

Priliminary Phytochemical Screening, Anthelmintic Activity of Methanolic and Aqueous Extract of *Syzygium Cumini* Linn. Bark (Myrtaceae).

Kannabiran kavitha*, Mariappan Murali, Kuncha Jayachandra

Department of Biochemistry, Jaya College of Paramedical Sciences College of Pharmacy, Chennai-602024, Tamil Nadu, India.

Abstract:

Preliminary Phytochemical investigation was carried out on the methanolic extract of *Syzygium cumini* Linn. Bark. It indices presence of Carbohydrates, Amino acids, Tannins, Saponins, phytosterols, Terpenoids, phenols and flavonoids. We are also quantitatively estimated total phenolic content, tannins and favanioids by using spectrophotometer. The total phenolic content was 580.23 ± 3.03 mg/g, tannin content was 534 ± 4.03 mg/ g while the flavonoid content was 315.42 ± 4.52 mg/g. The Methanolic and aqueous extract of *Syzygium cumini* Linn. Bark (Myrtaceae) was investigated for activity against Indian earthworms *Pheretima posthuma*. Various concentrations of each extract (25-100 mg/ml) were tested, which involved determination of time of paralysis and time of death of the worms. Both extracts (aqueous and methanolic) at the tested dose (25-100 mg/ml) were produced significant activity. The maximum activity of Methanolic extract of *Syzygium cumini* bark was 36.58 minutes as time of paralysis, 70.58 minutes as time of death at dose of 100 mg/ml. At the same concentration aqueous extract were showed 76.25 minutes as time of paralysis and 80.33 minutes as time of death. Albendazole (20 mg/ml) which is included as standard reference showed 21.16 minutes as time of paralysis, 23.5 minutes as time of death. Normal saline used as control there is no paralysis and death of earth worms. The present study indicates Methanolic extract of *Syzygium cumini* bark acts as potential usefulness of as an anthelmintic and aqueous extract acts as moderate anthelmintic activity. This is the first research report regarding Anthelmintic activity of Methanolic and aqueous extracts of *Syzygium cumini* bark. Furthermore, purification, isolation, characterization of phytoconstituents responsible for anthelmintic activity is in progress.

Keywords: Albendazole, Anthelmintic activity, Flavonoids, *Pheretima posthuma*, *Syzygium cumini* bark, Tannins, Total Phenol Content.

1. Introduction:

Parasitic diseases are a major infestation in the human beings like helminthiasis. The disease is caused by round worm, hook worm, thread worm, tape worm and filarial, guinea worm are found in intestine [1]. The worm is responsible for many type of disease; they harm the host by depriving of food, causing blood loss in stool, injury to organs, intestinal or lymphatic obstruction and by secreting the toxins [2]. Helminthiasis is really fatal, but it is a major cause of ill health [3]. Number of synthetic drugs used to control and prevent the infestation related to worms, the drugs like mebendazole, albendazole, piperazine and pyrantel, almost mebendazole used as broad spectrum anthelmintic drugs [4]. Adverse effect like tolerance, resistance, nausea, vomiting, drowsiness, dizziness, and abdominal pain occurred at long term used of synthetic medicine [5]. Therefore, overcome the problem associated with synthetic medicine, the natural compounds are selected. Naturally produced medicinal products offer as an alternate anthelmintic and therapeutic agents so as to overcome

some of these infestation and subsequently may be sustainable and environmentally acceptable because the natural or herbal compounds are free from adverse effect [6].

Syzygium cumini belonging to the family of Myrtaceae is a large evergreen tree. It has been valued in Ayurveda and Unani system of medication for possessing variety of therapeutic properties. Most of the plant parts are used in traditional system of medicine in India. According to Ayurveda, its bark is acrid, sweet, digestive and astringent to the bowels, anthelmintic and in good for sore throat, bronchitis, asthma, thirst, biliousness, dysentery, blood impurities and to cure ulcers [7]. In Unani medicine system the ash of leaves is used for strengthen the teeth and the gums, the seeds are astringent, diuretic, stops urinary discharge and remedy for diabetes and the bark showed good wound healing properties [8]. *Syzygium cumini* is a medicinal plant, whose parts were pharmacologically proved to possess hypoglycemic [9], antibacterial [10], antidiarrhoea effects

[11] and anti-inflammatory activity of leaf and barks [12,13].

However, No scientific data are available regarding methanolic and aqueous extracts of *syzygium cumini* bark usefulness as anthelmintic agent. Keeping the above information in view the present study was endeavour to ratify the anthelmintic activity of methanolic and aqueous extract of *syzygium cumini* on Indian earth worms (*Pheretima posthuma*).

2. Materials and Methods:

Plant Material

The fully mature, fresh stem bark of *syzygium cumini* was collected from Midhilaragam, Mellacheruvu village, Chittoor district, AndhraPradesh. The stem bark was identified and authenticated by Dr.S.B.Narasimha Reddy, Professor, Department of Botany, S.G.Govt.Degree college, Piler and voucher specimen (No.JCP/2010/153) was deposited in the Herbarium of the same department. The bark was air dried at room temperature (25°C) for 30 days and converted into fine powder with an automix blender, the powder was kept in a deep freezer until the time of use.

Preparation of Extracts

500 gm of dry fine powder was suspended in 1.5 liters of methanol and double distil water separately then stirred magnetically for 24 hours at room temperature. The extract were double filtered by using musline cloth and whatmann No. 1 filter paper. The filtrate was concentrated to dryness under reduced pressure at 40°C using rotary vacuum evaporator (Buchi labortechnik AG, Switzerland) to obtain crude extract. The dried MESC and AESC (Methanolic & Aqueous extracts of *Syzygium cumini*) was stored in vacuum desiccators under controlled conditions till it used for experimental purpose.

Preliminary Phytochemical Screening:

1 gm of the methanol extract of *syzygium cumini* bark were dissolved in 100 ml of its own mother solvent to obtain a stock of concentration 1% (w/v). The standard methodology of Harborne (1998) [14] and

Kokate (2001) [15] were adopted for the phytochemical screening.

Table 1: Preliminary Phytochemical screening of Methanolic extract of *Syzygium cumini* Bark

Phytochemicals	Methanolic extract
Alkaloids	-
Amino acids	+
Anthraquinones	-
Flavonoids	+
Carbohydrates	+
Phytosterols	+
Saponins	+
Tannins	+
Terpenoids	+
Phenols	+

+ Presence, - Absence

Determination of Total Phenolic content, Tannins and flavonoids

The total phenolic content in the extracts were determined using Folin-ciocalteu reagent according to the Malic and Singh (1980) [16]. Tannin content was determined by Folin-Denis reagent according to the method of Schandrel (1970) using tannic acid as standard [17]. The favonoids were estimated by earlier reported method (Ivan *et al.*, 2004) [18].

Assessment of Anthelmintic Activity

Experimental animals: (Earthworm Collection, Maintenance and Authentication)

The Indian adult earthworms *Pheretima posthuma* (Annelida) were collected from moist soil of the field and washed with normal water and saline solution to remove soil and fecal matter. Earthworms were indentified and authenticated from Dr.Sudhakar reddy Department of Zoology, Govt. Junior College, Piler. The Earth worms of 4-8 cm in length and 0.2-0.3 cm in width were used for all experimental parameters.

Drugs and Chemicals used:

Albendazole (Glasko Smith Kline) was used as reference standard purchased from local medical shop, thiruninravur, chennai.

Table 2: Anthelmintic activity of Methanolic and Aqueous Extracts of *Syzygium cumini*.Linn Bark on Indian Earthworms (*Pheretima posthuma*).

Name of the group	Name of the extract	Concentration (mg/ml)	Time taken for paralysis(min.)	Time taken for death(min.)
Group-I	1% gum acacia in saline		–	–
Group-II	Albendazole	20	21.16±1.83	23.5±1.37
Group-III	MESCB	25	72.75±0.81	88.16±3.80
		50	57.5±2.28	76.08±1.13
		75	44.58±1.73	70.33±0.63
		100	36.58±0.53	70.21±1.17
Group-IV	AESCB	25	91.66±1.43	100.91±2.81
		50	88.5±1.84	96.16±0.30
		75	80.66±1.43	88.5±1.42
		100	76.25±0.34	80.33±1.69

All the values are mean ± SEM (n=6). P<0.05 compared to Albendazole.

Chemicals Methanol (95% V/V) (S.D fine chemicals, Mumbai).

Preparation of test sample:

Samples for experiments were prepared by dissolving extract to obtain a stock solution of 100 mg/ml, from the stock solution, different working dilutions were prepared to get concentration range of 25, 50, 75 and 100 mg/ml of each extracts (MESCB&AESCB). For present study Albendazole taken as standard drug. The concentration of standard drug was prepared in 1% gum acacia in normal saline to give 20 mg/ml concentration.

Experimental Animals Groups Dividing:

The Indian adult Earth worms can be divided into ten groups. Each group consists six earth worms. Group-I is contain Vehicle (1% gum acacia in normal saline), Group-II containing Albendazole as a reference standard (20 mg/ml) , Group-III is having MESCB with different concentrations such as III_A (25 mg/ml), III_B (50 mg/ml), III_C (75 mg/ml), III_D (100 mg/ml) and Group-IV is having AESCB with different concentrations V_A (25 mg/ml), V_B (50 mg/ml), V_C (75 mg/ml), V_D (100 mg/ml).

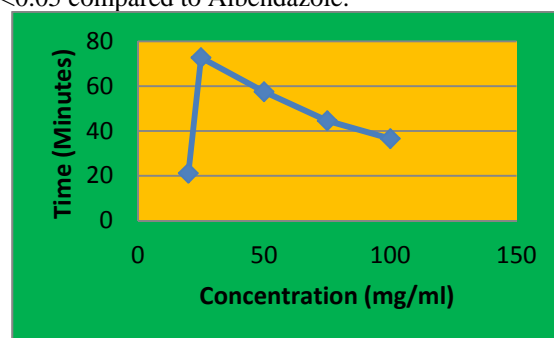


Figure 1: The time taken for paralysis of earthworm at different concentration of methanolic extract of *syzygium cumini* bark. [20 mg/ml is the conc. of Standard].

Evaluation of Anthelmintic Activity

The evaluation of anthelmintic activity was followed by earlier reported method (Bhusan,M., *et al.*, 2010) [19]. Anthelmintic activity was evaluated on adult Indian Earthworms (*pheretima posthuma*) due its anatomical and physiological resemblance with the intestinal round worm of human beings. Four different concentrations (as given earlier) were prepared and the group of six earthworms which having equal size were released into 50 ml of sample with desired concentration in petridish. Observations were made for the time taken to cause paralysis and death of the individual worms. Mean time for the paralysis in

minutes was noted when no movement of any sort could be observed, except when the worm was shaken vigorously.

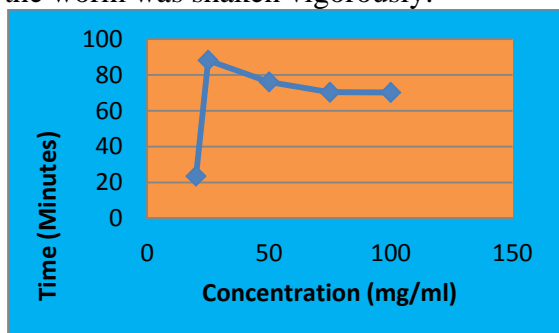


Figure 2: The time taken for death of earthworms at different concentrations of methanolic extract of *syzygium cumini* bark. [20 mg/ml is the conc. of Standard]. Time of death in minutes was recorded after ascertaining.

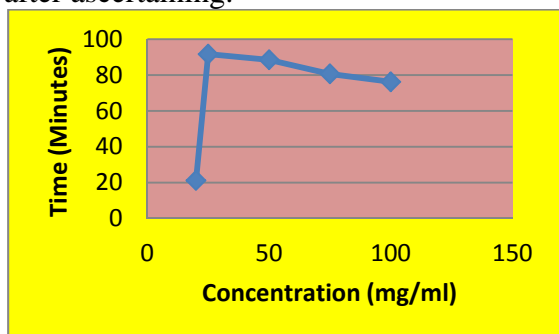


Figure 3: The time taken for paralysis of earthworms at different concentrations of aqueous extracts of *syzygium cumini* bark. [20 mg/ml is the conc. of Standard]. The worms neither moved when shaken vigorously nor when dipped in warm water (50°C). Paralysis is assumed to occur when they do not revive even in saline solution. Potency is inversely proportional to time taken for paralysis and / or death of parasite. Observations were shown in table 2, regarding the anthelmintic activity of methanolic and aqueous extract of *syzygium cumini* bark against Indian earthworms.

3. Result and Discussion:

Preliminary phytochemical analysis showed the presence of Phenols, Terpenoids, Tannins, Saponins, Phytosterols, Carbohydrates, Flavonoids, Aminoacids like phytoconstituents (Table-1) may be responsible to show a potent anthelmintic activity. Phenols are very

important plant constituents because of their radical scavenging ability due to their hydroxyl group [20]. The phenolic content may contribute directly to the antioxidant activity [21]. It has been suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans [22]. Consequently, the antioxidant activity of methanolic extract are often explained by their total phenolic content, tannins and flavonoid contents with good correlation. The total phenolic content in the methanolic extract of *Syzygium cumini* was 580.23 ± 3.03 mg/g, tannin content was 534 ± 4.03 mg/g while the flavonoid content was 315.42 ± 4.52 mg/g. These results demonstrate that tannins represents the main group of phenolic compounds in *Syzygium cumini* bark.

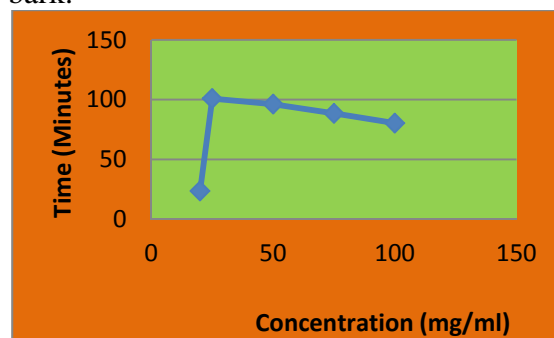


Figure 4: The time taken for death of earthworms at different concentrations of aqueous extracts of *syzygium cumini* bark. [20 mg/ml is the conc. of Standard].

Polyphenolic compounds shown anthelmintic activity and some synthetic phenolic anthelmintics are shown to interfere with energy generation in helminthic parasites by uncoupling oxidative phosphorylation [23]. Tannins possess antiparasitic activity [24]. Reported anthelmintic activity of tannin that they can bind to free protein in GIT of host animal or glycoprotein on the cuticle of the parasites and may cause death [25]. It is possible that tannin contained in the methanolic and aqueous extract of *Syzygium cumini* bark may produce similar effect. From the observation made all the extracts of bark of *syzygium cumini* was showed anthelmintic activity. After a brief

stimulant effect, earthworm lost their motility of exposure to crude extract of bark *Syzygium cumini* Linn. Each extract containing 25, 50, 75, and 100 mg/ml produced dose dependent paralysis ranging from loss of motility to loss of response to external stimuli, which eventually progressed to death.

As shown in graph no.1 and 2, methanolic extract of bark *Syzygium cumini* Linn. and its different fractions exhibited anthelmintic activity in dose dependent manner giving short time of paralysis 36.58 minutes, and time of death is 70.58 minutes respectively with 100 mg/ml. Also as shown in graph no.3 and 4, aqueous extract of *Syzygium cumini* Linn. bark and its different fractions exhibited anthelmintic activity in dose dependent manner giving time of paralysis was 76.25 minutes and time of death was 80.33 minutes respectively with 100mg/ml. Therefore potency of drug was found to be inversely proportional to the time taken for paralysis / death of worms (Table 2). The higher concentrations of each crude extract produced paralytic effect much earlier and necrotic spots were observed externally on the worms, with higher concentrations. The effect of each crude extract was compared with Albendazole as standard drug (20 mg/ml).

4. Conclusion:

Using the *pheretima Posthuma* as the animal models, we have shown that (Methanolic and Aqueous) crude bark powder of *Syzygium cumini* has potential to act against helminthiasis. Moreover, the extent of anthelmintic effect of the bark powder is comparable to that of standard drug, Albendazole being used against helminthiasis, in general. This observation unambiguously suggests that the bark powder of *Syzygium cumini* must contain lead compounds that may provide profound implications on designing de novo anthelmintic drugs. We are presently working on identifying and elucidating the three-dimensional structures of a few lead compounds from the crude bark powder.

We strongly believe that the outcomes of the study will trigger exciting research on addressing helmenthiasis diseases in a cost effective manner.

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