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Phytochemical analysis and in vitro anthelmintic evaluation of Wild Cinchona (Neolamarckia cadamba) fruits

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ABSTRACT

Neolamarckia cadamba Roxb., a plant commonly used in Indian traditional medicines, has been recognized for its anthelmintic properties. This study aimed to evaluate the *in vitro* anthelmintic activity of *N. cadamba* fruit extracts against *Ascaridia galli* (roundworm), *Raillietina spiralis* (tapeworm), and *Pheretima posthuma* (Indian adult earthworm). The hydro-methanolic extract of *N. cadamba* fruits exhibited superior anthelmintic activity compared to the ethyl acetate extract. Phytochemical analysis of the extracts revealed the presence of phenols, tannins, saponins, glycosides, phytosterols, flavonoids, and terpenoids. Furthermore, the contents of phenolics, flavonoids, proanthocyanidins, and anthocyanins in the fruit extracts were quantified. The quantitative analysis of total phenolic content of extracts revealved that hydromethanolic extract contains higher quantity of phenolics, flavonoids, proanthocyanidins, and anthocyanins in comparision to the ethylacetate extract. These findings support the observation that hydro-methanolic extract of *N. cadamba* fruits exhibited superior anthelmintic activity compared to the ethyl acetate extract because polyphenolic compounds are well established for their anthelmintic activity.

Keywords: *Neolamarckia cadamba*, phytochemical investigation, anthelmintic evaluation, anthelmintic mechanisms

INTRODUCTION

The plant *Neolamarckia cadamba* (Roxb.) Syn. *Anthocephalus cadamba* (Roxb.) (Hindi name: Kadam, Kadamb; English name: Wild cinchona; Family: Rubiaceae) is a component in the Indian system of traditional medicine. Indian subcontinent is rich in diversity of flora, which has been used in several traditional medicinal preparations since ancient time. *N. cadamba* (Roxb.) is among them and even cited in Indian hindu religion and mythologies. The Indian tribals and villagers have been using the bark, leaf, flower and fruit of *N. cadamba* (Roxb.) in various traditional medicinal preparations for treating various ailments including infections, uterine disorders, skin diseases, inflammation, diarrhea, anemia, leprosy, dysentery, stomatitis, diabetes, anemia, sour throat, fever and cough [1-5]. *N. cadamba* has been placed among the useful tropical plants [6].

Various research reports are available on phytochemical investigation and biological evaluation of bark, roots and leaves of *N. cadamba* but few attempts were constructed to explore the phytochemicals and biological potential of *N. cadamba* fruits [7, 8]. The medicinal activities of *N. cadamba* have been supported by previous studies. One study conducted by Dhingra et al. reported the antioxidant and hepatoprotective effects of *N. cadamba* bark extract [9]. Another study by Rajesh *et al*. demonstrated the antimicrobial and wound-healing properties of *N. cadamba* leaf extracts [10]. Furthermore, Sharma et al. and others investigated the anti-inflammatory and analgesic activities of *N. cadamba* stem bark extract [11]. In continuation of our research efforts on anthelmintics [12-13], we planned to perform the phytochemical investigation and *in vitro* anthelmintic evaluation of the extracts of Wild Cinchona (*N. cadamba* Roxb.) fruits.

To study the anthelmintic activity worms choosen were *Ascaridia galli (roundworm), Raillietina spiralis (tapeworm), and Pheretima posthuma (Indian adult earthworm). Raillietina spiralis*, commonly known as tapeworm, is a parasitic helminth that infects the digestive tract of various avian species, including chickens, pigeons, and ducks. The tapeworm causes several pathogenic effects on its host like intestinal damage causing damage to the intestinal lining, causing inflammation, ulceration, and erosion of the mucosal surfaces; nutritional defeciencies because it consumes the host's ingested nutrients, particularly vitamins, minerals, and proteins; impaired digestive functions; anemia and secondary infections [14]. *Ascaridia galli*, commonly known as roundworm, is a parasitic helminth that infects the digestive tract of various avian species, including chickens. The roundworm causes several pathogenic effects on its host like intestinal blockage & damage, nutritional defeciencies and respiratory issues [15]. *Pheretima posthuma*, commonly known as the Indian adult earthworm, is a type of annelid worm found in the soil. While it is not typically considered a pathogen, there are certain pathogenic effects associated with *Pheretima posthuma* infection like indirect disease transmission [16].

EXPERIMENTAL

Reagents: Solvents (HPLC grade) were purchased from Merck KGaA, Darmstadt, Germany and S D Fine-Chem Limited, India. Piperazine citrate (Medicine Grade) was purchased from CDH India.

Collection of N. cadamba fruits: The fruits of *N. cadamba* were collected from Guntur, A.P., India. The *N. cadamba* fruits were identified by Dr. Pramod Kumar, Dept. of Pharmacognosy, H.N.B. Garhwal University, Uttarakhand, India (Accession No. APPS/2019/88). The collected fruits were divided into small green fruits (immature) and pale yellow to slightly orange fruits (ripe) according to their level of maturity. The ripe *N. cadamba* fruit was reported to have higher phenolic contents and therefore used for extraction purpose.

Production of fruit extract: The fruits of *N. cadamba* were cleaned and sever into small pieces. These small pieces were dried by air and size reduced to a gritty powder. 25 g of *N. cadamba* powder was extracted with methanol:water (4:1, v:v) at room temperature for 72 hours. Another 25 g of powdered fruits of *N. cadamba* was extracted with ethyl acetate at room temperature for 72 hours. These extracts were allowed to pass through filter paper (Whatman® Grade 1). The filtrates were concentrated on under reduced pressure at 40 °C for 2h.

Fig. 1. Fruit of *Neolamarckia cadamba*

The crude extracts were dried under vacuum (25 mm Hg at 28 °C) and kept in a desiccator. The practical yield of hydromethanolic crude extract and ethylacetate crude extract was 4.8 g and 2.4 g, respectively.

Phytochemical screening: The fruit extracts of *N. cadamba* were qualitatively examined for existence of phytochemicals by using the given reagents and test: phenols with Ferric chloride; alkaloids with Hager's, Wagner's, Mayer's; terpenoids with Salkowski's; saponins with Frothing; glycosides with Legal's, Modified Borntrager's; phytosterols with Libermann Burchard's; fat and fixed oils with Stain test, water-acetone; tannins with Gelatin; amino acids and proteins with Xanthoproteic; flavonoids with Alkaline reagent, Lead acetate; carbohydrates with Fehling's, Benedict's, Molisch's reagent.

Estimation of secondary metabolites total phenolic content: The total contents of phenolics, flavonoids, proanthocynidins and anthocyanins in the extracts were successively determined by Folin-Ciocalteu method [17], spectrophotometric method [18], Vanillin-HCl method [19], and ammonia HCl test [20].

Worms used: The *in-vitro* anthelmintic efficacy of the extracts was evaluated by utilizing the roundworm (*Ascaridia galli*), tapeworms (*Raillietina spiralis*) and earthworms (*Pheretima posthuma*). *Raillietina* species and *Ascaridia galli* were suggested appropriate animals for evaluation of anthelmintic drugs [21]. Tapeworms and roundworms were isolated from the infested intestine of freshly slaughtered fowls and kept in normal saline. Earthworm were gathered from a nearby moist location and cleaned with regular saline. Earthworm resembles the human intestinal roundworm (*Ascaris lumbricoids*) in both anatomy and physiology [22] and therefore used for anthelmintic evaluation. The round worm, earthworm and tapeworm of size ranging 6-8, 7-9, and 6-8 cm respectively were used. Identification of helminths and earthworm was carried out in Dept. of Pharmacology, Agra Public Pharmacy College, Agra, India.

***Anthelmintic activity*:** The *in vitro* anthelmintic efficacy of the extracts was evaluated following the reported procedure [23]. Four test samples of concentration 25 mg/ml, 50 mg/ml, 75 mg/ml and 100 mg/ml prepared in distilled water for hydro-methanolic and ethyl acetate extract each of *N. cadamba* fruits were used. Six worms viz. *Ascaridia galli, Raillietina spiralis* and *Pheretima posthuma* of recommended shape and size were kept in separate petri dish, prefilled with 25 mL of extract to be tested. Piperazine citrate (10 mg/mL) served as the reference and distilled water served as the control. All tested animals were subjected to the same procedure. Standard drug and test solutions were freshly prepared and used in experiments. The length of time required for paralysis, when there was no movement other than when the worms were shaken forcefully. The length of time required for death was determined when the worms did not move after they were shaken forcefully or submerged in hot water (50 ºC). The data were given as mean ± SEM, with n equal to 6.

RESULTS AND DISCUSSIONS

Phytochemical investigation of N. cadamba fruits: The hydro-methanolic and ethyl acetate extracts of fruits of *N. cadamba* were tested for the existence of phytosterols, terpenoids, saponins, tannins, flavonoids and glycosides (Table 1).

Estimation of secondary metabolites: The quantitative analysis of the major class of compounds in the extracts were carried out. The results were shown in Table 2.

Table 1. Qualitative analysis of the extracts of *N.cadamba* fruits

Phytoconstituents	Test	Ethyl acetate extract	Hydro methanolic extract
Alkaloids	Wagner’ s Hager’ s Mayer’ s	- - -	- - -
Terpenoids	Salkowski.s	+	+
Carbohydrate	Molischs Benedict’s Fehling’s	- - -	+ + +
Glycoside	Legal’s Modified Borntrager’s	+ +	+ +
Flavonoids	Alkaline reagent Lead acetate	+ +	+ +
Phytosterols	Libermann Burchard’s	+	+
Saponins	Frothing	-	+
Phenols	Ferric chloride	+	+
Gums	Gums	-	-
Fats & Fixed Oils	Stain test	-	-
Proteins	Xanthoproteic	-	-
Tannins	Gelatin	+	+

Table 2. Secondary metabolites in extracts of *N cadamba* fruits

Secondary metabolites	Hydro-methanolic extract	Ethyl acetate extract
Total Phenolic content (mg GAE/g)	11.39±0.73	8.11±0.55
Total flavonoid content (mg QE/g)	9.10±0.79	7.03±0.65
Proanthocyanidins (mg CE/g)	28.11±0.47	17.05±0.79
Anthocyanin (mg QE/g)	16.03±0.57	13.03±0.77

Anthelminitic evaluation: Both, the hydro-methanolic and ethyl acetate extracts of *N. cadamba* fruits demonstrated notable anthelmintic potential compared to the standard drug. Hydro-methanolic extract showed higher anthelmintic activity than ethyl acetate extract. Each crude extract showed dose dependent anthelmintic action. Shortest time taken for paralysis (P, min) and death (D, min) were observed at concentration - 100 mg/mL. Standard drug, piperazine citrate also displayed dose dependent anthelmintic activity (Tables 3 and 4).

Phytochemical investigation of the fruits extracts of *N. cadamba* revealed existence of polyphenolic compounds as the major phytoconstituents. Moreover, the quantitative analysis of total phenolic content of extracts revealves that hydromethanolic extract contains higher quantity of phenolics, flavonoids, proanthocyanidins, and anthocyanins in comparision to the ethylacetate extract. In addition it was observed that anthelmintic activity shown by hydro-methanolic extract of fruits of *N. cadamba* was higher in comparision to ethylacetate extract. The higher content of phenolis observed in hydro-methanolic extract of *N. cadamba* fruits exhibited superior anthelmintic activity compared to the ethyl acetate extract because polyphenolic compounds are well established for their anthelmintic activity. Several reports indicated that polyphenolic compounds produce anthelmintic activity [24]. Even synthetic phenolic anthelmintics such as bithionol, oxyclozanide and niclosamide were documented to disrupt the helminth’s energy-producing process by

Table 3. *In-vitro* anthelmintic evaluation of hydro-methanolic extract of *N. cadamba* fruits (HMNC).

Groups	Conc. tested (mg/mL)	Time taken for paralysis (P, min) and death (D, min) of helminthes/worms					
		Pheretima posthuma (Earthworm)		*Ascaridia galli* (Roundworm)		*Raillietina spiralis* (Tapeworm)	
		P	D	P	D	P	D
Control (Distilled water)	-	x	x	x	x	x	x
HMNC	25	33.31 ± 0.76	48.93 ± 0.79	27.71 ± 1.11	56.88 ± 0.98	32.55 ± 1.23	63.18 ± 0.91
	50	22.11 ± 0.79	34.55 ± 0.51	19.55 ± 0.77	42.20 ± 0.67	27.17 ± 0.79	54.81 ± 0.77
	75	17.73 ± 0.91	29.33 ± 0.78	14.25 ± 0.96	30.18 ± 0.68	18.41 ± 0.77	37.51 ± 0.78
	100	09.11 ± 0.56	13.44 ± 0.76	07.17 ± 0.61	11.28 ± 1.11	11.23 ± 0.90	21.20 ± 0.79
Standard (Piperazine citrate)	10	16.13 ± 0.68	32.31 ± 0.88	12.11 ± 0.69	17.31 ± 0.76	25.07 ± 0.68	63.45± 0.91

Results were represented as Mean ± SEM (n=6). ‘x’ represents nil bioactivity within 24 hours of administration

Table 4. *In-vitro* anthelmintic evaluation of ethyl acetate extract of N. cadamba fruits (EANC)

Groups	Conc. tested (mg/mL)	Time taken for paralysis (P, min) and death (D, min) of helminthes/worms					
		Pheretima posthuma (Earthworm)		*Ascaridia galli* (Roundworm)		*Raillietina spiralis* (Tapeworm)	
		P	D	P	D	P	D
Control (Distilled water)	-	x	x	x	x	x	x
EANC	25	41.11 ± 1.11	56.34 ± 0.98	32.19 ± 1.53	55.18 ± 0.78	41.33 ± 1.11	65.16 ± 0.79
	50	29.31 ± 0.69	41.78 ± 1.13	27.11 ± 0.91	51.23 ± 0.97	37.17 ± 1.53	57.18 ± 0.57
	75	21.71 ± 1.11	33.98 ± 0.68	17.98 ± 0.76	33.67 ± 0.88	26.11 ± 1.53	46.55 ± 1.17
	100	17.23 ± 1.13	23.53 ±1.23	13.11 ± 0.69	21.53 ± 1.53	21.11 ± 1.23	37.56 ± 0.77
Standard (Piperazine citrate)	10	16.13 ± 0.68	32.31 ± 0.88	12.11 ± 0.69	17.31 ± 0.76	25.07 ± 0.68	63.45± 0.91

Results were represented as Mean ± SEM (n=6). 'x' represents nil bioactivity within 24 hours of administration

decoupling of electron transport-linked phosphorylation [25]. It was suggested that polyphenolic compounds present in *N. cadamba* fruits exhibited similar actions and produced anthelmintic activity. Cell membrane permeation, cell lysis and enzyme inhibition may contribute in anthelmintic action. Further, binding of polyphenolics with free proteins in alimentary canal of infested animal [26] or glycoproteins present on cuticle of the helminth [27] also add to the anthelmintic action.

CONCLUSIONS

In the hydro-methanolic and ethyl acetate extracts of *N. cadamba* fruits, various classes of natural compounds were detected. Polyphenolic compounds were the major components. Both extracts showed anthelmintic activity in a dose dependent manner. Further investigations can focus on isolating and characterizing specific bioactive compounds from *N. cadamba* for potential therapeutic applications in helminthic infections.

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