



Evaluation of *Bauhinia variegata* Linn stem bark for anthelmintic and antimicrobial properties

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Abstract

This research work was taken up to investigate the anthelmintic and antimicrobial potential of the stem bark of *Bauhinia variegata* Linn. The crude ethanolic extract of the plant was evaluated for anthelmintic activity using Indian earthworm *Pheretima posthuma* and *Ascardia galli* as test worms. Various concentrations (10-100 mg/ml) of ethanolic extract were tested in the bioassay, which involved determination of time of paralysis (P) and time of death (D) of the worms. Piperazine citrate (10mg/ml) included as reference standard. Antimicrobial activity of the crude ethanolic extract was evaluated by agar disc diffusion method and Minimum Inhibitory Concentration (MIC) was determined by broth dilution method by using some bacterial and fungal strains of microorganism. Ciprofloxacin (5µg/ml) and Cotrimazole (25µg/ml) were used as reference standard for the antibacterial and antifungal studies respectively. The ethanolic extract showed promising anthelmintic and antimicrobial activities and results were comparable with the standards included in the study.

Key words: *Bauhinia variegata*, anthelmintic activity, antimicrobial activity, Piperazine citrate, Ciprofloxacin, Cotrimazole

1. Introduction

The traditional medicines hold a great promise as source of easily available effective anthelmintic agents to the people, particularly in tropical developing countries, including India. It is in this context that the people consume several plants or plant-derived preparations to cure helminthic infections [1]. The origin of many effective drugs

is found in the traditional medicine practices and in view of this it is important to undertake studies pertaining to screening of the folklore medicinal plants for their proclaimed biological efficacy.

Bauhinia variegata Linn. (Family-Caesalpinaceae) popularly known as Raktakanchan, is a medium sized deciduous tree

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found throughout India, Burma and China [2]. The stem bark of the plant is of great medicinal importance and traditionally used as astringent, liver tonic and anthelmintic. It is claimed to be useful in the treatment of leucoderma, leprosy, asthma, wounds, tumor, ulcers and dysentery [3-5]. The phytochemical studies on the stem bark revealed the presence of flavonone glycoside, β -sitosterol, lupeol, quercetin, saponins and tannins [6-9]. Earlier reports on biological activities of stem bark are scarce. The vast ethnomedicinal uses prompted us to investigate the anthelmintic and antimicrobial properties of the plant in a scientific manner.

2. Materials and Methods

2.1 Plant material

The stem bark of *B. variegata* was collected from mature trees in the month of October and its botanical identification was confirmed from Botanical Survey of India (BSI), Koregaon Road, Pune where the voucher specimen (No.165415) has been deposited.

2.2 Preparation of extract

The plant material (stem bark) was dried for several days in shade and powdered with the help of an electric grinder. After defatting the bark powder (300 g) using petroleum ether (40-60°C), it was air dried and extracted exhaustively with 95% ethanol in a soxhlet apparatus. The liquid extract so obtained was filtered and distilled on water bath; a reddish brown syrupy mass was obtained. It was finally dried at low temperature under reduced pressure in a rotary evaporator. The ethanolic extract was subjected to preliminary phytochemical testing for the presence of different chemical classes of compounds [10, 11].

2.3 Worms Collection and Authentication

Indian earthworm *Pheretima posthuma* (Annelida) were collected from the water logged areas of soil and *Ascaridia galli* (Nematode) worms were obtained from freshly slaughtered

fowls (*Gallus gallus*). Both worm types were identified at the P.G. Department of Zoology, Pratap College, Amalner, Maharashtra.

2.4 Microorganisms used

For the present study, the microorganisms used include *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger*.

2.5 Anthelmintic Assay

The anthelmintic assay was carried as per the method of Ajaiyeoba *et al* [12] with necessary modifications. The assay was performed on adult Indian earthworm, *Pheretima posthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasite of human beings [13-16]. Because of easy availability, earthworms have been used widely for the initial evaluation of anthelmintic compounds *in vitro* [17-20]. *Ascaridia galli* worms are easily available from freshly slaughtered fowls and its use, as a suitable model for screening of anthelmintic drug was advocated earlier [21-23]. Fifty ml formulation containing different concentrations of crude ethanolic extract (10, 25, 50 and 100 mg/ml in distilled water) were prepared and six worms (same type) were placed in it. This was done for both types of worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50°C) [24-25]. Piperazine citrate (10 mg/ml) was used as reference standard while distilled water as control.

2.6 Antimicrobial activity

2.6.1. Determination of Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) of the ethanolic extract was performed by broth dilution method [26] at concentrations ranging

from 25 µg/ml to 300 µg/ml in dimethyl sulphoxide (DMSO) against the microorganisms used for the study.

2.6.2. Determination of zone of inhibition

The zone of inhibition was determined by agar disc diffusion method [27]. The extract was dissolved in DMSO at a concentrations of 5, 10 and 20 mg/ml. Ciprofloxacin (5 µg/ml) and Cotrimazole (25 µg/ml) were used as reference standards for the antibacterial and antifungal studies respectively. Solvent control (DMSO only) was also maintained throughout the study.

3. Results and discussion

Preliminary phytochemical screening of crude ethanolic extract revealed the presence of alkaloids, saponins, flavonoids and tannins. As shown in Table 1, ethanolic extract of *B. variegata* exhibited anthelmintic activity in dose-dependant manner giving shortest time of paralysis (P) and death (D) with 100 mg/ml concentration, for both types of worms. The ethanolic extract of *B. variegata* caused paralysis of 14 min and time of death of 29 min respectively against the earthworm *P. posthuma*. The reference drug Piperazine citrate showed the same at 20 and 89 min respectively.

Ascardia galli worms were also shown sensitivity to the ethanolic extract significantly at higher concentration of 100 mg/ml. The ethanolic extract caused paralysis of 10 min and time of death of 22 min respectively. Piperazine citrate did the same at 16 and 63 min respectively.

The predominant effect of Piperazine citrate on worm is to cause a flaccid paralysis that result in expulsion of the worm by peristalsis. Piperazine citrate by increasing chloride ion conductance of worm muscle membrane produces hyperpolarisation and reduced excitability that leads to muscle relaxation and flaccid paralysis [28]. The stem bark extract of *B. variegata* not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 100 mg/ml, in shorter time as compared to reference drug Piperazine citrate. Phytochemical analysis of the crude extracts has revealed tannins to be among the chemical constituent contained within them. Tannins were shown to produce anthelmintic activities [29]. Chemically tannins are polyphenolic compounds [30]. Some synthetic phenolic anthelmintic e.g. niclosamide, oxiclozanide, bithionol etc., are shown to interfere with energy generation in

Table 1: Anthelmintic activity of ethanolic extract of stem bark of *B. variegata*

Test substance	Concentration mg/ml	Time taken for Paralysis (P) and Death (D) of worms in minute			
		<i>P. posthuma</i>		<i>A. galli</i>	
		P	D	P	D
Vehicle	-	-	-	-	-
Ethanolic extract	10	49.25 ± 0.33	60.84 ± 0.47	57.16 ± 0.29	64.94 ± 0.33
Ethanolic extract	25	30.30 ± 0.49	50.28 ± 0.37	42.27 ± 0.36	55.14 ± 0.29
Ethanolic extract	50	21.86 ± 0.26	41.20 ± 0.35	30.14 ± 0.29	42.91 ± 0.36
Ethanolic extract	100	14.27 ± 0.41	29.93 ± 0.43	10.97 ± 0.33	22.13 ± 0.25
Piperazine citrate	10	20.10 ± 0.13	89.08 ± 0.14	16.17 ± 0.14	63.16 ± 0.19

Results expressed as Mean ± SEM of six observations

Table 2: MIC ($\mu\text{g/ml}$) and zone of inhibition (mm) of ethanolic extract of *B. variegata* stem bark

Microorganisms	Zone of inhibition (mm)*				Standard #
	MIC (µg/ml)	Extracts (mg/ml)			
		5	10	20	
<i>Staphylococcus aureus</i>	75	7.4	13.2	20.4	28.6
<i>Bacillus subtilis</i>	50	9.8	16.6	22.8	24.7
<i>Pseudomonas aeruginosa</i>	300	7.2	12.3	20.1	24.9
<i>Escherichia coli</i>	200	8.2	10.8	16.4	21.8
<i>Aspergillus niger</i>	250	8.7	12.3	18.2	22.9
<i>Candida albicans</i>	175	9.4	15.2	21.6	29.4

* Values are mean of three readings,

Standards: For antibacterial study-Ciprofloxacin 5 $\mu\text{g/ml}$
For antifungal study-Cotrimazole 25 $\mu\text{g/ml}$

helminth parasites by uncoupling oxidative phosphorylation [31]. It is possible that tannins contained in the extract of *B. variegata* produced similar effects. Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal [32] or glycoprotein on the cuticle of the parasite and cause death [33].

Table 2 depicts the antimicrobial activity of the ethanolic extract of stem bark of *B. variegata*. The results of MIC study revealed the antimicrobial activity of the extract against the tested strains of microorganisms between concentration ranges of 50 and 300 $\mu\text{g/ml}$. The results of zone of inhibition study revealed that the extract possess antimicrobial activity in a

concentration dependent manner against the test organisms and was comparable with the standard drugs. In general, commercial antibiotic and antifungal drugs cause side effects such as liver, kidney and gastrointestinal tract toxicity [34]. Severe hepatotoxicity had also been reported in patients undergoing anti-fungal drug therapy [35]. However, herbal remedies often do not produce any side effects [36]. Therefore, alternative medicine has become a popular remedy to various types of ailments.

In conclusion, though *B. variegata* has revealed significant anthelmintic and antimicrobial activity against test organisms used for the study, further phytochemical and clinical studies are required to understand its principles.

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