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PHYTOCHEMICAL AND ANTHELMINTIC SCREENING OF *MORINGA OLEIFERA* STEMETHANOLIC EXTRACT

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ABSTRACT

Helminthic infections are the most common infection in human beings affecting a large proportion of the world's population. Anthelmintic (Anthelmintic) drugs are the drugs which are used to kill or reduce the number of helminthic parasites in the intestinal tract or tissues of the body. *Moringa oleifera* belonging to the moringaceae family is a highly valued plant, distributed in many countries of the tropical and subtropical. The standard drugs such as piperazine citrate, albendazole, mebendazole and 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. The plant which shows muscle relaxant property may also shows antihelminthic activity. So we have selected *Moringa oleifera* which is an herbal drug and shows muscle relaxant property. Hence the present study was undertaken for phytochemical evaluation and to test the antihelminthic activity of ethanolic extract of *Moringa oleifera* stem. Preliminary phytochemical investigation indicates the presence of alkaloids, glycosides, flavonoids, steroids, carbohydrates and tannins. Indian adult earthworms (*Pheretima posthuma*) were used to study antihelminthic activity. The activity was checked in ethanolic extract with three different concentrations (100, 200, and 400mg/ml) and compared with the standard drug albendazole (40mg/ml) and control as distilled water. The result was expressed in the terms of paralysis time and death time of worms. Ethanolic extract of *Moringa oleifera* stem shows antihelminthic activity in dose dependent manner and maximum efficacy is seen at 400mg/ml concentration. Hence it was concluded that ethanolic extract of *Moringa oleifera* stem have antihelminthic activity

Keywords; *Moringa Oleifera*, Earthworms, Anthelmintic Activity, Albendazole, Paralysis, *Pheretima Posthuma*

INTRODUCTION

Anthelmintic or antihelminthics are the antiparasitic drugs that expel parasitic worms (helminths) and other internal parasites from the body by killing them and without causing significant damage to the host. They are also called as vermicides (those that kills) or vermifuges (those that stun). Anthelmintics are used to treat people who are infected by helminths, a condition called helminthiasis. These drugs are also used to treat infected animals. At the present time there are effective and broad spectrum of agents which cure or control infections caused by flukes and intestinal helminths. Due to the use of unsafe and repeated dose the parasite develop resistance towards the commercially available drugs.

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 helminth. They are nematodes and platyhelminths. Nematodes are also known as roundworms which includes the intestinal worms and filarial worms. The common roundworm infection is ascariasis. About 10 % of the developing world is infected with intestinal worms, according to world health organization (WHO). Ascariasis is an infection of the small intestine caused by a species of roundworm called *Ascaris lumbricoides*. The symptoms like nausea, vomiting, irregular stools, visible worms in the stool, stomach or abdominal pain and weight loss is seen in ascariasis. Some people with a large infection may have other symptoms such as fatigue and fever¹.

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The world health organization reveals that over two billion people suffering from parasitic worm infection². It is estimated that by the year 2025, about 57% of the population in developing countries will be influenced by parasitic worm infection³. The prevalence of parasitic helminths typically displays a negative binomial distribution with in an infected population such that relatively few persons carry heavy parasite burdens. Without treatment, those individual are likely to become ill and to perpetuate infection within their community⁴.

Anthelmintic are drugs that may act locally to expel worms from the GIT or systemically to eradicate adult helminths or development forms that effect organs and tissue of the body⁵ This helminths infection also causes acute as well as chronic ill health among the various human being and cattle's. More than half of the population is suffering from the worm infection and mostly cattle's suffers from this worm infection⁶. In most of the developing countries the major health concern is helminths infection because they predispose humans to other infection such as fungal and bacterial infections⁷. Intestinal infections with worm can easily treated because it can easily be killed by the drug and this drug needs not to be absorbed when given by oral route⁸.

This anthelmintic drug sometimes produces some side effects. This side effect includes abdominal pain, loss of appetite, nausea, vomiting, headache, diarrhoea⁹. Anthelmintic produces from the natural sources play key role in the treatment of this parasite infection¹⁰. In ethno medicine (study of the traditional medicine practice by various ethnic groups) at least 80% of the world population in developing countries uses plant material as there source of primary health care¹¹. Due to this it increases problems of development of resistance in helminths against anthelmintic. So the medicinal plants are used for anthelmintic activity¹². There is an increase in the anthelmintic resistance and the impact of conventional anthelmintic on the environment. So it is important to have alternative method against gastrointestinal nematode.

Moringa oleifera is the most widely cultivated spices belonging to the family moringaceae. It is the only genus in the family moringaceae. *Moringa oleifera* is the fast-growing, drought-resistant tree and it is widely cultivated



in tropical and subtropical area. It is native to Himalayas in north-western India. The leaves and young seeds pods of *Moringa oleifera* are used as vegetables. It is used in water purification and in herbal medicine. The leaf powder is used for washing hands. The plant which shows muscle relaxant property may also shows anthelmintic activity. So we have selected *Moringa oleifera* which is an herbal drug and shows muscle relaxant property.

Figure 1: *Moringa oleifera* tree.
Botanical classification:

Kingdom	Plantae
Class	Angiosperms
Order	Brassicales
Family	Moringaceae
Genus	<i>Moringa</i>
Species	<i>Oleifera</i>
Bionominal Name	<i>Moringa oleifera</i>

Table 1: Scientific classification of *Moringa oleifera*.

MATERIALS AND METHODS:

Plant collection:

The fresh stem of the plant was collected in the month of November and December from the local areas of Secunderabad, Telangana, India.

Preparation of extract:

- The stems were collected and washed thoroughly in water, chopped, air dried for a week and pulverized in electric grinder.
- 150 gm. of the powder subjected to soxhlet apparatus using ethanol as solvents for 8-12 hrs.
- The extract was stored at 4 °C until used.

- The prepared extract is used for the anthelmintic activity.

Phytochemical evaluation:

It is performed on plant extract or separated phytoconstituents to explore the possible primary and secondary active constituents present in the extract or phytochemicals. It includes:-

- Screening for primary metabolites
- Screening for secondary metabolites

Qualitative photochemical screening for primary metabolites:-

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

1. Test for carbohydrates: -

About 50mg of the extract is dissolved in 5ml distilled water and filtered. The filtrate is tested for the presence of carbohydrates.

Molisch test:-

2ml of filtrate, 2drops of alcoholic solution of α – naphthol is added. Mixture is shaken well and 1ml concentrated H_2SO_4 is added slowly along the sides of test tube and is observed for colour. The formation of violet ring at the junction of two liquids indicates the presence of carbohydrate.

Fehling's test:-

2ml of filtrate is neutralized with alkali. The mixture is heated with 1ml of Fehling's solution A and B each and observed for precipitate formation. Red precipitate formed indicates the presence of reducing sugars.

Barfoed's test:-

To 1ml of filtrate, 1ml of barfoed's reagent is added and heated on a boiling water bath for 2 minute and observed for precipitate formation. Red precipitate formed indicates the presence of reducing sugars.

Benedict's test:-

To 0.5ml filtrate, 0.5ml benedicts reagent is added. The mixture is heated on a boiling water bath for 2 minutes and observed for precipitate formation. Formation of orange red precipitate indicates the presence of reducing sugars.

2. Test for proteins and amino acids:-

About 100mg of extract is dissolved in 10ml of distilled water, filtered through wattmann number 1 filtrate paper and filtrate subjected to test for proteins and amino acids.

Millon's test:-

To 2 ml of filtrate, 2ml millons reagent is added heated to boil and observed for precipitate. The precipitate is again heated and observed formation of white precipitate which turns to red upon heating indicates the presence of proteins and amino acids.

Biuret test:-

To 1ml of filtrate. 1ml 10% NaOH solution is added heated to boil. To this a drop of $CuSO_4$ is added and observed for colour.

Formation of purple violet colour indicates the presence of proteins.

Ninhydrin test:-

To 2ml of filtrate, few drops of 0.5N ninhydrin reagent is added and boiled for few minutes observed for colour. Formation of violet-blue colour indicates the presence of amino acids. Test of fixed oils and fats :- Saponification

test:-

Treat the extract with few drops of 0.5N alcoholic KOH 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 alkali indicates the presence of fatty material.

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 present in it.

3. Test for alkaloids: -

About 50mg of solvent free extract is dissolved in the same solvent used for extraction and filtered. The filtrate is tested for the presence of alkaloids.

Mayer's test: -

To 0.5ml of filtrate, 2drops of mayer's reagent (solution of potassium mercuric iodide) is added along the sides of the test tube and observed for precipitate. The formation of creamy precipitate indicates the presence of alkaloids.

Wagner's test: -

To 0.5ml of filtrate, 2 drops of wagner's reagent (solution of iodine in potassium iodide) is added along the sides of the test tube and observed for the precipitate. The formation of reddish brown precipitate indicates the presence of alkaloids.

Dragendroff's test: -

To 0.5ml of filtrate, 2drops of dragendroff's reagent is added and observed for precipitate. The formation of prominent reddish brown colour precipitate indicates the presence of alkaloids.

Hager's test: -

To 0.5ml of filtrate, 1ml of hager's reagent (saturated picric acid solution) is added and observed for precipitate. Formation of prominent yellow colour precipitate indicates the presence of alkaloids.

4. Test for glycosides: -

For detection of glycosides about 50mg of extract is hydrolysed with concentrated HCl for 2hrs on a water bath and filtered.

The hydrolyzed is subjected to following test.

Bortragers test: -

To 2ml of hydrolysate, 3 ml of chloroform is added and shaken well. To the separated chloroform layer, 1ml of 10% ammoniasolution is added and observed for colour. Formation of pink colour indicates the presence of anthraquinone glycosides.

Keller-killiani test: -

about 50mg of the extract is dissolved in 2 ml of glacial acetic acid and 2drops of 5% ferric chloride solution is added and mixed to this 1 ml of H_2SO_4 is added, reddish brown colour appears at the junction of two liquid layers and the upper layer appears bluish green colour indicating the

presence of steroidal glycosides. Test for steroids and terpenoids: - Libermann-Burchard's test: -

To the 50 mg of extract dissolved in 2 ml of chloroform is treated with 2 drops of acetic anhydride, 2 drops of concentrated H₂SO₄ is then added along the sides of the test tube and observed for colour. Red, pink or violet colour at the junction of the liquids indicates the presence of steroids and triterpenoids and their glycosides.

Salkowski test: -

50 mg of extract in 2 ml of CHCl₃ is treated with 2 drops of concentrated H₂SO₄, shaken well and allowed to stand and observed for colour. The formation of yellow coloured layer indicates the presence of triterpenes and formation of reddish brown coloured layer indicates the presence of steroids.

Test f₃ test: -

About 50 mg of extract is dissolved in 2 ml of distilled water and then 2 drops of neutral 5% FeCl₃ solution is added and observed for colour. Formation of blue, green or black colour indicates the presence of phenolic compounds and tannins.

Lead acetate test: -

About 50 mg of extract is dissolved in 2 ml of distilled water and to this 3 ml of 10% lead acetate solution is added and observed for the precipitate. The formation of white precipitate indicates the presence of phenolic compounds and tannins.

Bromine water test: -

About 50 mg of the extract is dissolved in 2 ml of distilled water. 1 ml of bromine water is added and observed for the decolouration of bromine water. Discoloration of bromine water indicates the presence of phenolic compounds and tannins.

Test for flavonoids: - Schinoda test: -

10 mg of extract is dissolved in 2 ml of alcohol, to these 2 fragments of magnesium turnings and 0.5 ml of concentrated HCl were added and observed for the colour. Formation of magenta colour/ crimson red colour indicates the presence of flavonoids.

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 the colouration. 2 drops of dilute HCl is added and again observed for the discolouration. The formation of an intense yellow colour which turns to colourless on addition of dilute HCl indicates the presence of flavonoids.

5. Test for saponin glycosides: -

Saponin glycosides contain either steroids or triterpenoids aglycone and therefore they always give positive Libermann-Burchard's test. Saponin glycosides give positive results with foam test and haemolysis test.

Foam test: -

Shake 2 ml solution of test sample in a test tube for half minute. Stable foam is formed indicating presence of saponin glycosides.

Haemolysis test: -

Treat 2 ml solution of test sample prepared in normal saline with 0.2 ml of blood in normal saline and mix well. Saponins cause complete haemolysis of the blood.

Worm collection:

Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal saline to remove all faecal matter which was used for the antihelminthic study. The earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used for all the experimental protocol due to their anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.

METHOD:

- Five groups of approximately equal sized Indian earthworms consisting of four earthworms in each group were released into 10 ml of desired formulation.
- Group first serve as control, receive only water (negative). Group second serve as standard receive standard drug albendazole (positive) of 400 mg concentration. Group third serve as low dose of extract. Group fourth serve as medium dose and Group fifth serve as high dose.
- Observations were made for the time taken to paralysis and death of individual worms.
- Maximum time is 120 minutes.
- Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when the worms lost their motility followed with fading away of their body colour.

RESULTS AND DISCUSSION

Preliminary phytochemical screening has shown the presence of carbohydrates, alkaloids, glycosides, steroids, terpenoids, tannins and phenolic compounds in ethanolic extracts of plants was illustrated in Table 2.

Table 2: Phytochemical screening for ethanolic extract of *Moringa oleifera* stem.

TEST

Test for carbohydrates

- Molish's test
- Fehling's test
- Benedict's test
- Barfoed's test

Test for proteins and amino acids

- Million's test
- Biuret test
- Ninhydrin test

Test for fixed oils and fats

- Saponification test

Test for alkaloids	
• Mayers test	+ve
• Wagners test	+ve
• Dragendroff's test	+ve
• Hagers test	+ve
Test for glycosides	
• Kellar killani test	+ve
• Borntragers test	-ve
Test for steroids and terpenoids	
• Liebermann-burchard test	+ve
• Salkowski test	+ve
Test for tannins and phenolic compounds	
• Ferric chloride test	+ve
• Lead acetate test	+ve
• Bromine water	+ve
Test for flavonoids	
• Shinoda test	-ve
• Alkaline reagent test	-ve
Test for saponin glycosides	
• Foam test	-ve
• Haemolysis test	-ve



control



albendazole



low dose



medium dose



high dose

Figure 2: photographs of antihelminthic activity of ethanolic extract of *Moringa oleifera* stem before experiment.



control



albendazole



low dose



medium dose



high dose

Figure 3: Photographs of antihelminthic activity of ethanolic extract of *Moringa oleifera* stem after experiment.

Table 3: Time of paralysis.

Group	Treatment	Concentration	Paralysis(min)
1	Standard (albendazole)	40mg	71.25±1.10 ^a
2	Low dose of extract	100mg	89.25±0.47 ^c
3	Medium dose of extract	200mg	84.75±1.70 ^d
4	High dose of extract	400mg	80.5±2.02 ^{bc}
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 5 with group 4, ^c P<0.0001, indicates comparison of group 1 with group 2, ^d P<0.0001, indicates comparison with group 1 with group 3 and ^e P-0.0012, indicates comparison with group 1 with group 4.

Antihelminthic activity of ethanolic extract of *Moringa oleifera* stem (time of paralysis)

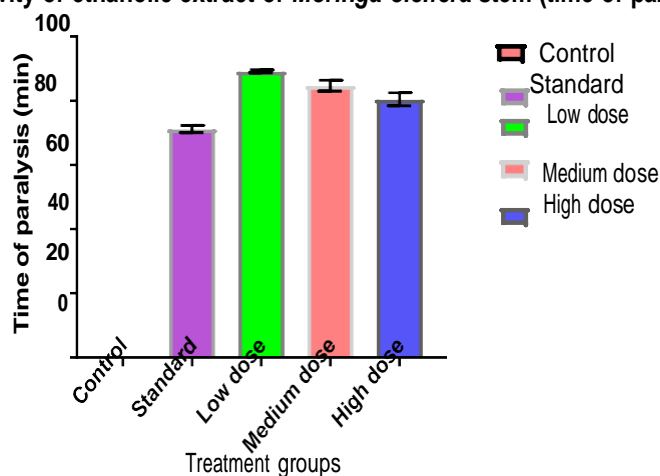


Figure 4: Paralysis time.

Table 4: Time of death.

Group	Treatment	Concentration	Death (min)
1	Standard (albendazole)	40mg	80.75±0.85 ^a
2	Low dose of extract	100mg	104.3±2.17 ^b
3	Medium dose of extract	200mg	91±0.70 ^c
4	High dose of extract	400mg	86.25±1.10 ^{dc}
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.

Anthelmintic activity of ethanolic extract of *Moringa oleifera* stem (time of death)

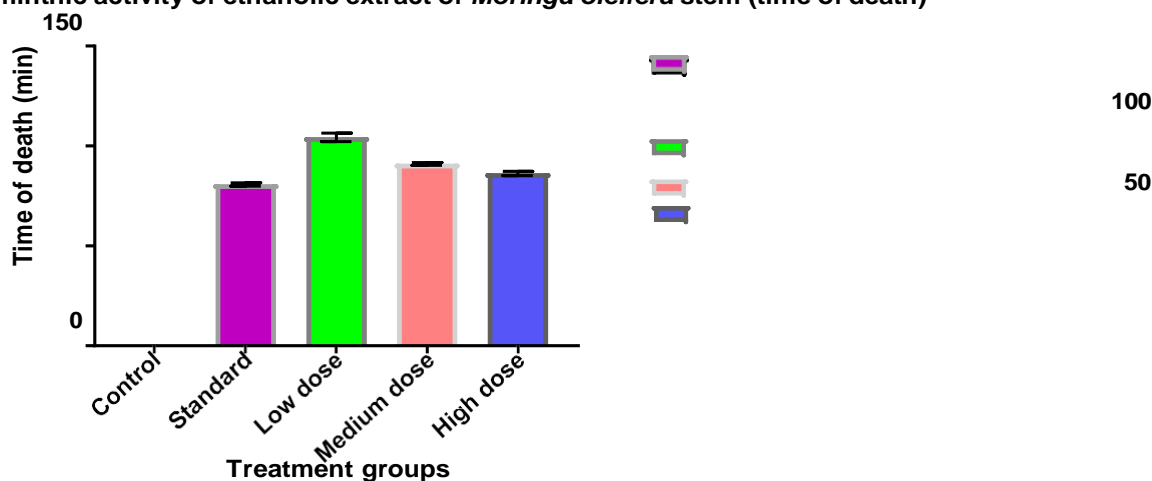


Figure 5: Death time.

Moringa oleifera 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 3.33% w/w. Phytochemical screening was performed on ethanolic extract of *Moringa oleifera*. It shows the presences of carbohydrates, alkaloids, glycosides, steroids and terpenoids, tannins and phenolic compounds (Table 2). 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 was illustrated in Table 3 and 4 respectively. 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 is $71.25 \pm 1.10 > 80.5 \pm 2.02 > 84.25 \pm 1.70 > 89.25 \pm 0.47$ it represents standard drug, high dose, medium dose, low dose respectively. The statistical values for time of death is $80.75 \pm 0.85 > 86.25 \pm 1.10 > 91 \pm 0.70 > 104.3 \pm 2.17$ it represents 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 *Moringa oleifera* shows anthelmintic activity in dose-dependent manner showing maximum efficacy at high dose (400mg/ml concentration). Anthelmintic activity of the extract was compared with the standard drug albendazole. From the above results, we can conclude that *Moringa oleifera* 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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