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Research Article

Pharmacognostical, Phytochemical and *In-Vitro* Anthelmintic Activity of *Cassia roxburghii* Seed and *Boerhaavia diffusa* Root against *Pheritima posthuma* Model

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Plants have served human beings as a natural source for treatments and therapies from ancient times, amongst them medicinal herbs have gain attention because of its wide use and less side effects. In the recent years plant research has increased throughout the world and a huge amount of evidences have been collected to show immense potential of medicinal plants used in various traditional systems. The objective of this study was to investigate pharmacognostical, phytochemical features and anthelmintic action of *Cassia roxburghii* (seeds) and *Boerhaavia diffusa* (roots). The different pharmacognostical parameters were evaluated as per standard protocols with some modifications. Qualitative analysis of various phytochemical constituents was determined by the well-known test protocol available in the literature. Phytochemical analysis revealed the presence of alkaloids, glycosides, flavonoids, steroids, saponins, triterpenoids and carbohydrate. Three different doses (10, 25, & 50 mg/ml) of each extracts were studied, which involves the determination of time of paralysis and time of death of worm. All the extracts showed a dose dependent increase in the anthelmintic action. Out of all the extracts, the methanol extracts showed highest activity in both plants followed by ethyl and aqueous extracts. At the concentration of 50 mg/ml the methanol showed a remarkable anthelmintic activity which was even greater than the standard drug (Albendazole) at the same concentration.

Keywords: *Cassia roxburghii*, *Boerhaavia diffusa*, *Pheritima posthuma*, Anthelmintic action, Paralysis, Albendazole.

INTRODUCTION

Helminth infections are among the most widespread infections in humans, distressing a huge population of the world. Although the majority of infections due to helminths are generally restricted to tropical regions and cause enormous hazard to health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia¹. Parasitic diseases cause ruthless morbidity affecting principally population in endemic areas². The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs therefore there is a foremost problem in treatment of helminthes diseases³. The development of integrated programs to control helminths is vital, but such control programs require viable alternatives to the use of anthelmintics⁴. Hence there is an increasing demand towards natural anthelmintics⁵. Medicinal plants have served through ages, as a constant source of medicaments for the exposure of a variety of diseases. The history of herbal medicine is almost as old as human civilization. The plants are known to provide a rich source of botanical anthelmintics, antibacterials and insecticides^{6,7}. A number of medicinal plants have been used to treat parasitic infections in man and animals⁸⁻¹⁰. *Cassia* species family (Fabaceae) *Cassia roxburghii* Linn, commonly known as Ceylon senna (red cassia), belongs to the family

Fabaceae/Leguminosae (subfamily Caesalpiniaceae). Synonyms Red Cassia, Ceylon Senna L., *Roxburghii's Cassia*. It is a fairly large "shower" tree with feather like pinnately compound leaves and twigs covered with a dense carpet of fine and soft hair. *Cassia* species are annual under shrub grows all over the tropical countries (throughout India) the species is native to the Indian subcontinent and adjacent regions of Southeast Asia. It ranges through South India and Sri Lanka^{11,12}. The red cassia is a medium-sized tree, growing to 15-20 m (49-66ft) tall with spreading, drooping branches. The leaves are clusters of pink, rosé or orange flowers, 15-60 cm. (5.9- 23.6 in) long, and pinnate with three to eight pairs of leaflets, each leaflet 7-21 cm. (2.8-8.3 in) long and 4-9 cm. (1.6-3.5 in) broad. The flowers are produced in pendulous racemes 20-40cm. (7.9-15.7 in) long, each flower 4-7cm. (1.6-2.8 in) diameter with red to pinkish petals. The fruit is a legume. *C. roxburghii* Linn has been of keen interest in phytochemical and pharmacological research due to their excellent medicinal values¹³. *C. roxburghii* Linn are extensively used in traditional medicine in various parts of the world against a wide range of ailments various species of cassia are reported to have anthelmintic activity, antiviral, antimalarial, antioxidant activity, antidiabetic activity, Treating skin disease, Healing wounds, hepatoprotective and anticancer properties^{14,15}. *Boerhaavia Diffusa*: *Boerhaavia diffusa*

(Nyctaginaceae) commonly known as Raktapunarnava, Shothaghni, Kathillaka, Kshudra, Varshabhu, Raktapushpa, Varshaketu, Shilatika¹⁶ is a herbaceous plant species growing prostrate or ascending upward in habitats like grasslands, agricultural fields, fallow lands, wastelands and residential compounds. The plant was named in honor of Hermann Boerhaave, a famous Dutch physician of the 18th century¹⁷. The plant is mentioned in the Atharvaveda with the name 'Punarnava', because the top of the plant dries up during the summer season and regenerates again during the rainy season. Thus the plant generally perennates through the roots in the soil¹⁸. *Boerhaavia diffusa* (Nyctaginaceae) is one of the most famous medicinal plants in India; it is a perennial herbaceous plant growing in tropical regions such as the Antilles, South America, India and Africa¹⁹. The roots are very variable diffusely branched low spreading or creeping herbaceous perennial with an elongated fusiform or tapering tap root. The stems are numerous; 1-2 m long arises from the crown of the root, slender, round, nodose, jointed and often reddish or purplish in color. The leaves are simple, opposite, short petiolate, exstipulate, unequal in each pair, 2.5-5 cm long by 1-4.5 cm wide, oblong or suborbicular, acute, obtuse or rounded at apex, cordate, rounded or truncate at base, entire or wavy along the margin, subfleshy, glabrous or sparingly hairy above, silvery white beneath, petioles 0.7-3 cm long, slender, deeply grooved above. The flowers are small, regular, sessile or subsessile, pale rose to pink, in irregular clusters of 4-10, small umbels on extra axillary peduncles. Perianth-short, tubular about 3mm long and deeply constricted at the middle. The lower tubular part is greenish, persistent and is covered with glandular hairs. The upper limb is light red, funnel shaped and 5 lobed. The stamens are 2 or 3, Filaments united in the ovary at the base and not exerted outside of the perianth. The anthers are small and 2 celled. The ovaries are small completely covered by the perianth. The fruits are very small, one seeded and enclosed in persistent lower half the perianth. The perianth is covered with sticky glandular hairs²⁰. Its various parts and especially roots have been used for gastrointestinal, anthelmintic activity, hepatoprotective and gynecological indications in above mentioned parts of the world and also throughout India²¹. The aim of this work was to determine the quality (types), quantity (amount) of bioactive compounds and *in vitro* anthelmintic activity of *Cassia roxburghii* (seeds) and *Boerhaavia diffusa* (roots) in Bhopal region of Madhya Pradesh.

MATERIAL AND METHODS

Collection and identification of plant

The seed of *Cassia roxburghii* and root of *Boerhaavia diffusa* were collected from rural area of Bilaspur District in the month of February, 2020. The identification and authentication of plant was done by Dr. Ashwini Kumar, Dixit, Botanist, from the Department of Botany, Guru Ghasidas Vishwavidhyalaya CG India. A voucher specimen number GGV/BOT/2019/AKD/14 was kept in Department of Botany, Guru Ghasidas Vishwavidhyalaya CG India for future reference.

Drugs and chemicals

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Standard drug Albendazole obtained from local market (Vindhayanchal Medical Store, Bilaspur (C.G.)). All the chemicals and solvent used in this study were of analytical grade.

Preparation of extract

Preparation of *Cassia roxburghii* (seed), *Boerhaavia diffusa* (root) plants were done by Soxhlet extraction method. 250gm of powdered crude drug of *Cassia roxburghii* seed and

Boerhaavia diffusa root were subjected to Soxhletion (continuous hot extraction) with 1000 ml of ethyl acetate, 1000 ml of methanol and 1000 ml of water after pretreatment with petroleum ether. Soxhlet apparatus (boiling point range 60-80°C) for 8 h. The extract was concentrated under reduced pressure of 22-26mm Hg. At 450°C and the residue obtained was stored at 40°C until used. The crude extract was dissolved in distilled water to required concentrations and used for the experiments. The crude extracts were subjected to quantitative tests for the identification of various active constituents.

Macroscopical evaluation

Macroscopical study is the morphological description of the *Cassia roxburghii* (seed), *Boerhaavia diffusa* (root) which can be seen by naked eyes and it was performed by following the standard methods to determine the taste, size, color and odor of the *Cassia roxburghii* (seed), *Boerhaavia diffusa* (root)²².

Physicochemical parameters

Loss on drying

Ten gm of the powdered drug was accurately weighed in a tarred petridish. It was dried at 105°C for 1 hour in hot air oven and then reweighed. Loss on drying was determined by calculating the initial and final weight.

Total ash value

Five gm of powdered drug was incinerated in a silica dish at a temperature not exceeding 450°C until free from carbon in muffle furnace. It was then cooled and weighed. The percentage w/w of ash with reference to the air-dried drug was calculated.

Alcohol soluble extractive value

Five gm of coarsely powdered air-dried drug was macerated with 100 ml of alcohol in a closed flask for 24 hour, shaken frequently for six hours and allowed to stand for eighteen hours. It was then filtered rapidly taking precaution against loss of alcohol. 25 ml of the filtrate was evaporated to dryness in tared flat-bottomed shallow dish, dried at 105°C and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air-dried drug.

Water soluble extractive value

Five gm of coarsely powdered air-dried drug was macerated with 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently for six hours and allowed to stand for eighteen hours. It was then filtered rapidly taking precautions against loss of chloroform water. 25ml of the filtrate was evaporated to dryness in tared flat-bottomed dish dried at 105°C and weighed.

Qualitative analysis of phytochemicals

The extracts prepared for the study were subjected to preliminary phytochemical screening by using different reagents for identifying the presence or absence of various phytoconstituents viz., carbohydrates, proteins, alkaloids, tannins, steroid, flavonoids and terpenoids in methanolic, ethyl acetate and aqueous extracts of *Cassia roxburghii* (seed), *Boerhaavia diffusa* (root). The above phytoconstituents were tested as per the standard method^{23,24}.

Test organism

Indian adult earthworms (*pheritima posthuma*) collected from moist soil of the botanical garden were washed with normal saline and used for the anthelmintic study. The earthworms (4-6 c.m. in length and 0.1- 0.2 c.m. in width) earthworms were identified by Dr. LVKS Bhaskar professor and head Dean

(School of Life Sciences) Guru Ghasidas Vishwavidhyalaya A central University Dept. of zoology Reference No. 458/200/Zoology/21 Koni (Bilaspur) Chhattisgarh.

Investigation of in-vitro anthelmintic activity

Indian adult earthworms (*Pheritima posthuma*) collected from moist soil and washed with normal saline to remove all faecal matter were used for the anthelmintic study. The worms were acclimatized to the laboratory conditions before experimentation eight groups, with six earthworms each Indian adult earthworms were collected and groups were divided into twelve containing four earthworms in each group. Each group was treated with any one of 10 ml of standard drug Albendazole (10mg/ml, 25mg/ml, 50mg/ml), Ethyl Acetate (10mg/ml, 25mg/ml, 50mg/ml) Methanolic solvent (10mg/ml, 25mg/ml, 50mg/ml) or Aqueous solvent(10mg/ml, 25mg/ml, 50mg/ml) in eight different petri dishes.

Motility of the worms

The earthworms were placed in the petri dishes after they have been washed with 0.9%w/v of normal saline solution. Motility of the worms was observed and time for paralysis and time for death of the worms were noted down. Time for paralysis was noted when the worms showed no movement when shaken vigorously. Time for death was recorded when the worms showed no movement even when dipped in warm water at 50-60°C temperature and also with fading away of their body colour^{25, 26}.

RESULTS

The crude extracts so obtained after the soxhlet extraction process was further concentrated on water bath for evaporate the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important

phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts obtained from sample using ethyl acetate, methanol and water as solvents are depicted in the Table 1. Morphological characteristics of *Cassia roxburghii* seed and *Boerhaavia diffusa* root are summarized in Table 2. *Cassia roxburghii* seed and *Boerhaavia diffusa* root were shade dried and turned to powder for various physiochemical parameters like loss on drying, total ash value, alcohol soluble extractive, water soluble extractive and foaming index which are summarized in Table 3. The results of qualitative phytochemical analysis of the crude powder of *Cassia roxburghii* seed and *Boerhaavia diffusa* root were shown in Table 4. Methanolic extract of *Cassia roxburghii* seed and *Boerhaavia diffusa* root showed the presence of alkaloids, flavonoids, glycoside, carbohydrates, triterpenoids, saponins and tannins. Two worms (same type) were both placed in 9 cm Petri dishes in solutions of crude extracts in three different concentrations (10, 25, and 50 mg/ml in distilled water), respectively. This was done in duplicates for all the worm types. Mean times for paralysis (in minutes) were taken when no movement of any sort could be observed, except when the worms were shaken vigorously. Times of death of worms (in minutes) were recorded after ascertaining that worms neither moved when shaken vigorously nor when dipped in warm water (50 °C) and also with fading away of their body colour. Albendazole (10, 25 and 50 mg/ml) was included as standard compound, while Normal saline was included as control. Each group was treated with any one of 10 ml of standard drug Albendazole (10mg/ml, 25mg/ml, 50mg/ml), Ethyl Acetate (10mg/ml, 25mg/ml, 50mg/ml) Methanolic solvent (10mg/ml, 25mg/ml, 50mg/ml) or Aqueous solvent(10mg/ml, 25mg/ml, 50mg/ml) in eight different petridishes²⁷ Table 5, 6 & Figure 1.

Table 1: Yield of crude extracts of *Cassia roxburghii* seed and *Boerhaavia diffusa* root

S. No.	Parts	Ethyl acetate % Yield (W/W)	Methanol % Yield (W/W)	Aqueous % Yield (W/W)
1.	<i>Cassia roxburghii</i>	9.40	11.66	5.88
2.	<i>Boerhaavia diffusa</i>	8.50	7.45	10.6

Table 2: Morphological characteristic of *Cassia roxburghii* seed and *Boerhaavia diffusa* root

Organoleptic Evaluation				
S. No.	<i>Cassia roxburghii</i> (seed)		<i>Boerhaavia diffusa</i> (root)	
	Features	Observation	Features	Observation
1	Colour:	Pale brown in colour	Colour:	Yellowish brown in colour
2	Odour:	Odorless	Odour:	Odorless
3	Taste:	Mucilagenous	Taste:	Slightly bitter taste
4	Shape:	More or less spherical	Shape:	cylindrical and longitudinally striated roots
5	Surface:	Glossy and smooth	Fracture:	fibrous, powder colour is yellowish brown

Table 3: Physiochemical analysis of powder of *Cassia roxburghii* seed and *Boerhaavia diffusa* root

Parameters	Observations	
	<i>Cassia roxburghii</i> seed	<i>Boerhaavia diffusa</i> root
Water soluble extractive value	20.7%	23.61%
Alcohol soluble extractive value	5.1%	15.20%
Total ash	2.8041%	10.11%
Water soluble ash	1.6663%	14.9 %
Acid insoluble ash	0.4626%	1.9%
Loss on drying (LOD) % w/w	8.7973	5.50
Foaming index	21 ml	23ml

Table 4: Phytochemical screening of *Cassia roxburghii* seed and *Boerhaavia diffusa* root extracts

Phytochemical Analysis of <i>Cassia roxburghii</i> (seed)				
S. No.	Phytoconstituents	Ethyl Acetate	Methanol	Aqueous
1	Alkaloids	+ve	+ve	+ve
2	Flavonoids	-ve	+ve	-ve
3	Glycoside	-ve	+ve	-ve
4	Carbohydrates	-ve	+ve	-ve
5	Triterpenoids	-ve	+ve	-ve
6	Saponins	-ve	-ve	-ve
Phytochemical Analysis of <i>Boerhaavia diffusa</i> (root)				
S. No.	Phytoconstituents	Ethyl Acetate	Methanol	Aqueous
1	Alkaloids	+ve	+ve	+ve
2	Flavonoids	+ve	-ve	-ve
3	Glycoside	+ve	+ve	+ve
4	Carbohydrates	+ve	+ve	-ve
5	Tannins	-ve	+ve	+ve
6	Saponins	-ve	+ve	+ve

Table 5: In-vitro anthelmintic activity of different extract *Cassia roxburghii*

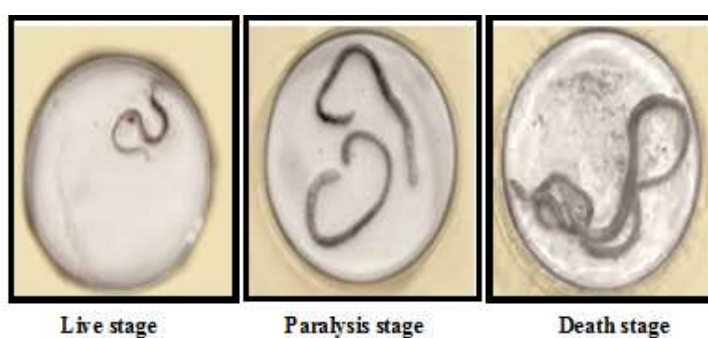
S. No.	Drug (Treatment)	Dose(mg/ml)	Time taken for paralysis(min)	Time Taken for Death(min)
1	Control [Normal saline]	-	-	-
2	Standard [Albendazole]	10	50	57
		25	55	60
		50	62	65
3	Ethyl Acetate extract of <i>Cassia roxburghii</i>	10	70	82
		25	55	61
		50	48	59
4	Methanolic extract of <i>Cassia roxburghii</i>	10	52	63
		25	49	59
		50	43	52
5	Aqueous extract of <i>Cassia roxburghii</i>	10	54	68
		25	53	60
		50	45	55

Table 6: In vitro anthelmintic activity of different extract *Boerhaavia diffusa*

S. No.	Drug (Treatment)	Dose(mg/ml)	Time taken for paralysis(min)	Time Taken for Death(min)
1	Control [Normal saline]	-	-	-
2	Standard [Albendazole]	10	53	55
		25	62	67
		50	72	77
3	Ethyl Acetate extract of <i>Boerhaavia diffusa</i>	10	56	61
		25	64	69
		50	71	76
4	Methanolic extract of <i>Boerhaavia diffusa</i>	10	69	74
		25	73	78
		50	81	86
5	Aqueous extract of <i>Boerhaavia diffusa</i>	10	58	63
		25	66	70
		50	74	80



(A)



Live stage

Paralysis stage

Death stage

(B)

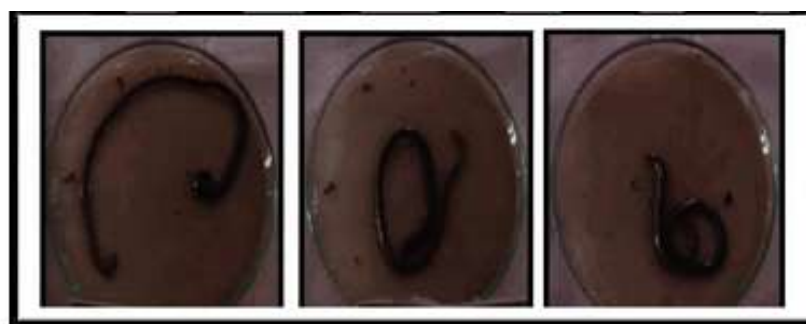


Live stage

Paralysis stage

Death stage

(C)



Live stage

Paralysis stage

Death stage

(D)

Figure 1: Anthelmintic activity of (A) normal saline (control) (B) standard drug (Albendazole) (C) *Cassia roxburghii* seed extract (D) *Boerhaavia diffusa* root extract

DISCUSSIONS

Different species of worm's viz. *Ascaris Nippostrongylus* and *Heterakis* has been used in the screening of anthelmintic agents. But of all the worms, earthworms have been widely accepted for in vitro studies mainly because of their resemblance to intestinal roundworms in reaction to anthelmintics and due to their easy availability. It is observed that all anthelmintics that are toxic to earthworms are worthy to be investigated upon as potent anthelmintic agents. In this assay we have evaluated the of *Cassia roxburghii* and *Boerhaavia diffusa*. The result have been summarized in Table no.4 & 5 it is seen that all the extracts in their different concentrations namely; 10mg/ml, 25mg/ml, 50mg/ml showed anthelmintic activity comparable with the standard drug Albendazole in their respective concentrations; 10mg/ml, 25 mg/ml, 50mg/ml. All the extracts showed a dose dependent increase in the anthelmintic action. Out of all the extracts, the methanol extracts showed highest activity in both plants followed by ethyl and aqueous extracts. At the concentration of 50 mg/ml the methanol showed a remarkable anthelmintic activity which was even greater than the standard drug Albendazole at the same concentration with time of paralysis and death of the worms less of the former then the letter. Therefore from this study we can presume that out of the many phytoconstituents present, one or few are responsible for the exhibited anthelmintic activity. Therefore further isolation and characterization of the methanol extract needs to be carried out in order to establish the possible active compound responsible for the activity. Hence, it was observed that the cassia roxburghii showed better activity in methanolic extract and *Boerhaavia diffusa* showed better activity in ethyl acetate extract. In vitro studies could also be carried out in the future in order to establish the effectiveness of this plant as an anthelmintic drug.

CONCLUSION

The present study enabled us to conclude the potential use of ethyl acetate, aqueous and methanolic extract of both *Cassia roxburghii* and *Boerhaavia diffusa* as anthelmintic agents against *Pherithema posthuma*. And hence it suggests that the cassia roxburghii showed better activity in methanolic extract and boerhaavia diffusa showed better activity in ethyl acetate extract.

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