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Combinational Effect of *Andrographis paniculata, Azadirachta indica* and *Carica papaya* latex for Anthelmintic Potential on *Pontoscolex corethrurus*

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Abstract

The present study was done with the aim to screen the anthelmintic activity of decoction containing *Andrographis paniculata* (Kalmegh), *Azadirachta indica* (Neem), *Carica papaya* latex using *Pontoscolex corethrurus*. The different concentrations (40%, 60% and 80%) of decoction were evaluated for determining the time of paralysis and the time of death of earthworms. Albendazole (10mg/ml) used as standard reference and normal saline as control. The data of the study revealed that the decoction at 80% concentration showed better anthelmintic activity compared to standard drug Albendazole.

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Introduction

Helminthiasis is habitual and global disease affecting human beings in emerging countries. Parasitic illness is caused by intestinal helminths and protozoans cause transience and anguish in native countries [1,2]. Parasitic worms residing in the body given the specific word “helminths" means “worm". Usually, helminths hardly ever cause demise but affect mental and physical health of children [1, 3].

Drugs that drive out helminths by killing or stunning them are known as anti-helminthics or anthelmintics [4]. Parasitic worms develop resistance towards anthelmintics henceforth an ample number of medicinal herbs and plants used to treat parasitic infection [5,6]. Medicinal plants showing anthelmintic activity have attain a great focus due to the ability of compounds to produce more pharmacological effect and less side effects towards the human body [7].

Andrographis paniculata known as "king of bitters" reported as having anthelmintic activity. *Andrographis paniculata* used by decoction, infusion, or powder or with the combination form with other medicinal plants to improve the activity [8]. *Azadirachta indica* (neem) is a highly versatile plant used in India for more than 2000 years due to its spectrum of biological activity. The active ingredients present in neem can be used for treating helminths [9]. Crude latex of *Carica papaya* has potential and benefits towards human health. Since it contains various proteins and enzymes which directly impact the host which results in killing and stunning the host organism by growth inhibition, mortality, and physiological damages [10].

2. Materials and methods

2.1. Collection of plant materials

The powders of Kalmegh and Neem were procured from local market of Mangaluru city, Karnataka, India.

2.2. Collection of latex

Unripe matured fruit was tapped early in the morning before making incisions. Vertical cut of 1-2 mm deep was made using a stainless-steel knife. A plastic container was used to collect and to store the latex. Latex adhering to the fruit was carefully scraped off and transferred to the collecting box using plastic spoon. Latex was then subjected for oven drying at 40°C for 45 minutes [11].

2.3. Authentication

The powder samples of Kalmegh and Neem were identified in Department of Pharmacognosy at Karavali college of Pharmacy, Mangaluru, Karnataka.

2.4. Pharmacognostic evaluation

The collected plant materials were studied for organoleptic and powder microscopical characteristics.

2.5. Preparation of decoction

In separate beakers, 25g of each weighed powder sample (Kalmegh and Neem) was thoroughly mixed with 100ml of distilled water and boiled for 15 minutes. The prepared decoction was cooled at room temperature and filtered. The filtrate obtained from both the samples were mixed together [8].

2.6. Dissolution of latex

10g of dried latex was weighed accurately. Mixed with 50ml of distilled water and filtered. The dissolved latex solution was

then mixed with the prepared decoction and this was used for further studies.

2.7. Phytochemical screening

Phytochemical analysis was carried out on decoction containing Kalmegh, Neem and Papaya latex for detection of carbohydrates, reducing sugars, proteins and amino acids, alkaloids, glycosides, phenolic compounds, flavonoids, tannins, saponins and phytosterols [12].

Detection of carbohydrates

Molisch's test

To the 2ml of decoction, 2-3 drops of Molisch's reagent was added followed by 1ml of sulphuric acid along the side of test tube. Formation of violet ring indicates the presence of carbohydrates.

Barfoed's test

To the 1ml of decoction, 1ml of Barfoed's reagent was added and heated for 2 minutes. Formation of red precipitate indicates the presence of monosaccharide.

Detection of reducing sugar

Benedict's test

To 0.5ml of decoction, 0.5ml of Benedict's reagent was added and boiled for 2 minutes. Appearance of green/yellow colour indicates the presence of reducing sugar.

Fehling's test

1ml of decoction was added to 1ml Fehling's reagent A & B and boiled in water bath. Formation of red precipitate indicates the presence of reducing sugar.

Detection of Proteins and amino acids

Millon's test

To the 2ml of decoction add 2ml of Millon's reagent. Formation of pink coloured solution indicate presence of protein.

Biuret test

To 2ml of decoction add few drops of 2% sodium hydroxide and 10% Copper sulphate solution. Appearance of violet colour indicate presence of amino acid.

Detection of alkaloids

Wagner's test

To the few ml of decoction, 1-2 drops of Wagner's reagent was added (along the sides of the test tube). Formation of brown or reddish precipitate indicates the presence of alkaloids.

Picric acid test

To few ml of decoction, 3-4 drops of 2% picric acid solution was added. Formation of yellow colour indicates the presence of alkaloids.

Detection of glycosides

Concentrated sulphuric acid test

To 5ml of decoction, 2ml of glacial acetic acid and 1 drop of 5% ferric chloride solution was added. To this, add few drops of concentrated sulphuric acid. Formation of brown ring at the junction of two layers indicate the presence of glycosides.

Borntrager's test

3ml of chloroform was added to 2ml of filtrated hydrolysate. Shake well and allow to separate the chloroform layer and add 10% of ammonia solution. Formation of pink coloured solution indicates the presence of anthraquinone glycosides.

Detection of cardiac glycosides

Keller-killani test

To 5ml of decoction, 2ml of glacial acetic acid and 1 drop of 5% ferric chloride solution was added. To this, add few drops of concentrated sulphuric acid. Formation of blue coloured solution (in acetic acid layer) indicates the presence of cardiac glycosides.

Bromine water test

To the sample solution add few ml of bromine water. Formation of yellow precipitate indicates presence of cardiac glycoside.

Detection of Phenolic compound:

Lead acetate test

To 1ml of sample solution add few ml of lead acetate solution. Formation of yellow precipitate indicate presence of phenolic compound.

Detection of flavonoids

Shinoda test

To the sample solution add 5ml of alcohol and few magnesium turnings. To this add few drops of concentrated hydrochloric acid. Appearance of pink to crimson colour solution indicate the presence of flavonoids.

Detection of tannins

Ferric chloride test

To aqueous extract add few drops of 5% ferric chloride solution. Appearance of dark green colour indicates the presence of tannins.

Lead subacetate test

To 1ml of decoction add few ml of lead subacetate. Formation of creamy gelatinous precipitate indicates the presence of tannins.

Detection of saponins

Foam test

Take few ml of sample and add few ml of water and shake well. Formation of foam indicates the presence of saponins.

Detection of phytosterol

Salkowski's test

To the decoction add few ml of concentrated sulphuric acid (shake well and allow to stand). Appearance of red colour in the lower layer indicates the presence of phytosterols [12].

2.8. Anthelmintic screening

Anthelmintic activity was studied for decoction containing Kalmegh powder, Neem powder, and Papaya latex using earthworm species *"Pontoscolex Corethrurus".*

2.9. Collection and identification of worms

Adult earthworms *(Pontoscolex corethrurus)* were collected (due to its anatomical and physiological resemblance with the intestinal parasites) from local garden of Vamanjoor-Mangaluru, Karnataka India. The collected earthworms were identified as *Pontoscolex Corethrurus* (Muller 1856) belonging to family *Rhinodrillidae.* The species were identified by Vivek Hasyagar, Research scholar, Department of applied zoology, Mangaluru university, Konaje-Mangaluru, Karnataka, India.

2.10. Screening method

Five groups of similar sized earthworms were selected. Each group consisted of four earthworms which were used for the study. First group acted as control and received only normal saline. The second group acted as the standard and received Albendazole (10mg/ml). Third, fourth and fifth group served as test and received test sample of 40%, 60% and 80% concentration respectively. The time of paralysis and death of

each individual earthworm was recorded. Paralysis was assumed to occur when the worms were non-motile when introduced into normal saline. The living worms were monitored closely and the time taken for complete death was noted. The death of worms was determined by transferring the motionless worms to warm water at 40°C. The warm environment stimulates and induces movement in the worms, if alive [4, 13].

3. Result & Discussion

The collected plant materials were studied for organoleptic and powder microscopical characteristics. Results are tabulated below

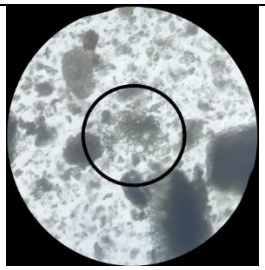
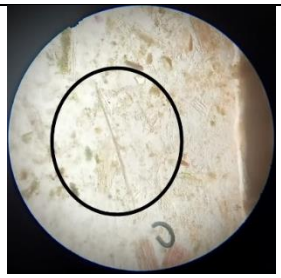
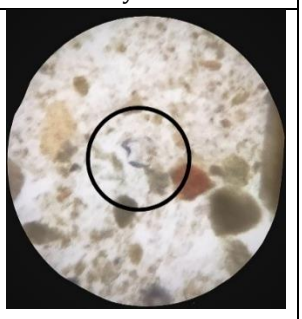
Table 01. Organoleptic evaluation

Sl. No	Organoleptic properties	Kalmegh	Neem
1	Colour	Pale green	Yellowish green
2	Odour	Characteristics	Characteristics
3	Taste	Bitter	Bitter

3.1. Powder microscopy:

The powder microscopy was carried out for powder sample of *Andrographis paniculata* and *Azadirachta indica*. *Andrographis paniculata* shows the presence of epidermis, xylem vessels and elongated fibres tapered at both ends. *Azadirachta indica* shows the presence of epidermis with anomocytic stomata and covering trichomes.

Table 02. Microscopic evaluation

Andrographis paniculata (Kalmegh)	*Azadirachta indica* (Neem)
 Epidermis	 Epidermis with anomocytic stomata
 Elongated fibres tapered at both ends	 Covering trichome

3.2. Phytochemical analysis:

The phytochemical studies were conducted based on qualitative analysis to identify the presence of bioactive Chemical constituents. Results are tabulated in the table below: -

Table 03. Qualitative analysis of phytochemical constituents.

Sl. No.	Phytochemicals	Result
1	Carbohydrates	+
2	Reducing sugars	+
3	Proteins & amino acids	-
4	Alkaloids	+
5	Glycosides	+
6	Phenolic compounds	+
7	Flavanoids	+
8	Tannins	+
9	Saponins	+
10	Phytosterols	+

(+) = Present ; (-) = Absent

Fig. 01. Qualitative tests for phytochemical screening.

3.3. Anthelmintic screening

The herbal preparation containing *Andrographis paniculata, Azadirachta indica* and *Carica papaya* latex shows significant anthelmintic activity against *Pontoscolex corethrurus.* The time of paralysis and time of death of earthworms after being treated with test sample are recorded in table no. 4. The activity of standard drug compared with test sample of different concentrations.

Table 04. Activity of Standard drug compared against test sample at different concentrations.

Sr.no	Samples	Concentration	Average time of paralysis (mins)	Average time of death (mins)
1.	Control	Normal saline	_	_
2.	Standard	10mg/ml	162.25	182.00
3.	Test sample	1) 40%	198.25	229.25
		2) 60%	171.00	194.00
		3) 80%	127.25	152.5

4. Conclusion

From the above result, it was concluded that the sample prepared from combination of *Andrographis paniculata*, *Azadirachta indica* and *Carica papaya* latex shows significant anthelmintic activity against earthworms *Pontoscolex corethrurus*. It was observed that sample with 80% concentration shows better anthelmintic activity as compared to standard drug Albendazole. So, it was concluded that the combination of these herbs has a good anthelmintic activity against the worms.

5. Acknowledgement

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6. Conflict of Interest

None

7. References

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