



Anthelmintic screening of some Plants used in Traditional Medicine

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ABSTRACT

In Indian medicinal plant literature, leaves of *Annona squamosa*, *Annona reticulata*, *Balanites aegyptica*, *Caesalpinia bonducella*, *Cleome gynandra* and *Diplocyclos palmatus* have been traditionally reported to be used as anthelmintics. Hence, it is essential to examine these plant species for anthelmintic potential to support traditional claim. The present study was carried out to determine and compare anthelmintic efficacies of these plants against the commercially available anthelmintic drug, nitazoxamide. Ethanol extracts of leaves of all studied plants were examined for anthelmintic activity against Indian adult earthworm. All extracts demonstrated highly significant dose-dependent anthelmintic activity at tested concentrations of 10, 20, 50 mg/ml, compared with reference standard nitazoxamide. In conclusion, all studied plants have potential application in the treatment of helminthes infections. The results of this study also validated the basis for the traditional use of these plants as anthelmintics in Indian medicine.

Keywords: Anthelmintic; Traditional medicine; Indian adult earthworm.

1. INTRODUCTION

Helminthic infections are among the most common infections in human being, affecting a large proportion of the world's population. In developing countries they pose a large threat to public health and contribute to the prevalence of malnutrition, anemia, eosinophilia and pneumonia. [1] They also have an immense impact on agricultural livestock and domesticated animals. [2] Increasing problems of development of resistance in helminths [3, 4] against anthelmintics have led to the proposal of screening medicinal plants for their anthelmintic activity. Anthelmintic plants offer a traditional alternative to manufactured anthelmintics that is both sustainable and environmentally acceptable. Such plants could have a more important role in the future control of helminth infections in the tropics. [5]

From the literature survey of traditional medicine, we undertook the present study in order to elucidate the ayurvedic and traditional use of the selected plants from the scientific view point. In the current study, six plant species were selected for investigation based upon their uses in gastrointestinal diseases. [6-9] The selected plants as

the subject to anthelmintic study are listed in table 1 with their family, local names and recorded utilizations in traditional Indian medicine. However, the anthelmintic activity of only a few of these species had previously been tested. [10-16]

2. MATERIALS AND METHODS

2.1. Plant collection

Fresh leaves of all plant species were collected from different locations in Western Ghats of Maharashtra State (MS), India. The plant species were identified with the help of Flora of Maharashtra [17, 18] and voucher specimens were deposited at the Postgraduate department of Botany, Sangamner College, Sangamner for the future reference. All leaves samples were washed, shade dried at room temperature for 15 days. The leaves were powdered with the help of electric grinder and stored in air tight bottles for further study.

2.2. Used organism

Indian adult earthworms (*Pheretima posthuma* Annelida; Megascolecidae) collected from water logged area of soil and identified at the department of Zoology, Sangamner

College, Sangamner. Later, it was washed with normal saline to remove all faecal matter and used for the anthelmintic study. The earthworms of 3-5 cm in length and 0.1- 0.2 cm in width were used for all the experimental protocol due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings. [19-22]

2.3. Preparation of extracts

Dried and coarsely powdered leaves of all species (50 g) were separately subjected to extraction in Soxhlet extractor using ethanol as a solvent. All the extracts were concentrated by evaporation in water bath and then dried in open air. The dried extract was stored in refrigerator for further use.

| Plant name and authors | Family | Local name | Part used | Traditional use (Lit.) |
|---|----------------|-----------------|-----------|--|
| <i>Annona squamosa</i> , L. | Annonaceae | Sitaphal | leaves | Leaves made into a paste without adding water are applied to unhealthy ulcers. Bruised leaves are used for destroying worms, also insecticidal, fish poison, used to remove lice in head. Fresh leaves crush between fingers and applied to nostrils [6,7]. |
| <i>Annona reticulata</i> L. | Annonaceae | Ramphal | leaves | Uses are same like those of <i>Annona squamosa</i> [6,7]. |
| <i>Balanites aegyptica</i> (L.) Del. | Simaroubaceae | Hinganbet | leaves | Leaves are pungent, bitter, purgative and anthelmintic and used in worms in children [6,8,9]. |
| <i>Caesalpinia bonducella</i> (L.) Fleming | Caesalpinaceae | Sagargota | leaves | Tender leaves are efficacious in disorders of liver and oil expressed from them. Leaves boiled with castor oil applied on painful swollen testicals [8]. Also used in swellings as emmenggouge,, febrifuge and anthelmintic [6,9]. |
| <i>Cleome gynandra</i> L. | Cleomaceae | Pandhari Tilvan | leaves | In Ayurveda and other system of medicine it is described as a curative medicine for neuralgia, headache, cough, wounds, anthelmintic, rubefacient, counterirritant and for snake bite and scorpion sting [6]. It's warm juice is a popular remedy for ear disease. The leaf juice is applied in skin disease. [7]. |
| <i>Diplocyclos palmatus</i> (L.) C. Jeffrey | | Shivlingi | leaves | Leaves are used as external application for inflammation. Also used for colic pains, stomach swelling and constipation. [6,9]. |

Table 1: Traditional use of the plant materials

2.4. Anthelmintic activity

In vitro anthelmintic activity was carried out according to the method described by Bhavar *et al.* [23] with some modifications. All the dried extracts of plant species were dissolved in minimum amount of DMSO and then volume is adjusted to 10 ml with saline water. All extracts and nitazoxamide solutions were freshly prepared before starting the experiment. In the set of experiment, fourteen groups of six earthworms were released in to 10 ml of desired formulations as follows: vehicle (5% DMSO in normal saline), nitazoxamide (10 mg/ml), and ethanol extract of leaves of plant species (10 mg/ml, 20 mg/ml, 50 mg/ml each) in normal saline containing 5% DMSO. Observations were made for the time taken to paralyse and death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body colours.

2.5. Statistical analysis

All values were expressed as mean \pm S.E.M. (n=6). Statistical analysis was carried out by one way ANOVA followed by Dunnett's test performed using GraphPad Prism 5.00.288, GraphPad Software Inc., San Diego,

California, USA. $P < 0.05$ was considered statistically significant.

3. RESULTS AND DISCUSSION

In order to select plant materials with possible anthelmintic activity, the database of ayurveda and ethnobotanical studies, which were published on Indian traditional medicine so far, were reviewed. Six plant materials selected for anthelmintic assay are listed in Table 1. *Pheretima posthuma* was used to assess the anthelmintic activity. The results of anthelmintic activity of ethanol extract of *A. squamosa*, *A. reticulata*, *B. aegyptica*, *C. bonducella*, *C. gynandra*, and *D. palmatus* are summarized in table 2. The results were compared with nitazoxamide as positive control and normal saline water as negative control. The data revealed that ethanol extracts of leaves of all studied six plant species showed significant dose dependent anthelmintic activity; when compared with standard drug nitazoxamide at the same concentrations. All the ethanol extracts of leaves of all studied six plant species were found to be more effective in causing the death of the worms as well as promoting paralysis.

| Plant | Concentration (mg/ml) | Paralysis time (min) | Death time (min) |
|-------------------------------|-----------------------|------------------------------|-----------------------------|
| <i>Annona squamosa</i> | 10 | 3.08 ± 0.03*** | 3.27 ± 0.09*** |
| | 20 | 2.14 ± 0.03*** | 2.76 ± 0.21*** |
| | 50 | 1.89 ± 0.20*** | 1.98 ± 0.03*** |
| <i>Annona reticulata</i> | 10 | 2.85 ± 0.15*** | 3.15 ± 0.12*** |
| | 20 | 2.46 ± 0.01*** | 3.26 ± 0.01*** |
| | 50 | 1.42 ± 0.06*** | 3.23 ± 0.01*** |
| <i>Caesalpinia bonducella</i> | 10 | 2.78 ± 0.21*** | 3.52 ± 0.25*** |
| | 20 | 2.16 ± 0.08*** | 2.69 ± 0.18*** |
| | 50 | 1.33 ± 0.13*** | 1.67 ± 0.09*** |
| <i>Balanites aegyptica</i> | 10 | 2.50 ± 0.08*** | 3.32 ± 0.15*** |
| | 20 | 2.62 ± 0.12*** | 3.18 ± 0.14*** |
| | 50 | 2.11 ± 0.06*** | 2.65 ± 0.18*** |
| <i>Cleome gynandra</i> | 10 | 2.446 ± 0.2033 ^{ns} | 6.46 ± 0.4042 ^{ns} |
| | 20 | 1.796 ± 0.2214** | 4.118 ± 0.1693*** |
| | 50 | 1.214 ± 0.03140*** | 2.378 ± 0.2455*** |
| <i>Diplocyclos palmatus</i> | 10 | 1.2421±0.09630*** | 3.7±0.4159*** |
| | 20 | 1.438±0.06873*** | 3.424±0.03458*** |
| | 50 | 0.904±0.1928*** | 2.102±0.1743*** |
| Nitazoxamide | 10 | 6.06 ± 0.06 | 7.79 ± 0.16 |
| Control | - | - | - |

Table 2: *In vitro* anthelmintic activity of ethanol extracts of leaves of some traditional medicinal plants

Each value represents mean ± SEM, One way ANOVA followed by Dunnett's 't' test, Note: n=6 in each group, **P<0.01, ***P<0.001.

Based on the findings of the present study it is concluded that, the ethanol extracts of leaves of all studied six plant species found to have strong anthelmintic activity; and found to be active as traditional

anthelmintics with regard to both paralyse and death times. However, further study is essential to ascertain their active constituents to lay down recommendation on scientific ground.

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Conflict of Interest: None Declared