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Research Article

Anthelmintic Activity of Alpha Amyrin of Monkey Jack from Western Ghats

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ABSTRACT

The petroleum ether, chloroform, ethanol extracts and pure compound of alpha amyrin from fruits of *Artocarpus gomezianus* was examined for anthelmintic efficacy utilizing earthworm (*Pheretima posthuma*). Diverse amounts (25–100 mg/mL) of fruit extraction were evaluated within the bioassay. Albendazole (25 mg/mL) served as the reference criteria medicine, while purified water functioned as the regulator. The paralysis and mortality times of the worms were documented. Extracts showed considerable anthelmintic efficacy at higher concentrations of 25 mg/mL. The extracts of petroleum ether, chloroform, and ethanol showed a dose-dependent anthelmintic effect in comparison to the conventional medication. The petroleum ether extract exhibited paralytic effects, with the time of death for *Pheretima posthuma* at doses of 25, 50, and 100 mg/mL resulting in 48.33 ± 0.62, 37.72 ± 0.55, and 27.87 ± 1.50 minutes, respectively. The corresponding times of death were 60.46 ± 0.43, 60.90 ± 0.77, and 60.31 ± 0.16 minutes, respectively. The chloroform extract demonstrated paralyzing effects and durations of death for *P. posthuma* at the dosage concentrations of 25, 50, and 100 mg/mL, which were identified to be 62.22 ± 1.93, 48.01 ± 0.82, and 37.69 ± 0.74 minutes, and 122.73 ± 2.08, 81.05 ± 0.87, and 60.85 ± 0.87 minutes, respectively. The ethanol extract exhibited paralytic effects and death time of *P. posthuma* with dose of 25, 50 and 100 mg/mL were found 56.08 ± 1.24, 47.94 ± 1.10, and 44.03 ± 0.89 and 120.48 ± 0.05, 90.42 ± 0.02 and 80.27 ± 0.85 minutes, respectively. The observation with standard albendazole showed that time of paralysis and death was 60.40 ± 0.02 and 120.40 ± 0.02 minutes, respectively. The α-amyrin showed an immobilizing duration of *Pheretima posthuma*. The average paralyzing duration of *P. posthuma* with a dose of 25, 50 and 100 mg/mL were ascertained to be 35.18 ± 0.12, 30.18 ± 0.15 and 20.17 ± 0.08 minutes, respectively. In the meantime, albendazole administered at a concentration of 25 mg/mL induced paralysis in the aforementioned helminthin 60.40 ± 0.02 minutes. In the average duration, albendazole at a dosage of 25 mg/mL resulted in a mortality time for the aforementioned worm. 120.40 ± 0.02 minutes. The α-amyrin showed more anthelmintic efficacy as compared to the conventional medication of albendazole.

Introduction

Research on natural compounds generated from plants aims to discover new medications by revealing novel pharmacological mechanisms. The bioactive components of plants are numerous. A significant number of plant species have been evaluated for their pharmacological and chemotherapeutic attributes, although a considerable array of edible medicinal plants remains to be utilized for their nutritional and therapeutic benefits.[1] Helminthiasis is a highly frequent illness and a significant public health concern globally. Globally, there are hundreds of millions of human infections caused by helminths, exacerbated by increased international travel and immigration from developing nations.[2] Helminths also impact millions of livestock, resulting in significant economic losses in both household and farm animals. An optimal anthelmintic should possess a wide range of efficacy. It should attain a substantial cure rate with a singular treatment dose. It must be devoid of toxicity to the host and economically viable. The plants are recognized as a substantial

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source of botanical anthelmintics.[3] Anthelmintics are pharmaceuticals that either eradicate fire or eliminate helminth infestations. The gastrointestinal tract harbors several helminths. Some also inhabit larvae of tissues that translocate into tissues. They detrimentally affect the host author by denying it of sustenance, inducing hemorrhage, inflicting organ damage, producing blockage of the intestinal or lymphatic systems, and secretion poisons. Helminthiasis is infrequently lethal; however, it significantly contributes to morbidity.[4] Many medicinal plants exhibit anthelmintic activity, meaning they can kill or expel intestinal parasites, such as worms. These plants often contain secondary metabolites, such as alkaloids, terpenoids, or polyphenols, that are responsible for their effects. The Eugenol, a component of Sacred basil (Tulsi), has shown potent anthelmintic activity against nematodes. Studies on plants like *Ficus glabrata* and *Turraeavogelii* have also demonstrated their effectiveness against various helminth species. [5]

Artocarpus gomezianus Wall. ex Trécul, usually referred to as Monkey jack, is an underutilized edible medicinal plant native to the Western Ghats of Karnataka and belongs to the Moraceae family. It is extensively dispersed throughout the Indian subcontinent and Southeast Asia. The fruit of the monkey jack, commonly referred to as lakoocha, is edible and found throughout the Western Ghats, a biodiversity hotspot. The fruits are utilized as a substitute for tamarind by local inhabitants. The various components of the plant are utilized in the formulation of herbal remedies. The bark, when applied externally, extracts purulent materials and facilitates the healing of boils, fissured skin, and pimples. Seeds possess purgative and hemagglutinating properties. The stem possesses vermifuge properties. The stem bark contains oxyresveratrol, which is used for treating tapeworm infections in Thailand. The lakoocha fruits are often consumed fresh. The consumable fruit pulp is thought to function as a liver tonic. The unprocessed fruits and male inflorescences are employed in pickles and chutneys. Pug-Haad, a brown powder in Thailand, is derived from the aqueous extraction of monkey jack, achieved by boiling wood chips and subsequently evaporating the water. This preparation has been completed and utilized as a conventional anthelmintic agent for the management of cestode infestation infections in Thailand. The wood is a valuable timber and the leaves are used as fodder. Attempts are made to study the anxiogenic, antibacterial, antioxidant, anthelmintic, and insecticidal efficacy of methanol extracts from fruit parts and leaves on mice, as well as the screening of selected biological activities of fruit pericarp extracts. The quantitative analysis of oxyresveratrol, the isolation and structural elucidation of 2-aryl benzofuran, and the antioxidant and toxicity activities of heartwood extract have been conducted. Lakoochins A and B, novel antimycobacterial stilbene compounds, were obtained after purification of a CH_2Cl_2 extract of roots of *A. gomezianus.* The phenolics with antiviral activity compounds are also obtained from *Erythrocalyx* and *A. gomezianus.*[1,6] The fruits comprise anti-inflammatory, antiviral, anticancer, antihelmintic activity and antiretroviral activity. The mature and differently altered walls of a plant ovary extract exhibit bactericidal and antioxidant effects that depend on dosage, antihelminthic, and insecticidal properties. The sap and bark of this plant have a crucial function in liver illness. This plant antioxidant plays a significant function in protecting against coronary heart disease and maintaining overall health. The World Health Organization assesses that around 80% of the worldwide populace is affected by conditions such as diabetes, cancer, coronary cardiovascular disease and neurodegenerative issues in the context of basic healthcare. Cytotoxic characteristics have been identified in medicinal plants that are utilized as therapeutic agents.[7] The number of investigators reported the anthelmintic activity of *Artocarpus lakoocha* using different extracts.[8-12] Hence, an attempt has been made to investigate the anthelmintic activity of crude petroleum ether, chloroform and ethanol extracts of unripe fruits of *A. gomezianus*. In addition, an active compound has also been isolated and characterized, with its structural elucidation and anthelmintic efficacy established.

Materials And Methods

The fruits of Monkey Jack were gathered from the Western Ghats at Banajalaya (lat.14.667 ^{0}N, long.75.0333 ^{0}E) located at an altitude 579 m. The voucher specimen (KUAB/AL – 3) was submitted at the "Department of Applied Botany, Kuvempu University Shankaraghatta".[1]

Preparation of Extracts

The shade-dried fruit substance was pulverized using a mixer grinder and underwent soxhlet extraction using petroleum ether, chloroform, 95% ethanol, and purified water for a duration of 18 hours, following the sequence of solvents in order of increasing polarity as previously documented.[1] The concentrated extracts were utilized to investigate anthelmintic efficacy against the earthworm (*P. posthuma).*

Extraction and Isolation of α-amyrin

Air dried and powdered Monkey Jack fruits (2.5 kg) were successively extracted with petroleum ether, chloroform, ethanol and water (each 3 L /18h) at room temperature to obtain 2.5 g of extract each petroleum ether and chloroform; 5.0 g for ethanol; and 7.5 g for water respectively. The petroleum ether extract was initially exposed to Column chromatography utilizing silica gel with a mesh size of 60 to 120. (petroleum ether: ethyl acetate gradient) to give fractions labeled A-F. Of the above fractions, fraction B gave 500 mg of pure compound. This fraction B was dried and recrystallized from CH_2Cl_2. The

pure compound was subjected to chromatogram studies (IR, LCMS and NMR spectra).

An IR spectrum was obtained on a JASCO FT/IR-5300 spectrophotometer. Mass spectra were obtained on aLCMS-2010A DATA REPORT SHIMADZU. NMR spectra were recorded using a BRUKER spectrometer (RR347 1H CDC13 and RR347 13CDC13). Column chromatography (CC) was conducted using silica gel 60-120. (MERCK, MJ9M592402, 60–120 mesh), alugramsil G/$UV_{254\ nm}$ (MacheryNagl GmbH, Germany) and Aluminum plates measuring 20×20 cm were utilized. The spectral data was obtained along with details of structural elucidation.

Determination of Anthelmintic Activity — Worms

The Indian worm (*Pheretima posthuma*) (*Annelida*) was procured from the Horticultural Department at N. R. Pura, Chikamagalur, Karnataka (latitude 13.55° N and longitude 75.35° E). The typical length of an Indian earthworm ranges from 6 to 8 centimeters. They were cleansed with water to eliminate filth.

Chemicals

Albendazole, Double-distilled water and saline solution (6% dextrose).

The study was conducted on mature Indian earthworms because of their morphological and physiological similarities to the human intestinal nematode parasite. [12-19] Due to their readily accessible nature, earthworms have been extensively utilized for the preliminary assessment of anthelmintic chemicals. The worms were acclimated to the laboratory conditions prior to research. The earthworms are split into five categories, each containing six worms allocated into eight Petri dishes holding the extraction solutions or controls. Medications as detailed below.

Gr. —1: Double distilled water serving as the control.

Gr. —2: Albendazole liquid formulation dosed at 25 mg/mL to serve in accordance with the standard. and

Gr.—3-5 representing Petroleum ether/chloroform/ethanol extract at a specified dosages of 100, 50 and 25 mg/mL, respectively.

At the same time, the isolated and purified α-amyrin compound was also subjected to determine its anthelmintic activity at the same concentration, and the other conditions were kept constant, as followed in the unrefined extracts of petroleum ether and chloroform and ethanol extracts. All petri dishes had maintained at ambient temperature. The functional worms were monitored meticulously. Measurements were recorded for the duration required to achieve paralysis (PT) and mortality (DT) in singular annelids. Every worm was regularly subjected to extrinsic stimuli that provoke and elicit motion within living earthworms. Paralysis was observed when the worms failed to move even in an isotonic saline solution. Death is determined when the worms cease their movement, accompanied by the discoloration of the body. The immobile worms were subsequently relocated to 40°C to verify their mortality.

Results And Discussion

Structural Elucidation of α-amyrin

The compound is isolated as a white crystalline powder. The isolated compound is sent to Radiant Service Pvt. Ltd., Bombay, for IR, LCMS, NMR, and structural conformation analysis of the compound. Based on the spectral data obtained from the Radiant Service Pvt. Ltd., the isolated compound is identified as α-amyrin. The compound had a molecular weight of 426, evidenced by its mass spectral peak at m/z 409 [M-H2O+H]+. The presence of a band at 1738 cm^{-1} in the IR spectrum infers that there is a carbonyl group and the peak at 1648 cm^{-1} shows the existence of a double bond. On the basis of the ^{1}H-NMRspectrums (Nuclear Magnetic Resonance). Table 1 showed six singles for the angular methyl group at d 1.07, 1.01, 0.98, 0.91, 0.79 and 0.80, two doublets at 0.84 and 0.87, an acetoxy methane group at d 4.50, a three proton broad signal at d 2.30 and a vinyledene group proton at d 5.12 indicating the presence of a pentacyclic triterpenoid in Table1. These signals were supported by the ^{13}C-NMR (13 carbon nuclear magnetic resonance) by revealing the presence of acetoxy methane carbon at d 80.57, unsaturated carbon atoms at d 124.38 and 145.19 and a carbonyl carbon at d 173.49 in Table 2. Based on the spectral, the isolated chemical is identified as alpha amyrin in Fig. 1.

Anthelmintic Activity of Crude Extract

From the above examine it was seen that petroleum, chloroform and ethanol extracts demonstrated dose-dependent anthelmintic action in relation to the common medicine albendazole in Table 3 and Fig. 2. Based on these results, the crude petroleum ether extracts which showed

Table 1: ^{1}H-NMR of isolated and purified compound, α-amyrin of fruits of *A. gomezianus*

Proton	*Signal*
H-3	4.50 (m, 1H)
H-12	5.12 (t,)
CH_3CO	2.30
C-Me	1.07
	1.01
	0.98
	0.91
	0.79
	0.80
	0.84
	0.87

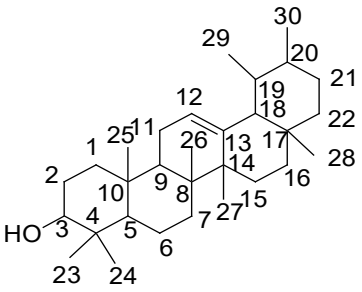

Fig. 1: Molecular structural of α-amyrin ($C_{30}H_{50}O$) isolated from mature unripe fruits of *A. gomezianus*

Table 2: ^{13}C-NMR of isolated and purified compound, α-amyrin of fruits of *A. gomezianus*

Carbon	*Signal*	*Carbon*	*Signal*
1	38.50	18	59.16
2	26.67	19	40.10
3	80.57	20	39.70
4	38.30	21	29.72
5	55.35	22	48.00
6	18.30	23	32.95
7	34.84	24	16.75
8	39.65	25	15.74
9	47.72	26	21.22
10	39.70	27	28.10
11	23.65	28	28.76
12	124.38	29	17.51
13	145.19	30	22.69
14	42.14	COO	173.49
15	28.16	CH_3CO	31.94
16	25.18		
17	36.85		

maximum activity was selected for isolation and structural elucidation of the compound. Accordingly, the bioactive compound α-amyrin was isolated, characterized, and its structure was also elucidated.

Anthelmintic Activity of α-amyrin

α-amyrin showed dose-dependent anthelmintic activity in comparison to the typical pharmaceutical albendazole in Table 3. The α-amyrin (25 mg/mL) demonstrated more anthelmintic activity as comparison to the criterion drug of albendazole in Fig. 3.

When the anthelmintic activity of crude extracts from petroleum ether, chloroform, and ethanol was compared with that of albendazole, a standard anthelmintic drug, all three crude extracts showed higher activity against helminthiasis. Furthermore, the increase in concentration of crude extracts also increased the anthelmintic activity also increased (Fig. 4). Therefore, all the crude extracts are anthelmintic and the extracts are arranged on the basis of anthelmintic activity as detailed below.

Petroleum ether > Chloroform > Ethanol

Similar to present study, Nirmal *et al.* [12] observed that the rhizome extracts of *Curcuma long* and *Zingiber officinale* showed anthelmintic activity accompanied by increased concentration of extract the anthelmintic activity is also increased. In addition, the combination of extracts showed synergetic effects of anthelmintic activity. The anthelmintic activity is due to phytochemical constituents, specifically alkaloids, saponins, flavonoids, terpenoids, and steroids etc. Sant *et al.* [13] observed that the ethanol extract of the flower of *Butea monosperma* exhibits greater potency than the aqueous extract, while both extracts possess notable anthelmintic properties. The anthelmintic efficacy is contingent upon the concentration of extracts. The efficacy of several extracts was determined to be inversely correlated with the duration required for the paralysis and demise of the worm. The study by Rabiu and Subhasish[15] demonstrated that the methanol extract of *Azadirachta indica* leaves displayed notable anthelmintic action at a dosage of 40 mg/mL. The duration for paralysis was 17 ± 0.32 minutes, while the duration for death was 30 ± 0.11 minutes. Ilango *et al.* [16] studied the anthelmintic efficacy of extracts from the aerial portions of *Tephrosia spinosa* was evaluated, revealing that both chloroform and methanol extracts demonstrated dose-dependent anthelmintic efficacy in relation to the standard medicine albendazole. The chloroform extract induced paralysis in the earthworm in the shortest duration (14.34 ± 0.0 minutes) and resulted in death (26.43 ± 0.3 minutes) more rapidly than the methanol extract, indicating either a larger quantity of anthelmintic compounds or a greater variety of such compounds in the chloroform extract. The study by Satyanarayana *et al.* [17] demonstrates that ethanolic extracts of *Annona squamosa* leaves have considerable anthelmintic activity in comparison to the usual medication, albendazole. They also found that *A. squamosa* leaf extracts of ethanolic demonstrated dose-dependent activity. Among the three extracts of ethyl acetate at a concentration of 200 mg/mL demonstrated a significantly elevated efficacy, eliminating the worm in 4.11 ± 0.09 minutes. The ethanolic extract shown substantial efficacy by eliminating the worm in 12.17 ± 0.16 minutes. The hexane extraction demonstrated slight substantial activity. The albendazole treatment criterion demonstrated worm mortality in 4.37 ± 0.66 minutes. The ethyl acetate extraction demonstrated efficacy comparable to that of the conventional medication albendazole. Therefore, the anthelmintic activity of the above three extracts of monkey jack and also a variation of anthelmintic activity in different extracts may be

Table 3: Anthelmintic activity of monkey jack (*A. gomezianus*) on different solvents (petroleum ether, chloroform and ethanol) extracts and alpha amyrin

Groups	*Concentration (mg/mL)*	*Pheretima posthuma (Indian earthworm)*	
		Paralyzing time (min)	*Death time (min)*
Distilled Water	-	-	-
Albendazole - standard	25	60.40 ± 0.02	120.40 ± 0.02
Petroleum ether extract	25	48.33 ± 0.62	60.46 ± 0.43
	50	37.72 ± 0.55	60.90 ± 0.77
	100	27.87 ± 1.50	60.31 ± 0.16
Chloroform extract	25	62.22 ± 1.93	122.73 ± 2.08
	50	48.01 ± 0.82	81.05 ± 0.68
	100	37.69 ± 0.74	60.85 ± 0.87
Ethanol extract	25	56.08 ± 1.24	120.48 ± 0.05
	50	47.94 ± 1.10	90.42 ± 0.02
	100	44.03 ± 0.89	80.27 ± 0.85
α-amyrin	25	35.18 ± 0.12	60.13 ± 0.04
	50	30.18 ± 0.15	52.18 ± 0.63
	100	20.17 ± 0.08	41.43 ± 1.08

attributed to the variation of phytochemical components and their variation. It is evident from the results that the phytochemical components of different extracts vary, as confirmed by the presence of variation in color spots along with different homologous restriction factor values for the different extracts. Tiuria *et al.* [18] studied the anthelmintic efficacy of *Phyllanthus niruri* Linn and demonstrated significant activity at a concentration of 1000 mg/mL. Significantly, the extracts of *P. niruri* at this concentration demonstrate potent anthelmintic action, compromising dissolving the cuticular layer of A. galli, hence impairing the locomotion of mature *A. galli*. These discoveries possess significant potential for the advancement of innovative anthelmintic therapies. Manaswini Dehuri cataloged indigenous medicinal plants with anthelmintic activity in both animals and humans. Due to the unfavorable economic conditions faced by livestock farmers and the high costs of anthelmintics, the common helminths in cattle can only be effectively addressed through the use of ethnoveterinary remedies. The integration of nanotechnology can also improve drug delivery and treatment outcomes. Omkar A. Devade stated that the extract of the entire plant of *Pisum sativum* was evaluated for anthelmintic action against Indian earthworms, in contrast to the conventional medicine albendazole. The concentrations of *P. sativum* extracts and albendazole were maintained at the same levels for comparison analysis.

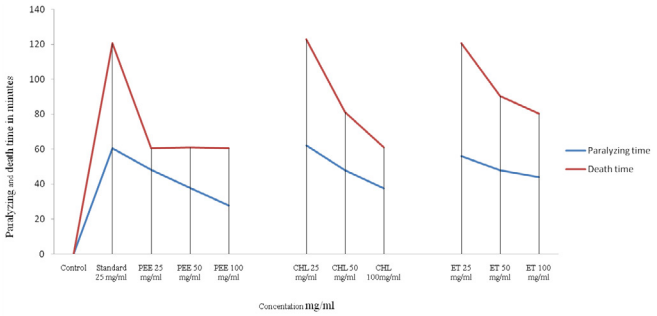

Fig. 2: *A. gomezianus* (Control: Distilled water, Standard drug: albendazole, PEE: Pet. ether extract, CHL: Chloroform extract, ET: Ethanol extract

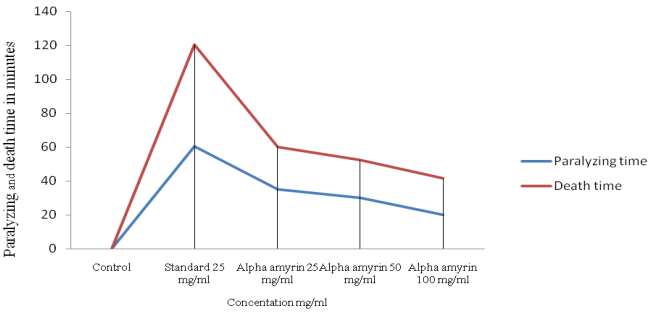

Fig. 3: Evaluation of anthelmintic efficacy between standard drug and α-amyrin

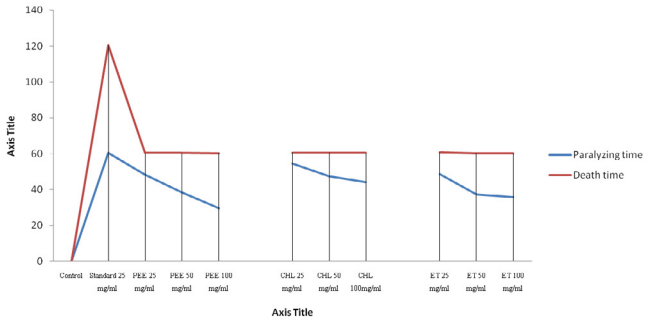

Fig. 4: Comparison of anthelmintic efficacy amidst the criterion medicine as well as crude extracts ofpetroleum ether, chloroform, and ethanol from immature fruit of Z. rugosa

Saline water served as the control. The anthelmintic efficacy was assessed by measuring the duration of paralysis and the time to death. Phytochemical analysis of plant extracts was conducted. The findings indicated that the anthelmintic efficacy of plant extracts was analogous to that of the reference medication, albendazole. Das *et al.* [19] conducted a study to assess the anthelmintic efficacy of ethanolic and aqueous extracts from the foliage and bark of *Tamarindus indica*, which demonstrated notable anthelmintic efficacy, as demonstrated by reduced paralysis and mortality times. The findings thus endorse the application of *T. indica* as an anthelmintic agent. Giash Uddin *et al.*[20] analyse the antioxidant, anti-inflammatory, anthelmintic, etc., activities of the methanolic extract of *Artocarpus lakoocha* foliage. Thrombolytic efficacy was

assessed utilizing *Perthima posthuma*. The antioxidant capacity was assessed by total phenolic content as well as DPPH free radical scavenging efficacy tests. The hypnotic solution and thermally induced hemolysis were assessed for anti-inflammatory efficacy. The methanolic extract of *A. lakoocha* exhibited considerable anthelmintic efficacy in a dose-dependent fashion, analogous to the conventional medication albendazole (10 mg/mL). Five distinct amounts (10, 20, 30, 40, and 50 mg/mL) were employed, with albendazole at 10 mg/ml serving as the reference. The total phenolic content (TPC) of the methanolic extract was determined to be 74.4 ± 0.291, using gallic acid as the criterion for comparison. The IC_{50} value for the DPPH assay was determined to be 26.95 ± 0.009. The botanical extract exhibited mild antioxidant properties. The methanolic extract (10 mg/mL) demonstrated the highest efficacy in the member stability experiment, exhibiting 15.06 and 20.16% prevention of hemolysis provoked by a hypotonic solution and thermal exposure, in that order. The pharmacological effects indicate the methanolic extract possesses prospective anthelmintic, antioxidant, and anti-inflammatory properties. Additional investigation would elucidate its full potential for the development of safer pharmaceuticals in medicinal science. Julienne Maria *et al.* [11] report that the rising resistance of helminths to chemical anthelmintics employed in goat farming necessitates investigation into the effectiveness of jackfruit and tamarind foliage decoctions as alternative anthelmintics, as well as the determination of the average efficacious dosage (ED_{50}) of various concentrations against the larval stage of gastrointestinal nematodes within caprines. Larvae were gathered, counted, and classified. The elevated concentration of Jack fruit and tamarind leaves decoction resulted in a higher mortality rate of larvae. Both jackfruit and tamarind extracts demonstrated significant efficiency in exterminating nematode larvae at elevated concentrations. ED_{50} was at a concentration of 40% for both infusions. The findings indicate Infusions of jackfruit and tamarind foliage may serve as anthelmintics for caprine species.

Hence, the increasing anthelmintic activity in the crude extraction of petroleum ether and the pure, isolated, and characterized α-amyrin may be attributed to the presence of phytochemicals and specific groups of phytochemicals that influence anthelmintic activity. The literature survey reveals that the extract (in methanol) of the fruit pericarp shows anthelmintic activity, and the traditional herbal formulations monkey jack, Ma-haad, and Pug-haad, which are obtained from the wood of monkey jack, are well-known anthelmintics in Thailand. Therefore, an attempt was made to evaluate the anthelmintic efficacy of crude extracts of petroleum ether, chloroform, and ethanol, along with the isolated, characterized, and purified compound α-amyrin. The study reveals that all the crude extract showed anthelmintic activity with comparatively higher efficacy. Therefore, α-amyrin, a triterpenoid, may act as an anthelmintic. The studies and oleoresin with respect to antimicrobial and antifungal are reported, whereas the anthelmintic activity of α- amyrin, particularly oleoresin general, may new reported for the anthelmintic activity of monkey jack. Further research and studies need to use alpha amyrin as a pure anthelmintic drug.

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