International Standard Serial Number (ISSN): 2319-8141
International Journal of Universal Pharmacy and Bio Sciences 5(1): January-February 2016

INTERNATIONAL JOURNAL OF UNIVERSAL PHARMACY AND BIO SCIENCES

*IMPACT FACTOR 2.093****
*ICV 5.13****

Pharmaceutical Sciences **RESEARCH ARTICLE ……!!!**

"ANTHELMINTIC ACTIVITY OF BARK AND LEAF EXTRACTS OF *KUNSTLERIA KERALENSIS*"

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KEYWORDS:

Carrageenan, Analgesic, Anti-inflammatory, *Kunstleria keralensis*, Tail flick.

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ABSTRACT

The hexane, chloroform and methanolic extracts of bark and leaf of the plant *"Kunstleria keralensis"* belongs to the family Fabaceae were screened for anthelmintic activity on Indian adult earth worms by *in vitro* method. The test sample of hexane extract of bark (HB), chloroform extract of bark (CB) and the hexane extract of leaf (HL) showed significant anthelmintic activity. The chloroform extract of leaf (CL) showed moderately significant activity. The methanol extract of bark (MB) and methanol extract of leaf (ML) showed insignificant anthelmintic activity.

INTRODUCTION:

The World Health Organization (WHO) defined health as "a complete state of physical, mental, and social well-being and not merely the absence of disease or infirmity". So during the past decade, traditional systems of medicine have become a topic of global importance[1]. India is a country with rich natural resources with variety of medicinal plants. In contrast to synthetic drugs, herbal drugs enjoy the advantages of comparatively less toxic than synthetic drugs, more harmony with the biological system and affordable to all classes of people. In the last few decades, herbs and plants have been in use as a source of therapeutic compounds in traditional medicinal system. Medicinal plants play an important role in traditional healthcare systems as well as in international herbal and pharmaceutical markets[2]. The history of herbal medicine is almost as old as human civilization and these served through ages as a constant source of medicaments for the treatment of a variety of diseases. Many medicinal plants are known to provide a rich source of antibacterial, insecticidal and anthelmintic compounds[3]. Helminthiasis or worm infestation is one of the most prevalent disease and one of the most serious public health problems in the world. Millions of humans and animals with infections by helminths exist worldwide, especially in the developing countries[4]. The helmintic control in domestic animals and human beings is widely based on the use of anthelmintic drugs. However, the efficacy of anthelmintic drugs used today has been reduced because of development of resistant nematode strains. Furthermore, the high cost of these drugs, residual concern in food, animals and environmental pollution have awaken interest in medicinal plants as an alternative source of anthelmintic drugs[5].

Kunstleria keralensis is a flowering plant belongs to the family Fabaceae, found in evergreen and semi-evergreen forest in the Southern Western Ghats of India. It is mainly distributed in the districts of Kerala such as Thiruvananthapuram, Kannur, Pallakad, Mallapuram, Thissur, Kasaragod and certain parts of Karnataka[6]. It is reported that the bark of the plant *Kunstleria keralensis* is used as a medicine by the tribal people of kerala to heal the body pain and also reported to have antifertility activity[7,8,9]. In view of its various medicinal properties, in the present study the solvent extracts of bark and leaf materials of *Kunstleria keralensis* were screened for anthelmintic activity.

2. MATERIALS AND METHODS

2.1 Collection and authentication of plant

The bark and leaves of *Kunstleria keralensis* were collected in the month of January 2012 in Agumbe forest region. The materials were shade dried, powdered and stored in air tight containers. The plant was identified and authenticated by botanist Dr. K.G. Bhat, Professor in botany, Poornapragna first grade college, Udupi, Karnataka. The herbarium of the identified plant was prepared and submitted to

the Department of Pharmacognosy, National College of Pharmacy, Shivamogga, Karnataka, India. The specimen number of the herbarium is NCP-14-2012-13 dated 22-11-12.

2.2 Preparation of plant extracts and evaluation of phytochemical tests

The bark and leaf extracts were made using the solvents hexane, chloroform and methanol by hot soxhlet and cold maceration methods[10,11,12,13]. The test samples were prepared and labeled the hexane extract as HB, chloroform extract as CB and methanol extract as MB. Similarly, the test samples of leaf extracts were also prepared and labeled the hexane extract as HL, chloroform extract as CL and methanol extract as ML respectively. The test samples of bark and leaf were analysed for various phytochemical constituents. The presence of various phytochemical constituents in these test samples has been reported earlier[14].

2.3 Worms

Indian adult earthworms (*Pheretima posthuma*) was collected from moist soil of the Bhadra Dam, B.R Project and identified by the zoologist, Department of Zoology, Kuvempu University, Shankarghatta. The earthworms were washed with normal saline to remove all the dirt matter and used for the anthelmintic study. The earthworms of 5-7cm in length and 0.2-0.3 cm in width were selected for the experiment. The earthworms were selected for the study due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.

2.4 Anthelminthic activity of bark and leaf extracts of *Kunstleria keralensis* by *in vitro* method

The test samples prepared from bark extract (HB, CB and MB) and leaf extract (HL, CL and ML) were tested for their anthelmintic activity by *in vitro* method. The adult Indian earthworms *Pheretima posthuma* were used to evaluate anthelmintic activity. The worms were divided into 15 groups of 6 worms each. The worms of group I were released into a plate containing 50ml of sterile distilled water which served as control. The worms of group II and III were released into a plate containing a solution of 50ml of Piperazine citrate syrup at a dose of 5 and 10mg/ml respectively which served as standard. The worms of group IV, VI and VIII were released into separate plates containing solution of 50ml of the test samples of bark HB, CB and MB at a dose of 5mg/ml. The worms of group V, VII and IX were released into separate plates containing solution of 50ml of the test samples of bark HB, CB and MB at a dose of 10mg/ml. Similarly, the worms of group X, XII and XIV were released into the separate plates containing solution of 50ml of the test samples of leaf HL, CL and ML at a dose of 5mg/ml. The worms of group XI, XIII and XV were released into the separate plates containing solution of 50ml of the test samples of leaf HL, CL and ML at a dose of 10mg/ml. All the test samples were prepared in sterile water. Observations were made for the time taken for paralysis and death of individual worms. Time for paralysis was noted when no movement of any sort even after transfer to

normal saline. Death was concluded when the worms lost their motility completely and failed to respond even after a touch with the needle followed by fading of their body colors[15, 16, 17].

2.5 Statistical analysis

The statistical analysis was carried out by one way analysis of variance (ANOVA). All the data were presented as Mean ± SEM.

3. RESULTS

3.1 Anthelmintic activity by *in vitro* method

Bark extracts

The worms released into the petriplates containing HB and CB test samples showed time for paralysis and death at 134±0.25, 175±0.26 and113±0.38, 145±0.48 minutes at a dose of 5mg/ml. The above same test samples showed paralysis and death time at 96±0.19, 120±0.14 and 78±0.19, 109±0.22 minutes at a dose of 10mg/ml respectively. The time for paralysis and death exhibited by test samples HB and CB were found to be have significant anthelmintic activity in comparison with standard drug Piperazine citrate which showed the time for paralysis and death of worms at 75±0.26, 120±0.38 and 56±0.19, 95±0.22 minutes at 5mg and 10mg/ml respectively. The worms released into the petriplates containing MB test sample took more time of 236±0.38 and 320±0.47 minutes for paralysis and death at a dose of 5mg/ml and 194±0.18 and 214±0.20 minutes at a dose of 10mg/ml respectively and their activity were found insignificant. The results obtained for the test samples of bark are shown in table 1.

Table 1. Anthelmintic activity of test samples of bark of *Kunstleria keralensis* by *in vitro* method

Drugs	Groups	Concentration (mg/ml)	Time taken for Paralysis of worms (in minutes)	Time taken for Death of worms (in minutes)
(Control) Water	**Group-I**	**----**	**----**	**----**
(Standard) Piperazine citrate	**Group-II** **Group-III**	5 10	75±0.26 56±0.19	120±0.38 95±0.22
HB	**Group-IV** **Group-V**	5 10	134±0.25 96±0.19	175±0.26 120±0.14
CB	**Group-VI** **Group-VII**	5 10	113±0.38 78±0.19	145±0.48 109±0.22
MB	**Group-VIII** **Group-IX**	5 10	236±0.38 194±0.18	320±0.47 214±0.20

Values are mean ± SEM, n = 6.

Note: HB (Hexane extract of bark), CB (Chloroform extract of bark), MB (Methanol extract of bark).

Leaf extracts

The worms released into the petriplates containing HL test sample showed time for paralysis and death at 123±0.18 and 168±0.26 minutes at a dose of 5mg/ml and 84±0.12 and 130±0.14 minutes at a dose of 10mg/ml respectively. It was found to have significant anthelmintic activity in comparison with standard drug Piperazine citrate which showed the time for paralysis and death of worms at 75±0.26, 120±0.38 and 56±0.19, 95±0.22 minutes at 5mg and 10mg/ml respectively. The worms released into the petriplates containing CL test sample showed moderately significant time for paralysis and death of worms at 185±0.38, 210±0.47 and 138±0.19, 198±0.21 minutes at 5mg and 10mg/ml respectively. The worms released into the petriplates containing ML test sample showed insignificant anthelmintic activity. The results obtained for the test samples of leaf are shown in table 2.

Table 2. Anthelmintic activity of test samples of leaf of *Kunstleria keralensis* by *invitro* method

Drugs	Groups	Concentration (mg/ml)	Time taken for Paralysis of worn (in minutes)	Time taken for Death of worms (in minutes)
Group-I (Control) Water	**Group-I**	----	----	----
Group-II (Standard) Piperazine citrate	**Group-II** **Group-III**	5 10	75±0.26 56±0.19	120±0.38 95±0.22
HL	**Group-X** **Group-XI**	5 10	123±0.18 84±0.12	168±0.26 130±0.14
CL	**Group-XII** **Group-XIII**	5 10	185±0.38 138±0.19	210±0.47 198±0.21
ML	**Group-XIV** **Group-XV**	5 10	236±0.42 200±0.24	292±0.46 243±0.35

Values are mean ± SEM, n = 6.

Note: HL (Hexane extract of leaf), CL (Chloroform extract of leaf), ML (Methanol extract of leaf).

4. DISCUSSION

Many plants species such as *Thespesia lampas, Asystasia gangeticum, Cassia auriculata, Cucurbita mexicana, Sterculia villosa, Albizia anthelmintica* showed anthelmintic activity[18, 19, 20, 21, 22, 23]. In the present study the test samples of bark and leaf extracts of plant *Kunsleria keralensis* belongs to the family Fabaceae were tested for anthelmintic activity. Several reports are available on many plant species belonging to the presently studied family Fabaceae with anthelmintic activity. Many plants of Fabaceae such as *Butea monosperma, Acacia nilotica, Tephrosia purpurea, Sesbania grandiflora, Dalbergiella welwitschii* have been reported with significant anthelmintic activity[24, 25, 26, 27, 28].

However, anthelmintic activity has not been reported for the metabolites of the plant *Kunsleria keralensis*. Hence, in the present study the extracts of *Kunsleria keralensis* has been evaluated for this activity.

Though both the bark and leaf extracts showed anthelmintic activity, the bark extracts exhibited comparatively more anthelmintic activity than leaf extracts. The anthelmintic activity of extracts of non-polar solvents such as HB, CB and HL of bark and leaf of *Kunstleria keralensis* showed significant activity. This may be due the presence of alkaloids, steroids and tritepenoids[29, 30]. The earlier studies revealed that the active principles in medicinal plants responsible for the anthelmintic activity are non-polar compounds. The extracts of polar solvents such as methanol and water have shown less activity or insignificant activity[31]. Many plant extracts of polar solvents were reported to have anthelmintic activity with the presence of Flavanoids, Tannins and Saponins[32, 33, 34, 35, 36]. Though the MB and ML test samples have phytoconstituents such as Flavanoids, Tannins and Saponins, these showed insignificant activity. This may be due the presences of less quantity of these phytoconstituents in these samples.

The further isolation, purification and the spectral analysis of pure compounds of bark extracts HB, CB and leaf extract HL may provide a potential anthelmintic lead molecule. The test samples can be evaluated further for other pharmacological properties because of the presence of various phytoconstituents.

ACKNOWLEDGEMENT

We sincerely thank Vision Group of Science and Technology, Government of Karnataka for granting fund under CISE to procure the analytical instruments to carry out the proposed research work.

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