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Preliminary phytochemical screening and *in vitro* evaluation of anthelmintic activity of leaves and stem bark of *Mangifera indica* L. (Anacardiaceae)

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Abstract
Human beings are afflicted with various diseases and infections. Among various types of infections, Helminthes infections are the most prevalent type of infection affecting a substantial fraction of the world's population. Among different herbs that provide healing affect to different infections. *Mangifera Indica* L. is one of those plants that have ethno medicinal claims against Helminthes. The present study is therefore aimed at validating the ethno medicinal claims for its use as an anthelmintic. The Preliminary phytochemical studies of the extract was carried out followed by determination of physicochemical parameters like moisture content, ash and extractive value. Anthelmintic activity of ethanolic extract was carried out on Indian earthworm *Pheretima Posthuma.* The Preliminary phytochemical studies of the extracts indicate the presence of flavonoids, tannins and steroids. Moisture content was 0.14 and 0.07% w/w for leaf and stem bark respectively; ash value 8% w/w and 9% w/w for leaf and stem bark; Water soluble extractive value (0.36% w/w and 0.44% w/w) and Alcohol soluble extractive value (0.6% w/w and 0.88% w/w. Anthelmintic activity showed significant anthelmintic properties at higher concentration. The ethanolic extract of *Mangifera indica* L. caused paralysis in 12.29 min and death in 17.32 min. in 80 mg/ml concentration while standard reference drug Albendazole at 40 mg/ml concentration has shown paralysis at 05.07 min and occurrence of death at 11.34 min. Further studies may be carried out to isolate active constituents from the extract to establish its mechanism of action.

Keywords: *Mangifera indica* L., *Pheretima Posthuma*, preliminary phytochemical studies, flavonoids, anthelmintic

Introduction
Nature is the best combinatorial chemist, and she may have the answers to all of humanity's diseases. Natural product chemicals found from medicinal plants (and their equivalents) have provided a number of clinically relevant medications up to this point. The usefulness of medicinal plants in human survival is immeasurable. The knowledge of how to use medicinal plants has been learned through millennia by monitoring nature, and such plants are still useful in human health care [1]. Medicinal plants are used for the treatment of various ailments from the Vedic period [2-4]. The Charaka Samhita, an ancient herbal medicinal text, mentions several herbs for the treatment of various ailments [5]. The alternative systems of medicine in India like Unani, Ayurveda, as well as Homeopathy are still been used by about 70% of India's population which resulted in increased demand in herbal preparation [6, 7].

Compounds of natural origin have grown in importance in recent years as a result of the tremendous chemical diversity they provide. Herbal drugs consist of various phytochemicals that are comparatively less toxic but act as curative agents for a variety of human diseases. They deserve modern scientific analyses such as phytochemical studies, determination of toxicological parameters, biological evaluation and establish the role of isolated phytochemicals. These natural substances can be further modified to produce newer and therapeutically more potent drugs [8, 9].

The major drawback for using drugs obtained from plant sources is lack of research work needed for the authentication, determining the quality, purity of the crude drug and standardising the various formulations [11]. Quality of herbal drug in term of chemical

constituents and their efficacy necessitates the need of quality control studies of raw drug materials using pharmacognostical standardization. World health organization (WHO) has also created awareness towards validation of plant-based drug to maintain the quality, safety and efficacy [12]. Rich and diversified heritage of these invaluable traditional knowledge systems on medicinal plants is of immense significance in the context of fast erosion of cultural diversity in different parts of the world especially in tropical countries like India. The diversity of medicinal plants and their rich therapeutic traditional knowledge in the country necessitated intensive research in this domain. But very few plants have been scientifically validated which provides us with therapeutically active compounds that leads to the development of newer drugs [13, 14].

Helminthes infections are the most prevalent type of infection in humans affecting a substantial fraction of the world's population. Recently, the use of anthelmintic has resulted in toxicity within humans. As a result, new technologies are being developed and discovered. Plants are being used to create anthelmintic compounds which are thought to be the richest source of bioactive compounds. Anthelmintic are medications used to treat infections caused by worms, flukes, nematodes, round worms, tapeworms, and other parasites. Parasitic worms also infect cattle and crops, thus impacting the environment. Food production also has an economic impact as a result of this [15].

Among a large number of herbal plants that grow around us, *Mangifera indica* L. is one of those plants having various ethnomedicinal claims for various medicinal uses. *Mangifera indica* L. is an evergreen dome-shaped tree; grows naturally in Indo-Malaysian tropical region especially in India and Myanmar [16].

The colour of the bark is dark brown to black, peeling off in uneven, rather thick pieces, superficially fractured or inconspicuously fissured. Small terminal bud with small, lanceolate sharp bud scales are found in this tree. Twigs are thin, smooth, apically angular, glabrous, shiny, and dark green. The tree possesses a long tap root ranging from 6-8m with a dense cluster of feeder roots on the surface providing anchorage to the plant. The leaves are simple, linear-oblong, lanceolate – elliptic, pointy at both ends, borne on 1-12.5 cm long petioles with leaf blades that are usually about 25 cm long and 8 cm wide and release an aromatic scent when crushed gradually grows to lustrous dark green on the upper surface.

In Ayurveda, *Mangifera indica* L. is sometimes combined with other drugs including Shatavari (*Asparagus racemosus*) and Guduchi (*Tinospora cordifolia*) in a Rasayana recipe (q.v.) to clear digestion and acidity caused by pitta (heat) [16]. Toothaches, gastrointestinal disorders, dysentery, diarrhoea, respiratory and urinary tract infections, sore gums, and sore throats are all popular uses of herbal formulations of *Mangifera indica* L. used in some Indian states [17]. Asthma, cough, diarrhoea, dysentery and malaria are all treated with *Mangifera indica* L. In India, the roots of *Mangifera indica* L. is used in the treatment of ulcers, syphilis, and leucorrhoea [18]. Gargling bark extracts mixed with water is used to treat diarrhoea and throat problems. Hiccups and infection in the pharynx are relieved by inhaling the fumes from burning leaves [17]. The flower of this plant is used to cure ulcers, diarrhoea, haemorrhage, anaemia, dyspepsia, and dysentery [18]. A drink produced from unripe mango fruit is used to treat tiredness and heat stroke in India. Gastrointestinal illnesses, bilious disorders, blood disorders, and scurvy are all treated with half-ripe fruit eaten with salt and honey. In gastrointestinal and dermatological illnesses, AIDS, cancer, and asthma, it has been advocated as a nutritional supplement (antioxidant) as well as an anti-inflammatory, analgesic, and immunomodulatory medicine to prevent disease progression or improve the patient's quality of life [19]. The natives of India use various parts of the plant as a cardiotonic, hypotensive, wound healing, dentifrice, antimicrobial, and other medicinal purposes like astringent, diaphoretic, stomachic, vermifuge, tonic, laxative, anaemia and rheumatism.

Mangifera indica L. has been shown to have a wide range of chemical substances mostly polyphenols like mangiferin, catechins, gallic acid, quercetin, kaempferol, ellagic acid, protocatechuic acid, rhamnetin, propyl and methyl gallate, as well as anthocyanins. Mangiferin is a naturally occurring xanthonoid polyphenol antioxidant that helps to reduce insulin resistance, modulate glucose metabolism, lower cholesterol levels. Carotenoids present in this plant are excellent free radical scavengers; concentration of carotenoids increases as the fruit ripens. Several terpenoids present include ocimene, careen, terpinolene, limonene and myrcene. It has also been reported that *Mangifera indica* L. contains tocopherols like alpha-tocopherol, beta-tocopherol, and gamma-tocopherol which are widely found in the peel and flesh of *Mangifera indica* L.

Anti-inflammatory, antidiabetic, anticancer, antioxidant, antifungal, antibacterial, anthelmintic, hepatoprotective, gastroprotective, antiplasmodial, antihyperlipemic, immune-modulatory, and many other properties have been reported in various portions of M. Indica trees [18]. But according to Literature review the anthelmintic activity in leaves have not yet been evaluated. So, in the present paper we report the anthelmintic properties of the leaves and stem bark of *Mangifera indica* L.

Materials and Methods

Plant Materials: The leaf and stem bark of the plant *Mangifera indica* L. was collected from mango garden situated at Naihati, North 24 Parganas, West Bengal during the month of March 2023 and authenticated by Dr. Suchandra Samanta Mandal, M.Sc. (Botany), KU, M Phil. (Education), KU, Assistant Professor, K. Bed College, Krishnanagar, West Bengal, India.

Chemicals and reagents: The chemicals, solvents and reagents used in the study were of standard analytical grade obtained from S.D Fine Chem Ltd., Mumbai and Loba Cheme, Mumbai.

Petroleum Ether, Chloroform, Ethanol, Mayer's reagent, Wagner's reagent, Dragendroff's Reagent (solution of Potassium Bismuth Iodide), Hager's reagent (saturated picric acid solution). Millon's reagent, Ninhydrine solution, α-

naphthol, concentrated Sulphuric acid, Barfoed's Reagent, Hydrochloric acid, Phloroglucinol, Potassium hydroxide, Phenolphthalein, Copper Sulphate, Sodium Hydroxide, Zinc dust, Fehling's solution A and B, Magnesium Acetate, Sodium Nitroprusside, Ferric chloride, Glacial Acetic acid, Ruthenium Red solution, Gelatin, Sodium chloride, Biuret reagent, Concentrated Nitric acid, Iodine solution, Acetic anhydride, Normal saline and DMF.

Animals: *Pheretima posthuma* (Adult Indian earth worms) of about 5-7 cm long and 0.3-0.4 cm in width were used for the present study.

Standard drug: To compare the test results, Albendazole 400 mg Tablet (Glaxo Smith Klime) was used as standard.

Preparation of extract: [20, 21]

The plant material was dried thoroughly in shade condition at room temperature. It was then subjected to size reduction. 250 gm of the plant material was defatted with petroleum ether. The marc was thoroughly dried and subjected to cold maceration process with different solvent (12 hrs each) like chloroform, ethanol and water. The extracts obtained were dried at temperature below 40° to obtain concentrate of the crude extract. The extract was kept in a suitable container with proper labelling inside a desiccator for further use.

Phytochemical Screening: Different qualitative tests were performed on the crude extracts to identify the various active constituents that are present in the leaf and stem bark of the plant *Mangifera indica L.* Phytochemical screenings were performed using standard procedures. The results are depicted in Table 1.

Determination of anthelmintic activity

The anthelmintic activity was investigated on mature Indian earthworm *Pheretima posthuma*, which shares morphological and physiological similarities with human intestinal roundworm parasites.

The anthelmintic method was carried as per the method of Pal *et al.* [22], with minor modifications. Sixteen groups each containing six earthworms of approximately equal size were released into 10 ml of desired formulation. Each group was treated with, albendazole, chloroform extract, ethanol extract and aqueous extract (40, 60 and 80 mg/ml) in normal saline with 5% DMF and in vehicle alone acting as control. Time for paralysis was noted when no movement could be observed with a slight pin prick method. Time for death of individual earth worms was recorded when the worms showed no movement either by vigorous shaking or by dipping in warm water.

Results and Discussion

Qualitative Chemical Tests of the Extracts:

Preliminary phytochemical analysis of different extracts indicated the presence of alkaloids, flavonoids, carbohydrates, tannins, and other compounds (Table 1).

Table 1: Qualitative chemical tests of the extracts

Test		Chloroform Extract	Ethanolic Extract	Aqueous Extract
Alkaloids		+	+	+
Amino Acids	Millon's Test	+	+	+
	Ninhydrine Test	+	-	+
Carbohydrates	Molisch's Test	+	+	+
	Barfoed's Test	+	+	+
	Test for Pentoses	+	+	+
Fats and Fixed oils	Saponification Test	-	+	-
Flavonoids	Alkaline reagent Test	+	+	+
	Zinc hydrochloride Test	+	+	+
Glycosides	General Tests	-	+	-
	Borntrager's Test	-	-	-
	Modified anthraquinones Test	-	+	-
	Baljet's Test	-	-	-
	Legal's Test	-	-	-
	Froth formation Test	-	+	-
Mucilage		-	-	-
Tannins	Chlorogenic acid Test	+	+	+
	Ferric Chloride Test	+	+	+
	Gelatin Test	+	+	+
Proteins	Xanthoproteic Test	+	+	+
Starch		+	+	+
Steroids	Libermann-Burchard Test	-	+	+
	Salkowski Test	-	+	+

["+" = Present and "-" = Absent]

Anthelmintic Activity: The result of Anthelmintic Activity is given in Table 2 and result plotted in Figure 1.

Natural product compounds derived from medicinal plants and their counterparts have provided a number of clinically relevant drugs. Natural source compounds have gained in relevance in recent years due to enormous chemical diversity they provide. The leaves and stem bark of *Mangifera indica* L. are chosen for its varied ethnomedicinal properties. The leaves and stem bark of the plant are subjected to extraction by cold maceration method. The chosen plant material is defatted with petroleum ether followed by different solvent of increasing polarity like chloroform, ethanol and water. Maceration is the process by which organized tissue is transformed into a suspension of intact cells. If the desired compound has a high solubility in a solvent, then this method is appropriate for extraction then a simple filtration can be used to separate the compound from the insoluble substance. Chloroform extract showed the presence of alkaloids, amino acids, carbohydrates, flavonoids, tannins, proteins and starch. Ethanolic and aqueous extracts also indicated the presence of alkaloids, amino acids, carbohydrates, fats and fixed oils, glycosides, tannins, proteins, starch and steroids. The result of anthelmintic activity on earthworm *Pheretima Posthuma* revealed that the ethanolic extract at different concentration has shown paralysis effect and has caused death of earthworms. Significant anthelmintic properties were observed at higher concentrations. The ethanolic extract of *Mangifera indica* L. caused paralysis in 12.29 min and death in 17.32 min. in 80 mg/ml concentration. The reference drug albendazole at 40 mg/ml concentration has shown paralysis at 05.07 min and occurrence of death at 11.34 min.

Table 2: Anthelmintic activity of different extract of the leaf and stem bark of the plant *Mangifera Indica* L

Group Treatment Dose			**Reaction time in (minutes)**	
			Time taken for paralysis (P)	**Time taken for death (D)**
Control	Normal Saline	-	0.00	0.00
Standard	Albendazole	40 mg/ml	5.07±0.05	11.34±0.03
		60 mg/ml	3.38±0.21	7.56±0.02
		80 mg/ml	2.54±0.03	5.67±0.04
Chloroform extract		40 mg/ml	41.35±0.03	51.41±0.22
		60 mg/ml	30.23±0.27	36.94±0.06
		80 mg/ml	25.32±0.14	30.35±0.05
Ethanol extract		40 mg/ml	24.58±0.06	34.64±0.04
		60 mg/ml	16.38±0.03	23.09±0.01
		80 mg/ml	12.29±0.05	17.32±0.16
Aqueous extract		40 mg/ml	63.32±0.04	73.38±1.22
		60 mg/ml	56.45±1.15	63.16±0.21
		80 mg/ml	51.28±0.09	56.31±0.06

Results are expressed as mean ±SEM of three observations. P- Paralysis; D-Death *mg: milligram, ml: millilitre*

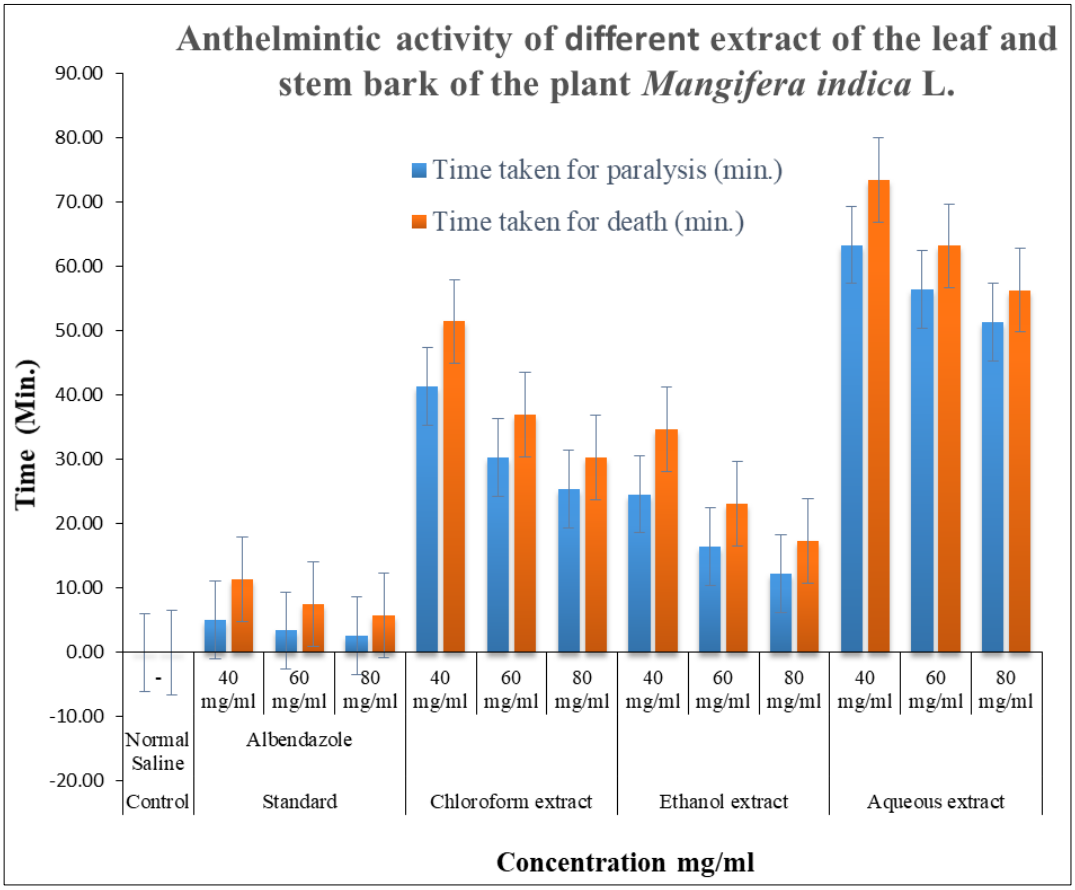

Fig 1: Values of paralysis and death time of *Pheretima posthuma* [Indian adult earth worms] plotted against varying concentration of different extract of leaves and stem bark of *Mangifera indica* compared with standard (Albendazole)

Conclusion

According to Ayurveda, *Mangifera indica* is a very potent and ethnomedicinally important plant. Literature review reveals that *Mangifera indica* L. has pharmacologically and medicinally essential compounds such as mangiferin, mangiferonic acid, hydroxymangiferin, polyphenols, and carotenes. The present study reveals the presence of various phytochemical constituents in the aqueous, chloroform and ethanolic extract of the leaves and stem of *Mangifera indica* L. The study has also revealed prominent anthelmintic activity of the ethanolic extract comparable to that of standard drug Albendazole; suggesting a need to isolate and evaluate the active constituents responsible for the exhibited biological activity. Further research may also be carried out to investigate this medicinal plant for various other folklore claims to establish its scientific evidence for the benefit of mankind.

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