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RESEARCH ARTICLE

Phytochemical Screening and Comparative Anthelmintic Activity of *Psidium guajava* L. Leaves extract against *Pheretima posthuma*

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ABSTRACT

Objective: *Psidium guajava* L. (Family: Myrtaceae.), commonly known as Guava, is a well-known traditional medicinal plant used in treatment of vomiting, diarrhea, dysentery, flatulence, gastroenteritis, abdominal distention and hyperglycemia. The present study was carried out to evaluate Phytochemical and Comparative *in vitro* anthelmintic activity of *P. guajava* leave extracts against Indian adult earthworm *Pheretima posthuma*. **Methods:** The traditional medicinal plant is used to control gastrointestinal infections. The phytochemical analysis and anthelmintic effects of extracts (Chloform, aqueous & methanol) and the standard drug (Piperazine citrate) were tested against Indian adult earthworm. The time taken to paralyze and death time of earthworm was considered for anthelmintic effects. **Results:** The phytochemical analysis revealed the presence of flavanoids, glycosides, alkaloids, steroids, and tannins. Methanolic extract of *P. guajava* leave extract was showed significant anthelmintic activity among all extracts. Methanolic extract have been found the highest rate of paralysis (1.820±0.242 min) and death (5.573±0.315 min) of earthworm against the standard drug Piperazine citrate. Conclusion: The wormicidal activity of *P. guajava* leaves extract suggests that it could be effective against parasitic infections of humans and validate the traditional use of *P. guajava* as a natural therapy for treatment of anthelmintic infection.

Keywords: Anthelmintic activity; Psidium guajava; Earthworm; Pheretima posthuma; Ethnomedicine

INTRODUCTION

Helminthic diseases are endemic across the globe causing gastro-intestinal infection in humans. Public health is greatly threatened in tropical and temperate regions, due to its moist and warm climate favoring survivability of parasitic eggs. Most of the chronic infections in human are due to Soil transmitted helminthes.[1,2] The transmission occurs though fomites or vectors in water lodging areas. Mostly unhygienic practices lead to transmission through various food items.[3] Several conditions like malnutrition, anemia, pneumonia and eosinophilia are being reported in the developing countries due to the prevailing infections, where India has not left behind so far.[4] By these, it causes huge economical losses, treatment costs and threat to public health, which recalls for prevention and control of the prevailing infections. Chemotherapy and chemoprophylaxis play a crucial role in this context. Several anthelmintic drugs are available that can either expel worms through faeces or kill the infecting helminths inside the body.[5] Due to rampant use of specific drugs, anthelmintic resistances have emerged out causing treatment failure and the parasitic control programs were limited to non-chemotherapeutic interventions like host selection, biological control and worm vaccines.[6] These led to introduction of ethnomedicine practices combating the drug resistance and treatment failure.

The traditional use plant *P. guajava* has been recognized since ancient era and its fruit is considered as poor man's apple. Presently many researchers have validated the anti-diarrhoeal, antiseptic, antibacterial and antioxidant properties of the plant extract.[7] The tree grows in tropical regions of the world i.e. Thailand, Brazil, West Indies, South America, India and Bangladesh.[8,9] According to IH Burkill, boiled water extract of *P. guajava* leaves and bark

are used in medicinal preparations, which were utilized as remedies for dysentery, diarrhea and upper respiratory tract infections in Florida, the West Indies and parts of South America.[9] In Malaysia, decoction of *P. guajava* leaf is used for stomachache and gastroenteritis.[10–12] Since the antidiarrheal property of *P. guajava* leave extract has been documented earlier, the present study was conducted to establish the anthelmintic property of it against earthworm "Pheretima posthuma".

MATERIAL AND METHODS

The present study was conducted during November To January where fresh leaves were collected in Raigarh district and earthworm in Raigarh, The extraction of leaves, Phytochemical study and anthelmintic activity and data analysis was done at Raigarh College of Pharmacy, Raigarh,C.G..

Collection of plant material

Fresh leaves of *P. guajava* were collected from Raigarh, Chhattisgarh (C.G.) in the month of November to January. The plant was identified and authenticated at Department of Botany, Gangadhar University, Sambalpur, Odisha, India. A voucher specimen has been preserved at Department of Pharmacy, Raigarh College of Pharmacy, Raigarh (C.G.) for future reference.

Extraction

The fresh and tender leaves of *P. guajava* were dried in a shade under room temperature and then crushed into coarse powder by using mechanical grinder (Bajaj electric mixer grinder, India). The coarse powder was again dried and passed through sieve no. 20 to get fine powder and stored in an airtight plastic bag until further required. Fifty gm of the *P. guajava* leave powder was weighed accurately and subjected to extraction in a Soxhlet apparatus at room temperature using with chloroform, methanol and aqueous as solvent. The crude extracts were filtered, and then concentrated to dryness in rotary flash evaporator (Eyela N-1001S-W, USA) at 45°C for 10h. Each crude extract was collected separately and stored in airtight containers and kept at room temperature for further studies. The percentage yield of the crude extract obtained [chloroform, (28.11%, w/w)], [methanol (56.23% w/w)], and [aqueous (62.34%, w/w)] were subjected to phytochemical analysis to find the presence of phyto-constituents.[13]

Selection of worm

Anthelmintic activity of the crude extracts was studied on earthworms *Pheretima posthuma* (*P. posthuma*, Annelida; Megascolecidae). The earthworms were collected from the water-logged areas of Raigarh, C.G. The identification and authentication of worms were done at Department of Pharmacology, Raigarh College of Pharmacy, Raigarh, Chhattisgarh. The collected earthworms were washed with normal saline solution to remove all the fecal matter. The earthworm *P. posthuma* having length of 4.3-5.1 cm and 0.2-0.3 cm in width and weighing about 0.9-3.09 g were used to screen Anthelmintic activity of crude extract. The collected *P. posthuma* earthworm was similar to intestinal roundworm parasites of human beings both anatomically and physiologically. Hence, the earthworm *P. posthuma* was used to study the anthelmintic activity for all the experimental protocol.[14]

Chemicals

Piperazine citrate (Mothercare, Raipur), chloroform and methanol solvents were purchased from Merck (Mumbai, India).

Phytochemical screening

The preliminary phytochemical screening of the chloroform, methanol and aqueous extract of *P. guajava* leaf was carried out as per the standard procedures for identification of primary chemical groups.[13–15]

Physical Evaluation of *P. guajava* leaves**:** The physical evaluation of *P. guajava* leaves was carried out by Ash values and Extractive values.[13] Ash value was a criterion to judge the identity and purity of leave and its extracts. Extractive value was significantly used to indicate the nature of chemical constituents and helps in the identification of adulterants.

Anthelmintic activity

The anthelmintic activity was carried as per the reported method[15] and was performed on Indian adult earthworm, *P. posthuma*. The collected earthworms were divided in to six groups containing six earthworms in each group. Extracts and standard solution (Piperazine citrate) were freshly prepared before performing the experiments. chloroform, aqueous & methanolic extracts of *P. Guajava* leaves and Piperazine citrate standard solution were poured into different petridishes. The test sample of all extracts (chloroform, aqueous & methanolic) containing in different concentrations (50, 100, 150,200 and 250 mg/mlin distilled water) were prepared. They were released in respective petri dishes. Observations were made for the time taken to paralyze (Paralysis was said to occur when the worm did not revive even in normal saline) and Death (Death was concluded when the worms lost their motility followed with their body colors fading away). The results are shown in Table 1.

Table 1: Phytochemical screening of *P. guajava* leave

Sl. No.	Test	Chloroform Extract	Aqueous Extract	Methanol Extract
1	Alka-loids	-	+	+
2	Carbo-hydrates	+	+	+
3	Amino acids	-	-	-
4	Proteins	-	-	-
5	Saponin	-	-	-
6	Glyco-sides	+	+	+
7	Tannins	+	+	+
8	Flavonoid	+	+	+

Note: '+'indicates presence of phyto-constituents,'-'Indicates absence of phytoconstituents

Statistical Analysis

Data of results are presented as mean± standard error of mean (SEM). The significant results were obtained when compared between standard versus treated group. Statistical analysis was performed by One-way analysis of variance (ANOVA) with Dunnett's post-hoc test by using Graph Pad Prism (version 3.00, San Diego California USA) Software for windows. The difference was considered to be statistically significance for P value $\leq$0.05.

RESULTS

The preliminary phytochemical investigation of the chloroform, aqueous and methanol extract of *P. guajava* leaves showed the presence of different phytoconstituents, and it has been described in Table 1.

The ash and extractive values of *P. guajava* leaves were recorded during the study. The total ash value (9.5%) indicates that the leave is comparatively rich in mineral elements. Acid insoluble and water-soluble ash values were 0.7% and 5.5% respectively (Table 2). The extractive values for chloroform, water and methanol were 0.25%, 7.0% and 9.1% respectively.

Table 2: Ash and extractive values of *P. guajava* leaves

Sl. No.	Physical constant		Leave (%)
1.	**Ash values**	Total ash value	9.5%
		Acid insoluble ash	0.7%
		Water soluble ash	5.5%
2.	**Extractive Values**	Methanol Soluble Extractive	9.1%
		Water Soluble Extractive	7.0%
		Chloroform Soluble Extractive	0.25%

The paralytic and wormicidal effect of different extracts and piperazine citrate against the earthworm were shown in the Table 3. The trend of both the effects was found to be higher in methanolic extract followed by water extract and less in chloroform extract. The methanol extract exhibited an increased paralytic as well as wormicide effect over Piperazine citrate at the given experimental concentrations.

Table 3: Anthelmintic activity of *P. guajava* leaves

Treatment	Concentra-tion (mg/ml)	Time takenfor paralysis (min)	Time takenfor death (min)
Control (Normal saline)	-	-	-
Chloroform	50	15.33±0.33	24.833±0.441
	100	12.167±0.291	20.167±0.328
	150	10.90±0.231	15.467±0.233
	200	8.733±0.203	11.676±0.145
	250	5.533±0.203	9.567±0.291
Water extract	50	14.467±0.260	21.4±0.173
	100	11.400±0.265	17.13±0.186
	150	9.800±0.153	13.5±0.289
	200	11.400±0.267	11.567±0.296
	250	3.633±0.233	7.033±0.240
Methanolic extract	50	12.33±0.491	20.467±0.441
	100	10.833±0.203	15.867±0.233
	150	7.967±0.120	11.983±0.164
	200	5.373±0.380	9.573±0.288
	250	1.820±0.242	5.573±0.315
Piperazine citrate (Standard)	10	21.333±0.524	20.300±0.462
	20	14.600±0.265	17.200±0.173
	30	9.570±0.290	12.253±0.163
	40	5.447±0.128	9.380±0.079
	50	2.567±0.093	6.800±0.153

DISCUSSION

The anthelmintic property of methanol extract of P. guavaja leaves was found to be more effective than other extracts and Piperazine. The phytochemical screening of methanolic extracts revealed the presence of flavonoids, glycosides, alkaloids, steroids and tannins, while amino acids, proteins & saponins were absent. Tannins are one of the main constituents that produce anthelmintic activity.[16,17] The ash values and extractive values are useful for checking of impurities present in a particular extract. Physico-chemical parameters of P. guavaja were investigated and were found to be in the permissible limit which indicated that the prepared plant extracts were of better quality. During comparative in vitro study of anthelmintic activity of *P. guajava* leaves extract against Indian adult earthworm *P.*

posthuman, the methanolic extract showed a significantly ($p \leq 0.05$) highest anthelmintic activity than that of aqueous extract, chloroform extract and Piperazine citrate. This may be due to the increased level of extraction of tannins in methanol followed by water and less in chloroform extracts. The methanolic extracts have been found to possess highest rate of paralytic (1.820±0.242 min) and death (5.573±0.315 min) of earthworm against the standard drug Piperazine citrate and other extracts. Chemically, tannins are polyphenolic compounds and some synthetic phenolic anthelmintic drugs (eg. Niclosamide & Oxyozanide)[18] interfere with energy generation in helminthes by uncoupling oxidative phosphorylation, it is possible that tannins contained in the extracts of *P. guajava* produced similar effects. Another possible anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein of the parasite and cause death. The results in this study can be corroborated to the different level of tannins present in different extracts, resulting into different degree of anthelmintic property. Our findings appraise the use ethnomedical practices, which can be an effort to combat the anthelmintic drug resistances.

CONCLUSION

The wormicidal activity of leaves extract of *P. guajava* suggests that it is effective against parasitic infections of humans. Further, in future it is necessary to identify and isolate the possible active phytoconstituents responsible for the anthelmintic activity and study its pharmacological actions.

CONFLICT OF INTEREST

All authors declare that there exists no conflict of interest.

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