ISSN : 2347 - 2243

Indo - American Journal of Life Sciences and Biotechnology




www.iajlb.com
Email : editor@iajlb.com or iajlb.editor@gamil.com

Indo-Am. J. of LifeSc & Bt.,2020- *ISSN 2347-2243 www.iajlb.com*

Indo-American Journal of Life Sciences and Biotechnology

Vol.8, Issue.4, Oct 2020

PHYTOCHEMICAL AND ANTHELMINTIC SCREENING OF *MORINGA OLEIFERA* STEMETHANOLIC EXTRACT

R. Mohana Priya, K. Sara Sirisha, Dr. G. Nagarajan

ABSTRACT

Helminthic infections are the most common infection in human beings affecting a large proportion of the world's population. Antihelminthic (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 indicatesthe 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). Antihelmintics 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 helminthis. 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 fever[1].

Pharmaceutical Chemistry
Dr.K.V. Subba Reddy Institute of Pharmacy
(Approved by AICTE,P.C.l New Delhi& Permanently Affiliated to JNTUA Anantapuramu
MOU with Government General Hospital &KMC, K urnool

The world health organization reveals that over two billion people suffering from parasitic worm infection[2]. It is estimated that by the year 2025, about 57% of the population in developing countries will be influenced by parasitic worm infection[3]. 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 community[4].

Antihelminthic 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 body[5] 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'ssuffers from this worm infection[6]. 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 infections[7]. 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 route[8].

This antihelminthic drug sometimes produces some side effects. This side effect includes abdominal pain, loss of appetite, nausea, vomiting, headache, diarrhoea[9]. Antihelminthic produces from the natural sources play key role in the treatment of this parasite infection[10]. 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 care[11]. Due to this it increases problems of development of resistance in helminths against antihelminthic. So the medicinal plants are used for antihelminthic activity[12]. There is an increase in the antihelminthic resistance and the impact of conventional antihelminthic 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, drough-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 antihelminthic 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	*Moringa*
Species	*Oleifera*
Bionominal Name	*Moringa oleifera*

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 ^{0}C until used.
- The prepared extract is used for the antihelminthic activity.

Phytochemical evaluation:

It is performed on plant extract or separated phytoconstituents to explore the possible primary and secondary activeconstituents 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 – napthol 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 forprecipitate 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 subjectedto 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 heatedand 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. Formationof 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 phenopthalein 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 ofprominent 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.

Borntragers 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-burchards test: -

To the 50 mg of extract dissolved in 2 ml of chloroform is treated with 2drops of acetic anhydride, 2 drops of concentrated H_2SO_4 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 tritepenoids and their glycosides.

Salkowski test: -

50mg of extract in 2ml of $CHCl_3$ is treated with 2 drops of concentrated H_2SO_4, 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 f_3 test: -

About 50mg of extract is dissolved in 2 ml of distilled water and then 2 drops of neutral 5% $FeCl_3$ solution is added and observed for colour. Formation of blur, green or black colour indicates the presence of phenolic compounds and tannins.

Lead acetate test: -

About 50mg of extract is dissolved in 2 ml of distilled water and to this 3ml 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 forlavonoids :-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 wereadded and observed for the colour. Formation of magenta colour/ crimson red colour indicates the presence of flavonoids.

Alkaline reagent test: -

10mg of extract is dissolved in 2 ml of water and treated with 1 ml of 10 % ammounium 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 400mg concentration. Group third serve as low dose of extract. Group forth 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

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

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 [be]
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.

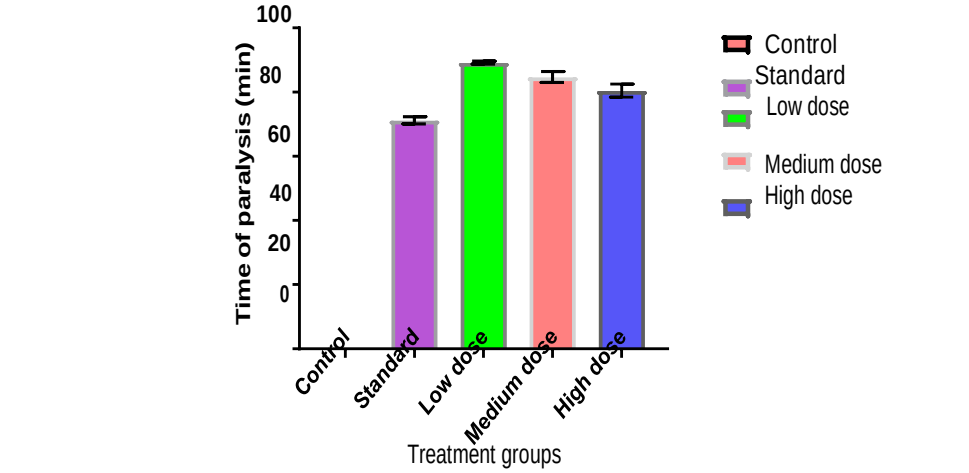

Figure 4: Paralysis time.

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 [de]
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.

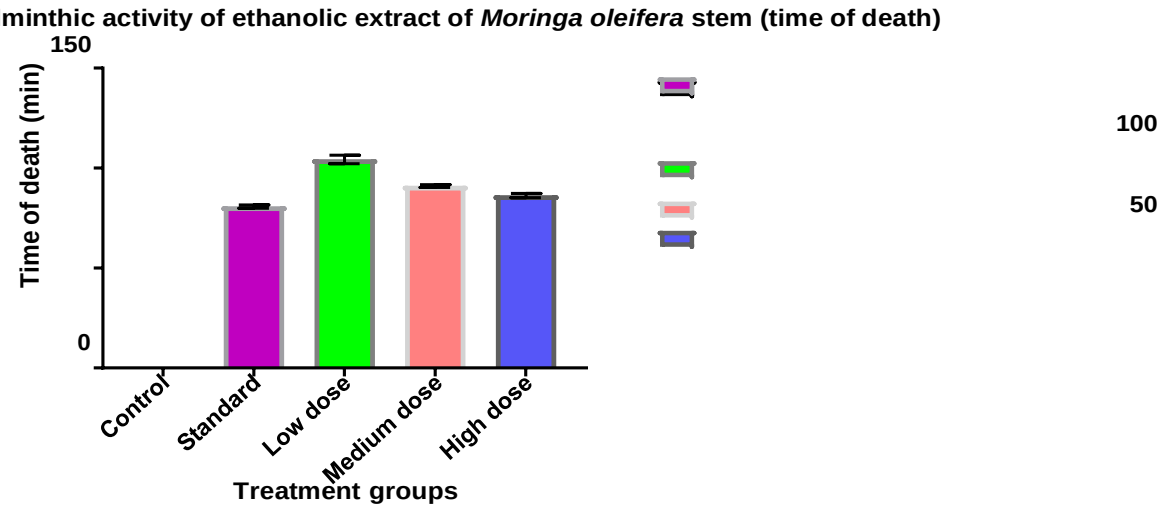

Figure 5: Death time.

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 antihelminthic activity. The evaluation of antihelminthic 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±1.10 > 80.5±2.02 > 84.25±1.70 > 89.25±0.47 it represents standard drug, high dose, medium dose, low dose respectively. The statistical values for time of death is 80.75±0.85 > 86.25 ±1.10 > 91±0.70 > 104.3±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 antihelminthic 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 antihelminthic activity in dose-dependent manner showing maximum efficacy at high dose (400mg/ml concentration). Antihelminthic 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 antihelminthic activity; therefore further study must be carried to know the active chemical constituents responsible for antihelminthic activity.

REFERENCES

1. Hotez PJ, Brindley PJ, Bethony JM, King CH, and Pearce EJ, Jacobson J. Helminth infections: the great neglected tropicaldiseases. The Journal of Clinical Investigation 2008; 118: 1311-1321.
2. Mulla WA, Thorat VS, Patil RV, Burade KB. Anthelmintic activity of leaves of *Alocasia indica* Linn. International Journal ofPharm Tech Research 2010; 2(1): 26-30.
3. Clewes CAN, Shaw C. Parasites. British Medical Bulletin 2000; 56(1): 193-208.
4. Barnabas BB, Mann A, Ogunrinola TS, Anyanwu PE. Screening for Anthelminthic activities from extracts of *Zanthoxylum zanthoxyloides*, *Neocarya macrophylla* and *Celosia laxa* against ascaris infection in rabbits. International Journal of Applied Research in Natural Products 2010; 3(4): 1- 4.
5. Clewes CAN, Shaw C. Parasites. British Medical Bulletin 2000; 56(1): 193-208.
6. Mohamed AAER, Ismail MAN, Mohamed AO, Moustafa FM. Cytotoxic effects of albendazole, antiparasitic drug, on the liver ofthe rat: Subchronic study. Egyptian journal of Biology 1999; 1: 16-29.
7. Dwivedi A, Dwivedi S, Sitoke AK, Patel R, Jhade D. Anthelmintic activity of a Polyherbal preparation. Ethnobotanical Leaflets2009; 13: 259-262.
8. Khandelwal, KR (2008), "Practical Pharmacognosy", 19th Ed, Nirali Prakashan, Pune, 149-156.
9. Aremu OA, Fawole JC, Chukwujekwu ME, Light JF, Finnie J, Staden V. *In vitro* antimicrobial, anthelmintic and cyclo- oxygenase inhibitory activities and phytochemical analysis of *Leucosidea sericea*. Journal of ethnopharmacology 2010; 131: 22- 27.
10. Velan SS, Suresh A, Suresh V, Kumar SN, Bright AJ, Karthikeyan P. In vitro anthelmintic activity of *Samanea saman* (Merr.) bark. Journal of Pharmacy Research 2010; 3(12), 2977-2978
11. Devi K, Indumathy S, Rathinambal V, Uma S, Kavimani S, Balu V. Anthelminthic Activity of Asta Churna. International Journal of Health Research 2009; 2(1): 101-103.
12. Aswar M, Aswar U, Watkar B, Vyas M, Wagh A, Gujar KN. Anthelmintic activity of *Ficus benghalensis*. International Journal of Green Pharmacy, 2008; 2: 170-172
13. Jabbar A, Raza MA, Iqbal Z, Khan MN. An inventory of the ethnobotanicals used as anthelmintics in the southern

Punjab (Pakistan). Journal of Ethnopharmacology 2006; 108: 152-154.
14. Iqbal Z, Lateef M, Ashraf M, Jabbar A. Anthelmintic activity of *Artemisia brevifolia* in sheep. Journal of Ethnopharmacology 2004; 93: 265-268.
15. Armando caceres, Ofyluz Cabrera, Ofelia morales, Patricia mollinedo, Patrica meundia. "Pharmacological properties of *Moringa oleifera*." Journal of ethnopharmacology 1991; 33(3): 213-216.
16. Armando caceres, Amarillis saravia, Sofia rizzo, Lorena zabala, edy de leon, Federico nave, "pharmacological properties of *Moringa oleifera*, screening for antispasmodic, anti-inflammatory and diuretic activity." Journal of ethnopharmacology 1992; 36(3): 233-237.
17. Sudarshana borah, Bibhuti bhusan kakati, Kabita mahato, Mritunjay kumar, "Investigation of *in-vitro* antihelminthic activity of *Calamus leptospadix* Griff. Shoot in Indian adult earthworm (*Pheretima posthuma)*" Journal of applied pharmaceutical science. 2013; 3(6): 156-159.
18. Narender Boggula et. al. Phytochemical evaluation and *in vitro* anti bacterial activity of dried seeds of *Abrus precatorius.*
International journal of pharmaceutical sciences review and research, 2017, 44(1):101-107.
19. Hennessy DR. Modifying the formulation or delivery mechanism to increase the activity of anthelmintic compounds. VeterinaryParasitology (1997); 72: 367-390.
20. Narender Boggula, Narender Bojjala, Thriveni Mandula, Shangati M Priyanka. Phytochemical investigation and *in-vitro* antibacterial activity of dried leaves of *Aerva lanata*. Indo American Journal of Pharmaceutical Sciences, 2016, 3(6):637-643.
21. Washington JA. Baron s, et al, eds. Principles of diagnosis : serodiagonosis in : baron's medical MB.(4^{th} edn) university of jexasmedical branch.(1996)
22. Nikhal SB, Dambe PA, Ghongade DB, Goupale DC. Hydroalcoholic extraction of *Mangifera indica* (leaves) by Soxhletion.International Journal of Pharmaceutical Sciences (2010); 2 (1): 30-32.
23. Roopashree TS, Dang R, Rani SRH, Narendra C. Antibacterial activity of anti-psoriatic herbs: *Cassia tora, Momordica charantia*
and *Calendula officinalis*. International Journal of Applied Research in Natural Products 2008; 1(3): 20-28.
24. Obasi NL, Egbuonu ACC, Ukoha PO, Ejikeme PM. Comparative phytochemical and antimicrobial screening of some solvent extracts of *Samanea saman* pods. African journal of pure and applied chemistry 2010; 4(9): 206-212.
25. Audu SA, Mohammed I, Kaita HA. Phytochemical screening of the leaves of *Lophira lanceolata* (Ochanaceae). Life Science Journal 2007; 4(4): 75- 79.
26. Narender Boggula, Ananda Kumar Chettupalli, Swetha Reddy Naram reddy, Vasudha Bakshi. Anti diabetic effect of *Alstonia scholaris* Linn. bark in Alloxan induced diabetic rats. Journal of Global Trends in Pharmaceutical Sciences, 2017, 8(1):3590 – 3598.
27. S.G. Mahajan, A.A.Mehta. J Immunotoxicol. 4(4), 287-294. [87] T. Rastogi, V. Bhutda, K. Moon, P.B. Aswar, S.S. Khadabadi. Asian J. Research Chem. 2009, 2(2), 181-182.
28. Narender Boggula. Evaluation of *in vitro* anti oxidant activity of dried roots of *abrus precatorius.* World Journal of Pharmaceutical Research, 2017, 6(3):1619-1628.
29. Prashant Tiwari , Bimlesh Kumar, Manoj Kumar, Mandeep Kaur , Jiban Debnath , Pardeep Sharma. Comparative anthelmintic activity of aqueous and ethanolic stem extract of *Tinospora cordifolia*. International Journal of Drug Development & Research,
2011; 3(1): 70-83.
30. Sajith Mohandas, Sreekumar T.R, Vishnu Prakash. Anthelmintic activity of vidangadi churna. Asian J Pharm Clin Res, 2013; 6(3): 94-95.
31. Yashaswini.T, Akshara, A.M.Krupanidhi , Prasad K, Kumar M.D, Akshaykumar K. Anthelmintic activity of seed extracts of
Artocarpus heterophyllus. IOSR Journal of Pharmacy and Biological Sciences, 2016; 11(5): 19-23.
32. Mulla WA, Thorat VS, Patil RV, Burade KB. Anthelmintic activity of leaves of *Alocasia indica Linn*. International Journal of Pharm Tech Research 2010; 2(1): 26-30.
33. Dwivedi A, Dwivedi S, Sitoke AK, Patel R, Jhade D. Anthelmintic activity of a Polyherbal preparation. Ethnobotanical Leaflets 2009; 13: 259-262.
34. Velan SS, Suresh A, Suresh V, Kumar SN, Bright AJ, Karthikeyan P. *In vitro* anthelmintic activity of Samanea saman (Merr) bark. Journal of Pharmacy Research 2010; 3(12), 2977-2978.
35. Devi K, Indumathy S, Rathinambal V, Uma S, Kavimani S, Balu V. Anthelminthic Activity of Asta Churna. International Journal of Health Research 2009; 2(1): 101-103.
36. Kubde MS, Khadabadi SS, Farooqui IA, Deore SL. *In-vitro* anthelmintic activity of *Colocasia esculenta*. Der Pharmacia Lettre 2010; 2(2): 82-85.
37. Vidyadhar S, Saidulu M, Gopal TK, Chamundeeswari D, Rao U, Banji D. In vitro anthelmintic activity of the whole plant of *Enicostemma littorale* by using various extracts. International journal of applied biology and pharmaceutical technology 2010; 1(3): 1119-1125.
38. Roy H. Preliminary phytochemical investigation and anthelmintic activity of *Acanthospermum hispidum* DC. Journal of Pharmaceutical Science and Technology 2010; 2(5): 217-221.
39. Cruz ASP. Anthelmintic effect of *Solanum lycocarpum* in mice infected with *Aspiculuris tetraptera*. The journal of American science 2008; 4(3): 75-79.
40. Narender Boggula, Himabindu Peddapalli. Phytochemical analysis and evaluation of *in vitro* anti oxidant activity of *Punica granatum* leaves. International Journal of Pharmacognosy and Phytochemical Research, 2017, 9(8); 1110-1118.