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Research Article

Pharmacognostic Evaluation Of Combretum Indicum And Formulation Of A Herbal Syrup With Anthelmintic And Anticancer Activity

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ABSTRACT

The setting of standards is a crucial step in determining the identity, purity, and quality of unprocessed plant-based drugs and formulations. In this case, a herbal syrup containing Combretum indicum, a common plant found in India's Western Ghats, was subjected to various preclinical studies. The drug was standardized according to WHO parameters through morphological study, microscopic investigation, and phytochemical screening. The results showed that the drug met the identification, purity, and quality criteria as per pharmacopeial standards. A herbarium specimen of Combretum indicum was prepared and authenticated by a botanist. The plant material was collected from the Holy Grace Campus and processed for the research project. Morphological analysis of the drug and the prepared syrup was conducted, which is important for identification. Phytochemical screening of the extracted drug revealed the presence of various constituents, including carbohydrates, tannins, flavonoids, and alkaloids. The herbal syrup was formulated and analysed for its physicochemical properties. The syrup was evaluated for its anthelmintic and anticancer activities. The anthelmintic activity was tested using Indian earthworms, Pheretima posthuma, in the pharmacognosy laboratory of Holy Grace Academy of Pharmacy. The results showed a dose-dependent increase in the effectiveness of the syrup, with the lowest dose causing death in 60 minutes. The anticancer activity was evaluated at the Amala Cancer Research Centre Society in Thrissur, and the results indicated a dose-dependent increase in the anticancer activity of the syrup.

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INTRODUCTION

Medicinal plants are important sources of bioactive compounds used in traditional and modern medicine. Pharmacognostic studies are essential for the identification, authentication, and quality control of crude drugs through morphological, microscopic, physicochemical, and phytochemical analyses **[1,2]**.

Combretum indicum (syn. Quisqualis indica), a member of the Combretaceae family, is a woody climbing shrub widely distributed in tropical regions **[18].** Various parts of the plant are traditionally used for treating intestinal worms, fever, skin diseases, and gastrointestinal disorders **[3]**. Phytochemical studies reveal the presence of alkaloids, flavonoids, tannins, glycosides, and phenolic compounds responsible for its therapeutic properties **[4]**.

Anthelmintic Activity

Helminth infections are common parasitic diseases, especially in tropical countries. Plant-derived compounds are widely investigated as alternative anthelmintic agents **[5]**. Extracts of *Combretum indicum* have shown anthelmintic activity against worms such as *Pheretima posthuma*, likely due to the presence of tannins and alkaloids that interfere with parasite metabolism **[6,19]**.

Anticancer Activity

Cancer remains a major global health problem, and natural products continue to be an important source of anticancer drugs **[7]**. Phytochemicals such as flavonoids and phenolic compounds present in *Combretum indicum* exhibit cytotoxic and antioxidant activities, suggesting potential anticancer effects **[8]**.

Herbal Syrup Formulation

Herbal syrups are commonly used oral formulations because they are palatable, stable, and easy to administer. They typically consist of a sucrose base containing plant extracts and are evaluated for physicochemical parameters such as pH, viscosity, and stability to ensure quality and therapeutic effectiveness **[9,10]**.

Medicinal Uses

Combretum indicum (syn. Quisqualis indica) is widely used in traditional medicine for the management of several ailments. The fruits and seeds possess **anthelmintic properties** and are traditionally used to treat intestinal parasites such as *Ascaris* species. Decoctions of the fruits and seeds are commonly administered as vermifuges and are also used in the treatment of nephritis. The seeds are sometimes given to children to control diarrhoea, while seeds macerated in oil are applied externally for parasitic skin diseases, boils and sores. Root decoctions are used as vermifuges and for rheumatism, whereas the leaf juice is applied for boils, ulcers and headache associated with fever. In traditional Philippine medicine, the plant is also used as a pectoral remedy, and in certain traditional practices it has been reported to be consumed as a method of birth control **[3,13,14,15,16]**.

MATERIALS AND METHODS

Plant Material Collection and Authentication

Fresh leaves of Combretum indicum were collected from the campus of Holy Grace Academy of Pharmacy, Kerala, India. The plant material was authenticated by a qualified botanist at St. Thomas College, Thrissur. A herbarium specimen was prepared and preserved for future reference following standard pharmacognostic procedures **[1]**.

Preparation of Plant Extract

The collected leaves were washed, shade dried, and powdered using a mechanical grinder. The powdered material was subjected to Soxhlet extraction using ethanol as the solvent. The extract was concentrated using a rotary evaporator and stored in airtight containers for further studies **[2]**.

Pharmacognostic Evaluation

Pharmacognostic studies including organoleptic evaluation (colour, odour, taste, and texture), macroscopic examination, and microscopic analysis were performed to confirm the identity and purity of the plant material according to standard pharmacognostic methods **[1,2]**.

Preliminary Phytochemical Screening

Qualitative phytochemical analysis of the plant extract was carried out to identify the presence of major secondary metabolites such as alkaloids, flavonoids, tannins, glycosides, saponins, carbohydrates, and phenolic compounds using standard procedures **[4]**.

Preparation of Herbal Syrup

A herbal syrup formulation containing the plant extract was prepared using sucrose as the base. The extract was incorporated into the syrup base with suitable flavoring agents and preservatives to obtain a uniform and palatable formulation **[9]**.

Evaluation of Herbal Syrup

The prepared formulation was evaluated for physicochemical parameters including colour, taste, pH, viscosity, and stability using standard pharmaceutical evaluation techniques **[9,17]**.

Anthelmintic Activity

Anthelmintic activity was evaluated using Indian earthworms (*Pheretima posthuma*), which are commonly used as experimental models for intestinal parasites. Different concentrations of the plant extract and herbal syrup were tested and compared with a standard drug. The time taken for paralysis and death of worms was recorded to determine the anthelmintic activity **[6]**.

Anticancer Activity

The anticancer activity of the plant extract was evaluated using Dalton's Lymphoma Ascites (DLA) cell line. The cytotoxic activity was assessed using the trypan blue exclusion method, where the percentage of viable and non-viable cells was determined after treatment with different concentrations of the extract **[11]**.

Statistical Analysis

All experimental results were expressed as mean ± standard deviation (SD). Statistical significance between groups was evaluated using appropriate statistical tests, and a value of $p < 0.05$ was considered statistically significant **[12]**.

RESULTS

Pharmacognososstic Evaluation

The pharmacognostic analysis of Combretum indicum revealed characteristic morphological and microscopic features useful for plant identification and authentication.

Phytochemical Screening

Preliminary phytochemical screening of the plant extract indicated the presence of several important secondary metabolites including alkaloids, flavonoids, tannins, glycosides, saponins, steroids, carbohydrates, proteins and phenolic compounds. These phytoconstituents are known to contribute

to various biological activities and therapeutic effects of medicinal plants.

Table 01: Phytochemical Components

COMPONENTS	Combretum indicum
Proteins	+
Carbohydrate	+
Tannins	+
Flavonoids	+
Alkaloids	+
Triterpenoids	+
Phenols	+
Saponins	-
Ellagic acids	-
Resin	+

Anthelmintic Activity

The plant extract demonstrated significant anthelmintic activity, as indicated by reduced paralysis and death time of test worms when compared with the control group. The activity increased with increasing concentration of the extract, suggesting dose-dependent anthelmintic potential.

Table 02 :Anthelminthic Activity

STD	CONC	PARALYSIS TIME	DEATH TIME	TEST	CONC	PARALYSIS TIME	DEATH TIME
1	2mg/ml	36 min	40 min	1	20mg/ml	36 min	1hr 6min
2	4mg/ml	31 min	35 min	3	60mg/ml	34 min	1hr 2min
3	6mg/ml	22 min	24 min	5	100mg/ml	33 min	1hr
4	8mg/ml	16 min	21 min				
5	10mg/ml	15 min	19 min				
NORMAL SALINE	0.09mg/ml	1hr 6min					
PLANE WATER		1hr 10min					

Anticancer Activity

In vitro cytotoxicity evaluation showed that the plant extract exhibited **moderate anticancer activity**, reducing the viability of cultured cancer cells in comparison with untreated controls. The observed activity may be attributed to the presence of bioactive phytochemicals such as flavonoids and phenolic compounds.

Table 03 :Table Containing DLA Cell line

Amala Cancer Research Centre Society
(A Society Registered T.C. Act, XII of 1955 SI. No. 56 of 1984)
Amala Nagar- 680 555, Thrissur, Kerala, India

Ref.No. ACRC/CYTO(9)/196/2024 Date: 30.05.2024

***IN VITRO* CYTOTOXICITY RESULTS**

The test compound was studied for short term *in vitro* cytotoxicity using Dalton's Lymphoma Ascites cells (DLA). The sample (16ABC) was dissolved in DMSO (1ml) and concentration range between 12.5-200µg/ml was used for the study.

The tumour cells aspirated from the peritoneal cavity of tumour bearing mice were washed thrice with PBS or normal cell line. Cell viability was determined by trypan blue exclusion method. Viable cells suspension ($1X10^6$ cells in 0.1ml) was added to tubes containing various concentrations of the test compounds and the volume was made up to 1ml using phosphate buffered cell line (PBS). Control tube contained only cell suspension. These assay mixture were incubated for 3 hour at 37 C. Further cell suspension was mixed with 0.1ml of 1% trypan blue and kept for 2-3 minutes and loaded on a haemocytometer. Dead cells take up the blue colour of trypan blue while live cells do not take up the dye. The number of stained and unstained cells was counted separately.

$$\% \text{ cytotoxicity} = \frac{\text{No. of dead cells}}{\text{No. of live cells} + \text{No. of dead cells}} \times 100$$

Drug concentration (µg/ml)	% Cell Death
	16ABC
12.5	7.8±1.2
25	11.7±1.6
50	18.21.9
100	35.3±1.5
150	41.7±1.4
200	55.8±3.1

Control tube contains 5 dead cells

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DLA CELL LINE						
	16ABC					
Drug con (µg/ml)						
200	1	2	3	4	AVG	SD
Live cells	48	42	47	50		
Dead cells	59	64	55	58		
Total no. of cells	107	106	102	108		
% cytotoxicity	55.1	60	54	54	55.8	3.1
150						
Live cells	64	65	62	66		
Dead cells	45	44	48	47		
Total no. of cells	109	109	110	113		
% cytotoxicity	41.3	40	44	42	41.7	1.4
100						
Live cells	72	70	74	67		
Dead cells	39	37	38	40		
Total no. of cells	111	107	112	107		
% cytotoxicity	35.1	35	34	37	35.3	1.5
50						
Live cells	87	90	89	84		
Dead cells	21	19	20	18		
Total no. of cells	108	109	109	102		
% cytotoxicity	19.4	17	18	18	18.2	0.9
25						
Live cells	97	102	98	102		
Dead cells	15	11	14	13		
Total no. of cells	112	113	112	115		
% cytotoxicity	13.4	9.7	13	11	11.7	1.6
12.5						
Live cells	107	105	104	104		
Dead cells	9	7	10	10		
Total no. of cells	116	112	114	114		
% cytotoxicity	7.76	6.3	8.8	8.8	7.89	1.2

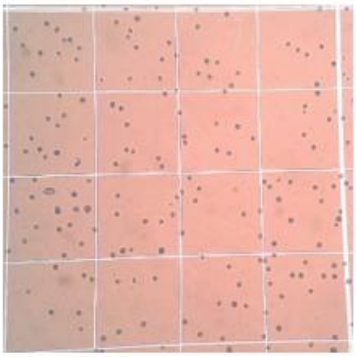

Figure1: 100% Dead Control

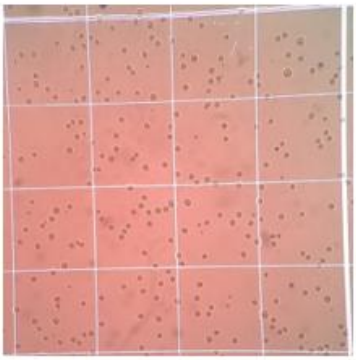

Figure 2:100% Live Control

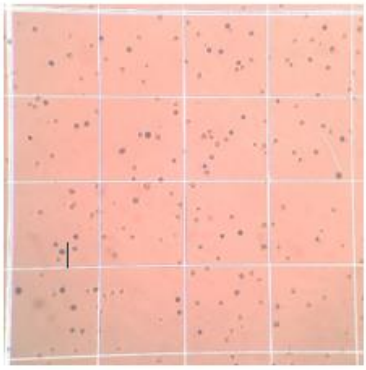

Figure 3: DLA 41%

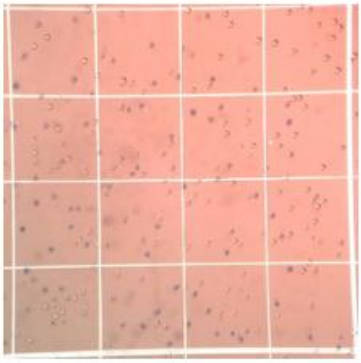

Figure 4: DLA 55.8%

Herbal Syrup Evaluation

The formulated herbal syrup prepared from the plant extract showed acceptable physicochemical properties, including suitable pH, viscosity, color, taste and stability. The formulation remained stable during the observation period and was found suitable for oral administration

DISCUSSION

The present study demonstrates the pharmacognostic standardization, phytochemical evaluation, and biological activities of Combretum indicum. The pharmacognostic analysis confirmed the identity and purity of the plant material through characteristic morphological and microscopic features, which are essential parameters for quality control of crude drugs.

Preliminary phytochemical screening revealed the presence of various secondary metabolites such as alkaloids, flavonoids, tannins, glycosides, and phenolic compounds. These constituents are known to contribute significantly to the biological activities of medicinal plants. Flavonoids and phenolic compounds are widely reported for their antioxidant and anticancer properties, while tannins and alkaloids are associated with anthelmintic activity.

The anthelmintic activity observed in this study showed a clear dose-dependent effect against Pheretima posthuma. The reduction in paralysis and death time of worms may be attributed to the presence of tannins, which can interfere with the energy metabolism of parasites or bind to proteins in the gastrointestinal tract, leading to death of the worms. Similar findings have been reported in earlier studies on plant-based anthelmintic agents.

The anticancer activity evaluated using the DLA cell line indicated moderate cytotoxic effects of the plant extract. The observed activity may be due to the presence of flavonoids and phenolic compounds, which are known to induce apoptosis and inhibit cell proliferation. Although the study provides preliminary evidence of anticancer potential, the exact mechanism of action remains unclear and requires further investigation using advanced techniques such as molecular assays and multiple cancer cell lines.

The formulated herbal syrup showed acceptable physicochemical properties, indicating its suitability for oral administration. The stability and palatability of the formulation further support its potential as a convenient dosage form.

Overall, the study provides scientific validation for the traditional use of Combretum indicum and highlights its potential as a source of bioactive compounds. However, further studies involving quantitative phytochemical analysis, in vivo models, and mechanistic evaluation are necessary to establish its therapeutic efficacy and safety.

CONCLUSION

The test compound was studied for short term invitro cytotoxicity in cancer cell line and for anthelmintic activity using *Pheretima posthuma* earthworm and it was concluded that the herbal formulation (syrup) was showing anti-cancer and anthelmintic properties.

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