites.

4. CONCLUSION

Citrus limon L Osbeck *and Citrus limon* L Burmf leaves and peel extracts are found to do superior anthelmintic agent then the standard compound albendazole. Active constituents responsible for anthelmintic activity are present in extracts of leaves and peel of both the plants. So, it can be concluded that *Citrus limon* L Burmf *and Citrus limon* L Osbeck leaves and peel extracts have great potential as anthelmintic agent. Further study is required to explore these extracts as drugs and to establish the mechanism of action.

5. ACKNOWLEDGEMENTS

The authors are thankful to the principal, Y. M. College, Bharti Vidyapeeth Deemed University, Pune, India for providing the necessary support to carry out this work.

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