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Assessment of Anthelmintic Activity of Ethanolic Extract of *Musa sapientum* Stem: An *In-Vitro* Approach

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ABSTRACT

Many medicinal plants claimed to possess anthelmintic activity. *Musa sapientum* belonging to the Musaceae family is a highly valued plant, distributed in many countries of the tropical and sub tropical. Its common names includes banana. Some standard drugs such as piperazine citrate, albendazole, mebendazole, thiabendazole possess some side effects such as nausea, vomiting, stomach and abdominal pain, headache, dizziness, and temporary hair loss etc. But the herbal drug shows fewer side effects. *Musa sapientum* L. (Musaceae) are mainly grown in the tropical and subtropical countries and are widely used for its nutritional values all over the world. Preliminary phytochemical investigation includes the presence of alkaloids, carbohydrates, glycosides, flavonoids, steroids and tannins. Indian adult earthworms (*Pheretima posthuma*) were used to study anthelmintic activity. The activity was checked in ethanolic extract with three different concentrations (100, 200, 300mg/ml) and compared with the standard drug Albendazole (10mg/ml) and control as distilled water. The result was expressed in the terms of paralysis time and death time of worms. Ethanolic extract of *Musa sapientum* stem shows anthelmintic activity in dose dependent manner and maximum efficacy is seen at 300mg/ml concentration. Hence it was concluded that ethanolic extract of *Musa sapientum* stem have anthelmintic property.

Keywords: *Musa sapientum,* earthworms, anthelmintic activity, albendazole, paralysis, *Pheretima posthuma.*

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INTRODUCTION

Man has been fascinated by nature since he evolved from his primitive ancestors, the apes. To start with, he hunted for food mainly by killing the wild animals, but if there was anything on which he could depend with any confidence towards its availability, it was the plant which provided him with food and they provided him with curative medicine and shelter. Because of this the primitive man was in love with the nature especially with plants because plants were the only source to fight with various diseases. From the plants they found various medicines and treatment practices to treat many diseases which put way for the modern treatment systems to save the human race. Today in this world traditional medicine plays a vital role in providing health care to large section of population, especially in developing countries. Medicinal plants are the important source for the production of synthetic and herbal drugs. The medicinal plants or the herbal drugs are used in many ways by the human[1-3].

Medicinal plants are frequently used in traditional medicine to treat different diseases in different areas of the world. This indigenous knowledge, passed down from generation to generation in various parts of the world, has significantly contributed to the development of different traditional systems of medicine as well as helped in exploration of different medicinal plants to find the scientific basis of their traditional uses. This exploration of biologically active natural products have played an important role in finding New Chemical Entities (NCEs) for example, approximately 28% of NCEs between 1981 and 2002 were natural products or natural product-derived[4,5].

Helminths are the parasitic worms which are most common infectious agents of humans in developing countries. It produces a global burden of diseases that exceeds conditions including malaria and tuberculosis. There are two major phyla of helminths. They are nematodes and platyhelminthes. Nematodes are also known as roundworms which includes the intestinal worms and filarial worms. Many herbal plants with muscle relaxant property shows the

anthelmintic activity. *Musa sapientum* is the one of those plants which shows the muscle relaxant property. So we have selected the *Musa sapientum* which is an herbal drug and shows the muscle relaxant property. *Musa sapientum* is a highly valued plant grown in tropical regions. It grows to the height of about 12 feet[6-8].

Banana is a familiar tropical fruit. From its native Southwestern pacific home, the banana plant spread to India by about 600 BC and later on it spread all over the tropical world. It is possibly the world's oldest cultivated crop. It even spread into the Islands of the Pacific and to the West Coast of Africa as early as 200-300 BC. *Musa sapientum* is a treelike perennial herb that grows 5-9m in height, with tuberous rhizome, hard, long pseudostem. The inflorescence is big with a reddish brown bract and is eaten as vegetables. The ripe fruits are sweet, juicy and full of seeds and the peel is thicker than other banana[9,10].

Taxonomical classification:

Kingdom : Plantae
Division : Magnoliophyta
Class : Liliopsida
Order : Zingiberales
Family : Musaceae
Genus : *Musa*
Species: *M. sapientum*

Cultivation and distribution:

In different countries about 300 varieties of bananas are grown, of which a vast majority have been growing in Asian, Indo Malaysian and Australian tropics and are now widely found throughout the tropical and subtropical countries. India, Philippines, China, Ecuador, Brazil, Indonesia, Mexico, Costa Rica, Colombia, Thailand are the top banana producing countries. It is extensively grown and cultivated as a fruit plant all over Bangladesh. The banana grows almost everywhere in the country throughout the year. The principal banana growing areas however, are Rangamati, Barisal, Rangpur, Dinajpur, Noakhali, Faridpur and Khulna[11,12].

The present aim of the research work is to assess the phytochemical constituents and anthelmintic potential of ethanolic extract of stem of *Musa sapientum*.

Figure 1: *Musa sapientum* plant

Figure 2: *Musa sapientum* stem

MATERIALS AND METHODS

Plant collection and authentication

The dried stems of *Musa sapientum*, belonging to Family Musaceae were collected from Hyderabad, Telangana, India, and authenticated by Dr. G. Baba Shankar Rao, Department of Pharmacognosy and Phytochemistry, School of Pharmacy, Anurag Group of Institutions, Venkatapur, Ghatkesar, Telangana, India.

Preparation of plant extract

Stems were dried at room temperature (25-35 ^{0}C) and powdered with the help of an electric grinder and sieved to obtain fine powder. The fine powder was subjected to extraction with ethanol in a Soxhlet apparatus (10-12 cycles). The extracts were cooled at room temperature, filtered and evaporated to dryness. A greenish semi-solid extract was obtained & kept in refrigeration for further use. The stem extract was used for evaluation of anthelmintic activity[13].

Preliminary qualitative analysis[14,15]

The crude drug extract from stem was investigated for the presence of various classes of phytochemicals by performing appropriate tests.

Phytochemical screening test for primary metabolites

Qualitative phytochemical screening for primary metabolites:

It is performed on plant extracts/phytoconstituents to explore carbohydrates, proteins, amino acids, fats & fixed oils present in it.

1. **Test for carbohydrates: -**about 50mg of extract was dissolves in 5ml of distilled water and filtered. Filtrate is tested for the preference of carbohydrates.

a. **Molisch test:** To 2ml of filtrate 2 drops of alcoholic solution of α-naphthol was added. The mixture was shaken well & 1ml of cone. H_2SO_4 was added slowly along the sides of the test tube and observe for colour. The formation of violet ring at the junction of two liquids in case of presence of carbohydrates.

b. **Fehling's test:** To 1ml of filtrate, 1ml of Fehling's reagent is added and heat in a boiling water bath for 2ml. Observe for ppt formation. The red ppt formed in presence of sugars.

c. **Barfoed's test:** To 1ml of filtrate, 1ml of Barfoed's reagent is added and heat in a boiling water bath for 2 min observe for ppt formation, the red ppt formed in presence of sugars.

d. **Benedict's test: -** To 0.5ml of Benedict's reagent is added. The mixture is heated on a boiling water bath for 2 minutes and observes for ppt formation. The formation

of orange, red ppt indicates the presence of reducing sugars.

2. **Test for proteins and amino acids:** About of 100mg of extract is dissolved in 10ml of distilled water, filtered through Whatman-1 filter paper & the filtrate is subjected to test the proteins and amino acids.

a. **Millon's test:** -To 2ml of filtrate, 2ml of millon's reagent is added heated to oil and observe for precipitate. The ppt was again heated and observed. The formation of white ppt which forms to red upon heating indicates the presences of proteins and amino acids.

b. **Biuret test:** -To 1ml of filtrate, 1ml of 10% sodium hydroxide solution is added and observe the colour formation of purple violet indicates the presence of proteins.

c. **Ninhydrin test:** -To 2ml of filtrate, few drops of 0.5% of Ninhydrin reagent is added and boil for few minutes and observe for colour formation of violet blue indicates the presence of amino acids.

3. **Test for fixed oils and fats:**

a. **Saponification test:** Treat the extract with few drops of 0.5N alcoholic potassium hydroxide solution and a drop of phenolphthalein solution, the resultant is heated on a water bath for about 1-2 hours, formation of soap due to neutralization of alkaline indicates the presence of fatty materials.

Phytochemical screening test for secondary metabolites[15-17]

Qualitative phytochemical screening for secondary metabolites:

It is performed on plant extract/phytoconstituents to explore alkaloids, glycosides, steroids, terpenoids, phenolic compounds, tannins, flavonoids and saponins.

1. Test for alkaloids: About 50mg of solvent free extract is distilled in the same solvent used for extraction and filter it. The filtrate is tested for the presence of alkaloids.

a. **Mayer's test:** To 0.5 ml of filtrate two drops of Mayer's reagent (solution of pet mercuric iodide) is added along the sides of the test tube and observe the ppt. The formation of creamy ppt indicates presence of alkaloids.

b. **Wagner's test:** To 0.5 ml of filtrate two drops of Wagner's reagent (solution of Iodine in pot iodide) is added along the sides of test tube is observed for ppt the formation of reddish brown ppt indicates the presence of alkaloids.

c. **Dragendroff's test:** To 0.5 ml of filtrate, two drops of Dragendroff's reagent is added and observe for ppt, the formation of a prominent reddish-brown colour ppt indicates the presence of alkaloids.

d. **Hager's test:** To 0.5ml of filtrate, 1ml of Hager's reagent (saturated picric acid solution) is added and observe for ppt formation of prominent yellow colour ppt indicates the presence of alkaloids.

2. Test for Glycosides: For detection of glycosides about 50 mg of extract is hydrolysed with concentrated HCl for two hours on a water bath and filter the hydrolysate is subjected to following test.

a. **Bontrager's test:** To 2 ml of hydrolysate, 3 ml of Chloroform is added and shaken well, to this separated chloroform layer 1 ml of 10% ammonia solution is added and observe for colour formation of pink colour indicates the presence of Anthraquinone glycosides.

b. **Keller-Kiliani test:** About 50mg of the extract is dissolved in 2 ml of glacial acetic acid and two drops of 5% ferric chloride solution is added and mixed, to this 1 ml of sulphuric acid is added. Reddish brown colour is appearing at the junction of two liquid layers and the upper layer appears bluish green colour indicating the presence of steroidal glycosides.

3. Test for Steroids and Terpenoids:

a. **Liebermann-Burchard test:** To the 50mg of extract dissolved in 2 ml of chloroform and is treated with two drops of acetic anhydride. Two drops of concentrated $H_2SO_4$is then added along the sides of test tube and observe for colour red, pink or violet colour at junction of liquids indicates the presence of Steroids/Triterpenoids and their glycosides.

b. **Salkowski test:** 50mg of extract in 2 ml of chloroform is treated with two drops of concentrated H_2SO_4 shaken well and allowed to stand and observe for colour, the formation of yellow coloured layer indicates the presence of Triterpenoids and formation of reddish-brown colour layer indicates the presence of steroids.

4. Test for Phenolic compounds and Tannins:

a. **Ferric chloride test:** About 50mg of extract is dissolved in 2 ml of distilled water and then two drops of neutral 5% Ferric chloride solution is added and observe for colour, the formation of blue, green or black colour indicates the presence of Phenolic compounds and tannins.

b. **Lead acetate test:** About 50mg of extract is dissolved in 2 ml of distilled water and observed for the precipitate, the formation of white ppt indicates the presence of Phenolic compound and Tannins.

c. **Bromine water test:** About 50mg of extract is dissolved in 2 ml of distilled water; 1 ml of Bromine water is added and observed for the decolouration of bromine water. Decolouration of bromine water indicates the presence of Phenolic compounds and Tannins.

5. Test for Flavonoids:

a. **Shinoda test:** 10mg of extract is dissolved in 2 ml of alcohol to these two fragments of Magnesium turnings and 0.05 ml of concentrated HCl were added and observed for colour formation of magenta colour or crimson red colour indicates presence of Flavonoids.

b. **Alkaline reagent test:** 10 mg of extract is dissolved in 2 ml of water and treated with 1 ml of 10% Ammonium hydroxide solution and observed for colour, two drops dilute HCl is added and again observed for discolouration. The formation of an intense yellow colour which turns into colourless on addition of dilute acid indicates the presence of Flavonoids.

6. **Test for Saponin Glycosides:** The saponin glycosides contain either steroids or Triterpenoids aglycon and therefore they always give positive Liebermann-Burchard test. Saponin glycosides give positive results with foam test and haemolysis test.

a. **Foam test:** Take 2 ml solution of test sample in a test tube for half minute, stable foam is formed indicating presence of saponin glycoside in it.

b. **Haemolysis test:** Take 2 ml of solution of test sample in a normal saline with 0.2 ml of blood in normal saline and

mix well. Saponin causes complete Haemolysis of the blood.

Animals required

Earthworms of about 5-7cm long were used for anthelmintic activity, collected from Jaya Biotech, Ghatkesar, Hyderabad, Telangana, India.

Grouping of animals

Earthworms were used for anthelmintic activity and grouped into control, standard. 6 animals in each group having length 8±1 cm. Albendazole was used as standard drug. 10 ml of each suspension poured in separate petridish. The earthworms were divided into 5 groups of six earthworms in each and placed in eight petridishes containing the extract solutions or drugs as mentioned below:

Group-1 : Received distilled water which served as control
Group-2 : Received albendazole which served as standard (10mg)
Group-3 : Treated with ethanol extract (100mg/ml)
Group-4 : Treated with ethanol extract (200mg/ml)
Group-5 : Treated with ethanol extract (300mg/ml)

Procedure

All petridishes were kept under room temperature. The living or viable worms were kept under close observation. Observation made for time taken to complete paralysis and death for individual worms. Earthworm was frequently applied with external stimuli which stimulates and induce movement in earthworms, if alive. Paralysis was said to occur when the worms do not revive even in normal saline. Death was concluded when the worms lose their motility followed with fading of body colour. The motionless worms were then transferred at 40 ^{0}C to confirm that they were dead[18,19].

RESULTS

Preliminary phytochemical screening has shown the presence of carbohydrates, alkaloids, glycosides, flavonoids and phenolic compounds in ethanolic extracts of stem of the plant (Table 1).

Table 1: Phytochemical screening for ethanolic extract of *Musa sapientum* stem

Tests	Stem
Carbohydrates	+ve
Proteins & Amino acids	-ve
Fixed oils & Fats	-ve
Alkaloids	+ve
Glycosides	+ve
Steroids & Terpenoids	-ve
Phenolic compounds & Tannins	+ve
Flavanoids	+ve
Saponin Glycosides	+ve

'+' indicates present, '-'indicates absent

Figure 3: Anthelmintic activity of ethanolic extract of *Musa sapientum* stem before experiment

Figure 4: Anthelmintic activity of ethanolic extract of *Musa sapientum* stem after experiment

Table 2: Anthelmintic activity of ethanolic extract of *Musa sapientum* stem

Group	Treatment	Concentration (mg/ml)	Paralysis (min.)	Death (min.)
1	Standard (Albendazole)	10	19.5±1.10[a]	61.25±0.85[a]
2	Low dose of extract	100	32.75±0.40[b]	93±2.40[b]
3	Medium dose of extract	200	18.25±1.70[c]	38.25±0.70[c]
4	High dose of extract	300	8.45±2.70[d]	24.5±1.10[d]
5	Control	-	-	-

All Values represents Mean±SEM; n=4 in each group

Significance of a is ****

Significance of b is ****

Significance of c is ****

Significance of d is ***

Significance of e is **

The values are expressed as mean±SEM of 4 worms. Superscript letters represents the statistical significance done by ANOVA, followed by Tukey's multiple comparison tests. [a] P<0.0001, indicates comparison of group 5 with group 1, [b] P<0.0001, indicates comparison of group 1 with group 2, [c] P-0.0002, indicates comparison of group 1 with group 3, [d] P-0.0364, indicates comparison with group 1 with group 4 and [e] P<0.001, indicates comparison with group 5 with group 4.

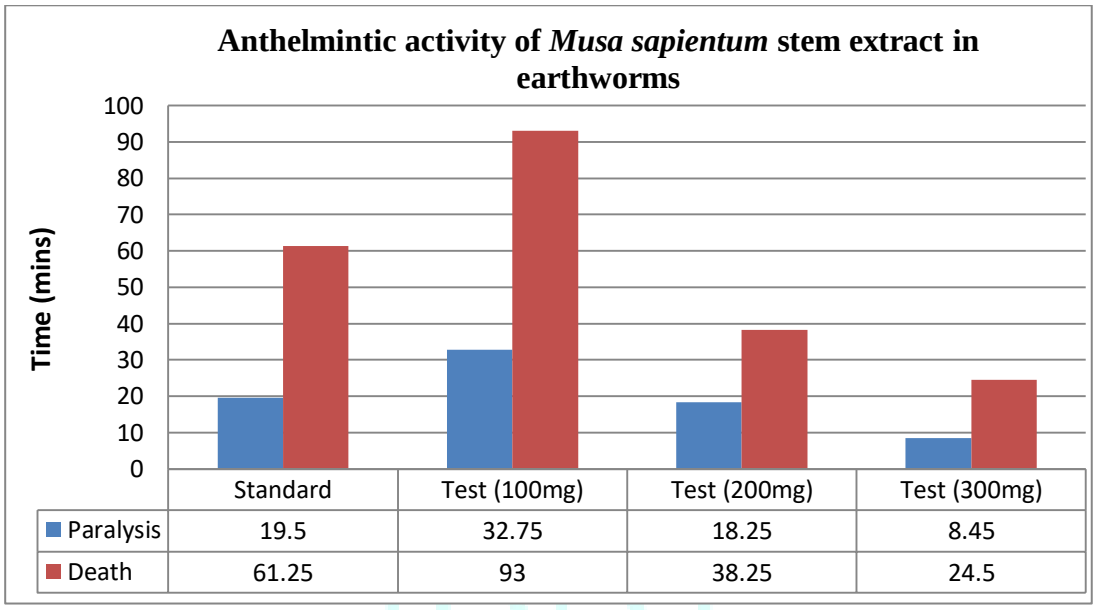

Figure 5: Anthelmintic activity of ethanolic extract of *Musa sapientum* stem

DISCUSSION

Musa sapientum was collected and dried for a week. The ethanolic extract was prepared by subjecting to soxhlet apparatus for 8-12 hours. The percentage yield of ethanolic extract was found to be 33.33% w/w. Phytochemical screening was performed on ethanolic extract of *Musa sapientum.* It shows the presence of the presence of carbohydrates, alkaloids, glycosides, phenolic compounds, tannins, flavonoids and saponin glycosides compounds in ethanolic extracts of plants. The presence of some of these phytochemical constituents may produce anthelmintic activity. The evaluation of anthelmintic activity was done. Five groups were taken consists of 4 earthworms in each group. Two parameters were observed that is time of paralysis and time of death. When the three concentrations of extract were compared with standard drug it shows activity in a dose-dependent manner showing maximum efficacy at high dose than to the medium dose followed by low dose.

The statistical values for time of paralysis of ethanolic extract of *Musa sapientum* stem is it represents 19.5±1.10, 8.45±2.70, 18.25±1.70, 32.75±0.40 standard drug, high dose, medium dose, low dose respectively.

The statistical values for time of death of ethanolic extract of Musa sapientum leaf is it represents 61.75±0.85, 24.5±1.10, 38.25±0.70, 93±2.40 standard drug, high dose, medium dose, low dose respectively. Further studies should be done to identify the active constituents responsible for the anthelmintic activity.

CONCLUSION

From our observations, higher concentration of extract produced paralytic effects much earlier and the time taken for death was shorter when compared with other two concentrations. Ethanolic extract of *Musa sapientum* shows anthelmintic activity in dose-dependent manner showing maximum efficacy at high dose (300mg/ml concentration). Anthelmintic activity of the extract was compared with the standard Albendazole. From the above results, we can conclude that *Musa sapientum* stem extract exhibited significant anthelmintic activity; therefore further study must be carried to know the active chemical constituents responsible for anthelmintic activity.

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AUTHORS CONTRIBUTION

All the authors contributed equally.

CONFLICT OF INTEREST

Author declares that there is no conflict of interest to disclose.

SPONSORSHIP

Nil.

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