

ESTIMATION OF IN VITRO ANTIOXIDANT, ANTI-INFLAMMATORY AND ANTHELMINTIC ACTIVITY OF FLOWER OF *Moringa Oleifera*

KEERTHANA.M¹, MADHUMITHA.K.R¹, Dr.P.DHASARATHAN*, Mr.V.SARAVANA PERUMAL²

¹Department of Biotechnology, Prathyusha Engineering College, Chennai

²Abbes biotech pvt ltd, kolathur, chennai

* Department of Biotechnology, Prathyusha Engineering College, Chennai

* Corresponding author

ABSTRACT

Moringa Oleifera is a fast-growing, drought-resistant tree of the family Moringaceae – (Muchenje et al., 2011), native to the India subcontinent. *Moringa Oleifera* is a plant that has been praised for its health benefits for thousands of years. Ethanolic extract of the dry pulverized flowers of *M. Oleifera* were obtained by Soxhlet extraction method. The ethanolic flower extract was subjected to preliminary phytochemical screening as the reported methods. The phytochemical screening of flower extract showed the presence of carbohydrates, proteins, glycosides, alkaloids, flavonoids, terpenoids, steroids and phenol. DPPH was used to determine in-vitro antioxidant activity and anti-inflammatory activity of flowers was investigated by hypotonic solution induced haemolysis assay. Anthelmintic activity was tested on Indian adult earthworm *Pheretima postuma*. different concentration (20, 40, 60, 80 mg/ml) of extract were tested for anthelmintic activity by determining the time of paralysis and time of death of the worms. The ethanolic extract showed anthelmintic activity at all concentrations and its activities are well comparable with standard drug, Albendazole (positive control). Tween 20 (1%) with saline was used as negative control did not show any anthelmintic activity.

Key Words: Albendazole, Anthelmintic, Extract, *Moringa Oleifera*, Phytochemical, *Pheretima postuma*

1. INTRODUCTION

Moringa Oleifera Lam. (*M. Oleifera*), commonly known as horse-radish or drumstick tree in English, belongs to family Moringaceae. It is a small sized tree, which is native to South Asia, Africa and Arabia and is used as traditional medicine in many tropical and subtropical countries (Muchenje et al., 2011). Pharmacological studies have demonstrated that *M. Oleifera* known to possess hypoglycemic, hypotensive, anti-microbial, hepatoprotective, immunomodulatory, antioxidant and

antitumor activities (Sudha et al., 2010). These biological activities could be attributed to the presence of secondary plant metabolites present in *M. Oleifera* such as carotenoids, vitamins, minerals, amino acids, sterols, glycosides, alkaloids, flavonoids and phenolics (Mahajan, 2010). The leaf extract of *M. Oleifera* have been reported to exhibit antioxidant activity both in vitro and in vivo due to abundant phenolic acids and flavonoids (Sadiq et al., 2013). The leaves as well as flowers, roots, gums and fruits are extensively used for treating inflammation. Flowers of *M. Oleifera* are rich in calcium, potassium and antioxidants (α and γ tocopherol), and are used in human diet, mainly in the Philippines (Napoleao et al., 2012). *Lactococcus lactis*.

Antioxidants are of great importance in preventing stress that may cause several degenerative diseases (Helen et al., 2000). On the other hand, antioxidants are molecules capable of stabilizing or deactivating free radicals before they attack cells (Kaliora et al., 2006). Antioxidant activity is defined "as an limitation of the oxidation of proteins, lipids, DNA or other molecules that occurs by blocking the propagation stage in oxidative chain reactions" and primary antioxidants directly scavenge free radicals, while secondary antioxidants indirectly prevent the formation of free radicals through Fenton's reaction (Huang et al., 2005). Several antioxidant plant compounds have been found in the Flower of *Moringa Oleifera*. *Moringa Oleifera* is rich in various antioxidants, including quercetin and chlorogenic acid. DPPH is 2, 2-diphenyl-1-picrylhydrazyl, a stable free radical scavenged by antioxidants from the extract through the donation of a proton to form reduced DPPH.

Inflammation is considered as primary physiologic defense mechanism that helps the body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses. The anti-inflammatory drugs with modern system of medicine have been reported for many severe side effects. The plant part of *Moringa Oleifera* Lam. have been successfully used in many diseases of liver, spleen, kidney, stomach etc. by the traditional

communities. Since, little work has been performed regarding the anti-inflammatory activity. So, an attempt was made to explore the anti-inflammatory potential on *Moringa Oleifera* Lam. Flower.

Anthelmintics or antihelminthics are a group of antiparasitic drugs that expel parasitic worms (helminths) and other internal parasites from the body by either stunning or killing them and without causing significant damage to the host. They may also be called vermifuges (those that stun) or vermicides (those that kill). Anthelmintics are used to treat people who are infected by helminths, a condition called helminthiasis. These drugs are also used to treat infected animals. Pills containing anthelmintics are used in mass deworming campaigns of school-aged children in many developing countries. The drugs of choice for soil-transmitted helminths are mebendazole and albendazole.

2. MATERIALS AND METHODS

Plant Sample Collection And Processing

The Fresh Flower of *Moringa Oleifera* were collected during early morning from (12°49'21.3"N 79°42'28.7"E) Kanchipuram (Fig.1). The collected Flower were washed twice with distilled water to remove the dust particles. Washed flowers were shade dried for a period of 1 week, it was shade dried in order to prevent the denaturation of phytochemicals due to heat and degradation of secondary metabolites. After a period of 1 week, the flowers are completely dried and attained a crispy stage. Thus, the flowers were homogenized into powder by using mortar and pestle. The mortar and pestle were chosen to grind the flowers because the heat generation during grinding is lower compared to other grinding procedure. The obtained dry powder was about 20g and it was stored for further usage.



Fig.1 Collection of flower of *Moringa OLEIFERA*

Extraction Of Plant Material

A Soxhlet extraction (Fig.2) procedure usually involves the following steps. First, the Flower powder was packed in filter paper and placed in the thimble. Next, vapors of a fresh solvent Ethanol, produced in a

distillation flask, passed through the thimble containing the material to be extracted and are liquefied in the condenser. When the liquid reaches the overflow level in the thimble, a siphon aspirates the solution, and the liquid falls back into the distillation flask, carrying the extracted solutes into the bulk liquid. The separation of solute from solvent takes place in the distillation flask. Then solute is left in the flask and fresh solvent vapours pass back into the solid bed of sample material. The operation is repeated until complete extraction is achieved.



Fig.2 Soxhlet extraction of flower of *Moringa Oleifera*

DETERMINATION OF PLANT EXTRACT YIELD

Flower powder (20g) was packed in filter paper and placed in the thimble. Then the Soxhlet extraction is performed. The packed material was allowed to dry and weighed as 4gram. Based on dry weight, the yields of Flower extract were calculated using following equation:

$$\text{Yield (g/100 g)} = (W1 \times 100) / W2$$

where, Y= Yield (g/100 g of dry plant material)

W1 = the weight of the extract after the solvent evaporation

W2 = the weight of the dry plant material

PHYTOCHEMICAL SCREENING OF FLOWER EXTRACT OF *M.OLEIFERA*

DETECTION OF ALKALOIDS

(a) DRAGENDROFF'S TEST

2ml of Dragendroff's reagent was added to 3ml of Flower extract. Appearance of orange brown precipitates reveals the presence of alkaloids.

(b) MAYER'S TEST

To 2 mL Flower extract, 1ml of Mayer's reagent were added. Appearance of red precipitates reveals the presence of alkaloids.

DETECTION OF FLAVANOIDS

To 1ml of Flower extract, few drops of NaOH and few drops of dilute HCl was added. Appearance of yellow color reveals the presence of Flavanoids.

DETECTION OF SAPONINS

FOAM TEST:

5ml of distilled water was added to 1ml of Flower extract and agitated for 15 minutes. Appearance of 1cm foam layer at top of extract reveals the presence of saponins.

DETECTION OF TERPENOIDS

2ml of chloroform was added to the 1 ml of extract and mixture was evaporated to Dryness. Later 2 ml of conc sulphuric acid was added and heated for 2 minutes. Appearance of grayish color reveals the presence of terpenoids.

DETECTION OF GLYCOSIDES

2ml of chloroform was added to the 1ml of extract followed by gentle addition of conc sulphuric acid. Appearance of reddish brown color reveals the presence of glycosides.

DETECTION OF STEROIDS

1ml of extract was mixed with 2ml of chloroform followed by gentle addition of conc sulphuric acid. Formation of red color in the lower chloroform region reveals the presence of steroids.

DETECTION OF CARBOHYDRATES

BENEDICT'S TEST

1ml extract was mixed with 2ml of Benedict's reagent and heated for 2 minutes. Appearance of reddish brown precipitate.

DETECTION OF PROTEIN

BIURET'S TEST

2ml of Biuret reagent was mixed with 1ml extract and heated for 2 minutes. Appearance of Violet color reveals the presence of Protein.

DETECTION OF PHENOL

0.5ml of extract was treated with 5% ferric chloride. Formation of deep blue or black color indicates the presence of phenols.

DETERMINATION OF INVITRO ANTIOXIDANT ACTIVITY

DPPH ANTIOXIDANT ASSAY

The free radical scavenging activity of the *Moringa Oleifera* was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH (Blois, 1958). Reagents used are 0.2 mM DPPH, Ethanol, Ascorbic acid (Standard). Solution of DPPH in ethanol (0.1 mM) was prepared and 1.0 ml of this solution was added to 2.0 ml of *Moringa Oleifera* Flower extract at different concentrations (100–500 µg/ml). After, thirty minutes, the absorbance was measured at 517nm. Ascorbic acid was used as the positive control. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The percentage DPPH inhibition was calculated by

$$\text{Inhibition(\%)} = [1 - (\text{Abs sample} / \text{Abs control})] \times 100$$

HYDROGEN PEROXIDE SCAVENGING ASSAY

The hydrogen peroxide scavenging was determined according to the method of Ruch *et al.* (1989). Reagents used are 0.1M Phosphate buffer (pH 7.4), 4 mM H₂O₂ in phosphate buffer, Ascorbic acid (Standard). A solution of H₂O₂ was prepared in phosphate buffer and their concentration was determined spectrophotometrically from the absorption at 230 nm. Various concentrations of *Moringa Oleifera* were added to H₂O₂ and incubated for 10 min. The absorbance at 230 nm was determined against a blank containing phosphate buffer without H₂O₂. The percentage scavenging of H₂O₂ and standard ascorbic acid was calculated using the formula:

$$\text{Inhibition(\%)} = [1 - (\text{Abs sample} / \text{Abs control})] \times 100$$

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY

To evaluate the anti-inflammatory effects of the extracts, the protocol described by Padmanabhan and Jangle and Elias and Rao was used with small modifications. A volume of 1 ml of extracts (ethanolic) or of diclofenac sodium at different concentrations (100, 200, 500, and 1000 g/ml) was homogenised with 1 ml of aqueous solution of bovine serum albumin (5%) and incubated at 27°C for 15 minutes. The mixture of distilled water and BSA constituted the control tube. Denaturation of the proteins was caused by placing the mixture in a water

bath for 10 minutes at 70°C The mixture was cooling inside the ambient room temperature, and the activity each mixture was measured at 660 nm.

The following formula was used to calculated inhibition percentage

$$\text{Inhibition (\%)} = \frac{(AC - AS)}{AC} \times 100,$$

AC is the absorbance of control, and AS is the absorbance of sample

EVALUATION OF ANTHELMINTIC ACTIVITY

The earthworm *Pheretima postuma* was divided into five groups consisting of two equal sized earthworms in each group (in triplicates) was released into 30 ml of the experimental formulation kept in a petri dish. The first group served as normal control which is treated only with normal saline, second group was treated with tween 20 along with normal saline served as negative control.

The third group served as standard drug, containing albendazole at 20, 40, 60 and 80 mg/ml in tween 20 (1%) diluted with normal saline. The ethanol extracts at different concentrations (20, 40, 60 and 80 mg/ml) constituted the fourth and fifth group.

All the test solutions and standard solutions were prepared freshly before starting the experiment. The mean paralysis time was noted when no movement of earthworm could be observed and the death time was recorded in minutes after confirming that worms neither moved when shaken nor when an external stimuli given by putting the motionless worms in 50°C hot water. Deaths of the worms were confirmed when the worms were unable to move and the appearance of a white secretion and fading of their body colour around its body. The paralysis and death time were expressed as mean \pm standard error for mean (\pm SEM). $P < 0.05$ -0.01 considered as statistically significant.

3.RESULTS

PLANT SAMPLE COLLECTION AND PROCESSING

The Flower sample of *Moringa OLEIFERA* were collected and shade dried for a period of 1 week. The shade dried Flowers (Fig.3) were homogenized into powder by using Mortar and pestle. The Powdered plant flower were stored for further usage (Fig.4).



Fig.3 Shade Dried Flower



Fig.4 Powdered Flower sample of *Moringa OLEIFERA*

EXTRACTION OF PLANT MATERIAL

The Flower powder was packed in filter paper and placed in the thimble. Next, vapors of a fresh solvent Ethanol, produced in a distillation flask, passed through the thimble containing the material to be extracted and are liquefied in the condenser. Then solute is left in the flask and fresh solvent vapors pass back into the solid bed of sample material. The operation is repeated until complete extraction is achieved. The flower Extract was collected (Fig.5).



Fig.5 Flower Extract of *Moringa Oleifera*

DETERMINATION OF PLANT EXTRACT YIELD

Based on dry weight, the yields of Flower extract were calculated using following equation:

$$\text{Yield (g/100 g)} = \frac{(W1 \times 100)}{W2}$$

The weight of the extract after the solvent evaporation (W1) = 5gram

The weight of the dry plant material (W2) = 20 gram

$$\text{Yield} = \frac{500}{20}$$

Yield of flower extract = 10%

PHYTOCHEMICAL SCREENING PLANT EXTRACT

DETECTION OF ALKALOIDS

DRAGENDROFF'S TEST

2ml of Dragendroff's reagent was added to 3ml of Flower extract. Appearance of orange brown precipitates (Fig.6) was observed. Presence of Alkaloids was confirmed.



Fig.6 Detection of Alkaloids - Dragendroff's test

MAYER'S TEST

To 2 mL Flower extract, 1ml of Mayer's reagent were added. Appearance of red precipitates (Fig.7) was observed. Presence of Alkaloids was confirmed.



Fig.7 Detection of Alkaloids - Mayer's test

DETECTION OF FLAVONOIDS

To 1ml of Flower extract, few drops of NaOH and few drops of dilute HCl was added. Appearance of yellow color (Fig.8) was observed. Presence of Flavonoids was confirmed.



Fig.8 Detection of Flavonoids - Sodium Hydroxide Test

DETECTION OF SAPONINS

FOAM TEST:

5ml of distilled water was added to 1ml of Flower extract and agitated for 15 minutes. Appearance of 1cm foam layer at top of extract was not observed (Fig.9) and Absence of Saponins.



Fig.9 Detection of Saponins - Foam test

DETECTION OF TERPENOIDS

2ml of chloroform was added to the 1ml of extract and mixture was evaporated to Dryness. Later 2 ml of conc sulphuric acid was added and heated for 2 minutes. Mild grayish color was appeared (Fig.10) and presence of terpenoids was observed.



Fig.10 Detection of Terpenoids - Salkowshi's Test

DETECTION OF GLYCOSIDES

2ml of chloroform was added to the 1ml of extract followed by gentle addition of conc sulphuric acid. Appearance of reddish-brown color was observed (Fig.11.) and presence of glycosides was confirmed.



Fig.11 Detection of Glycosides - Liebermann's Test

DETECTION OF STEROIDS

1ml of extract was mixed with 2ml of chloroform followed by gentle addition of conc sulphuric acid. Formation of red color in the lower chloroform region was observed (Fig.12) and the presence of steroids was observed.



Fig.12 Detection of Steroids - Liebermann-Burchard test

DETECTION OF CARBOHYDRATES BENEDICT'S TEST

1ml extract was mixed with 2ml of Benedict's reagent and heated for 2 minutes. Appearance of reddish brown precipitate was observed (Fig.13) and Presence of Carbohydrates was confirmed.



Fig.13 Detection of Carbohydrates - Benedict's test

DETECTION OF PROTEIN

BIURET'S TEST

2ml of Biuret reagent was mixed with 1ml extract and heated for 2 minutes. Violet color was not appeared (Fig.14) and absence of Protein was confirmed.



Fig.14 Detection of Proteins - Biuret's Test

DETECTION OF PHENOL

0.5ml of extract was treated with 5% ferric chloride. Formation of black color was observed and presence of phenols was confirmed.

S.No	PHYTOCHEMICAL TEST	NAME OF THE TEST	FLOWER EXTRACT
1.	Alkaloids	Dragendroff's Test, Mayer's Test	+
2.	Flavonoids	Sodium Hydroxide Test	+
3.	Saponins	Foam test	-
4.	Terpenoids	Salkowski's Test	+
5.	Glycosides	Liebermann's Test	+
6.	Steroids	Liebermann-Burchard test	+
7.	Carbohydrates	Benedict's test	+
8.	Proteins	Biuret's Test	-
9.	Phenols	Ferric chloride test	+

Table 1 Preliminary phytochemical analysis of *M. Oleifera* flower extract.

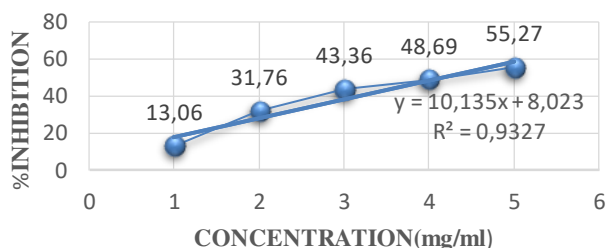
+ → Present ; - → Absent

INVITRO ANTIOXIDANT ACTIVITY

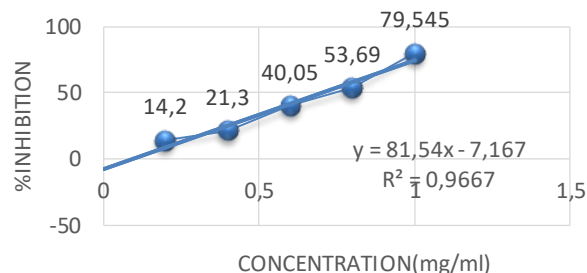
DPPH FREE RADICAL SCAVENGING ACTIVITY

DPPH is a stable free radical scavenged by antioxidants from the extract through the donation of a proton to form reduced DPPH. The complete reduction of a proton to form reduced DPPH and the complete reduction reaction of this stable free radical is indicated by change in colour from purple to pale yellow. The Absorbance was measured at 517nm as shown in GRAPH-1.

GRAPH-1:DPPH INHIBITION OF ANTIOXIDANT ACTIVITY OF FLOWER EXTRACT OF *Moringa olifera*



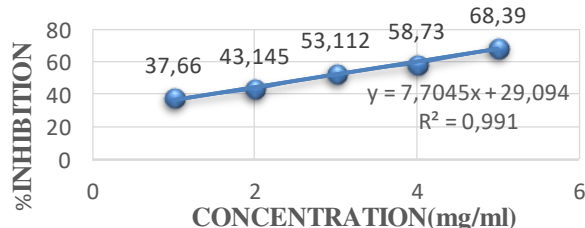
GRAPH-3 INHIBITORY EFFECT OF *Moringa olifera* FLOWER EXTRACT ON PROTEIN DENATURATION



HYDROGEN PEROXIDE SCAVENGING ACTIVITY

A solution of H₂O₂ was prepared in phosphate buffer and their concentration was determined spectrophotometrically from the absorption at 230 nm, decrease in absorbance of H₂O₂ upon oxidation of H₂O₂. The Absorbance was measured at 230nm as shown in GRAPH -2 and the percentage scavenging of H₂O₂ was calculated.

GRAPH-2: H₂O₂ INHIBITION OF ANTIOXIDANT ACTIVITY OF FLOWER EXTRACT OF *Moringa olifera*



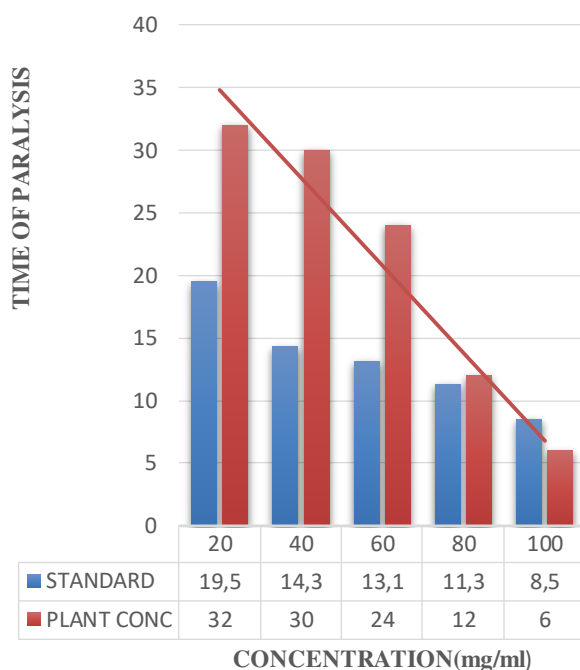
ANTHELMINTIC ACTIVITY

The anthelmintic activity of Ethanolic extract of *Moringa OLEIFERA* Flower are given in Table 2. The Ethanolic extract showed maximum activity and the time taken for the paralysis and death vary with different concentrations. The earthworms exposed to 80% ethanolic extract showed paralysis at 12 minutes and death at 27 minutes. Whereas the control drug at 80% concentration exhibited 12 minutes for paralysis and 16 minutes for death. Lesser concentrations took a long time for paralysis and death (Table 2) and the anthelmintic activity of ethanolic extracts increased with increase in concentration. All the data obtained was presented as Mean + SEM and were analyzed with student-t test. Ethanolic extract of *Moringa Oleifera* flower showed the significant anthelmintic effect carrying the death of worms at the concentrations (20, 40, 60, 80 and 100 mg/ml), as compared to worms, which were treated with standard (albendazole) at the concentration (20, 40, 60, 80 and 100 mg/ml). In the case of ethanolic extracts of the *Moringa Oleifera* flower produced better anthelmintic activity. Evaluations of anthelmintic activity of alcoholic extracts of flower of *M. Oleifera* on earthworms were given in the graph 4 and 5.

ANTI-INFLAMMATORY ACTIVITY

A volume of 1 ml of extracts (ethanolic) at different concentrations was homogenised with 1 ml of aqueous solution of bovine serum albumin (5%) and incubated at 27°C for 15 minutes. The mixture of distilled water and BSA constituted the control tube. Denaturation of the proteins was caused by placing the mixture in a water bath for 10 minutes at 70°C. The mixture was cooling inside the ambient room temperature, and absorbance was measured at 660 nm are shown in GRAPH-3. The inhibitory effect of different concentrations of *M. Oleifera* flower extract on protein denaturation are shown in GRAPH-3.

GRAPH-4:ANTHELMINTIC ACTIVITY (TIME OF PARALYSIS)



S.NO	EXTRACT	DOSAGE (mg/ml)	PARALYSIS (MINUTES)	DEATH (MINUTES)
1	Normal Control	-	-	-
2	Negative Control	-	-	-
3	Standard (Albendazole)	20	19.5 \pm 5.12	36.00 \pm 26.13
		40	14.3 \pm 5.77	30.50 \pm 15.21
		60	13.10 \pm 4.12	22.30 \pm 9.01
		80	11.30 \pm 4.50	16.10 \pm 5.86
		100	8.50 \pm 1.86	14.00 \pm 6.44
4	Ethanol	20	32 \pm 23.01	47 \pm 27.70
		40	30 \pm 19.12	42 \pm 25.47
		60	24 \pm 13.14	31 \pm 18.50
		80	12 \pm 8.75	27 \pm 10.50
		100	6 \pm 0.54	18 \pm 7.30

GRAPH-5:ANTHELMINTIC ACTIVITY (TIME OF DEATH)

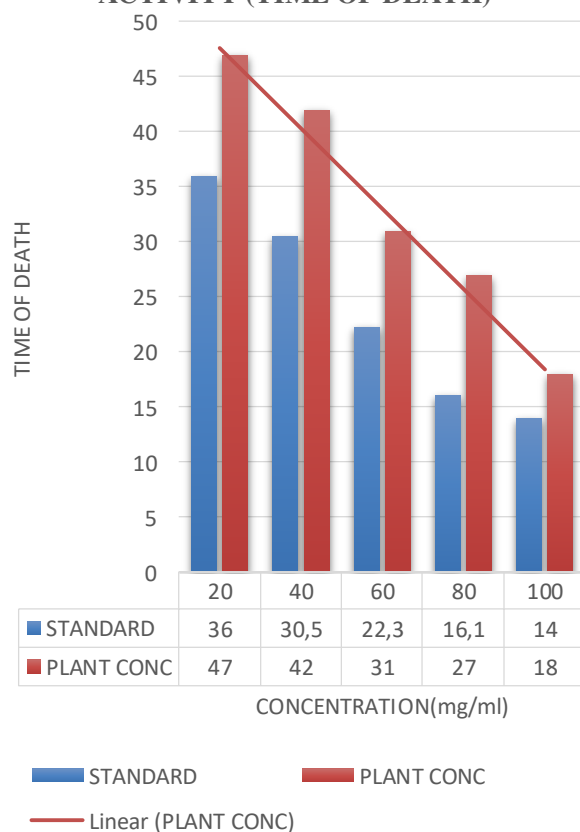


TABLE:2 THE ANTHELMINTIC ACTIVITY OF ETHANOLIC EXTRACT OF *Moringa Oleifera* FLOWER

4.DISCUSSION

Medicinal plants since ancient time are lauded for their diverse pharmacological actions which could be attributed to the presence of secondary plant metabolites such as alkaloids, flavonoids, glycosides, tannins, steroids etc. Some of these plants are important source of natural antioxidants that have been shown to reduce the risk and progression of certain acute and chronic diseases such as cancer, heartdiseases and stroke by scavenging free radicals which are implicated in the pathogenesis of many diseases(Ali SSet al.,2008 ;Pham-HuyLAet al.,2008

Moringa Oleifera Lam. (*M. Oleifera*), is used as traditional medicine in many tropical and subtropical countries (Muchenje V *et al.*, 2011). Pharmacological studies have demonstrated that *M. Oleifera* known to possess hypoglycemic, hypotensive, anti-microbial, hepatoprotective, immunomodulatory, antioxidant and antitumor activities (Sudha P *et al.*, 2010). Different plants would have different chemical constitution and the composition of these chemicals may vary from one part to another part within the same plant.

The phytochemicals present in the plant can be dissolved in various solvents depending on its solubility. More phytochemicals and the highest percentage extractive (11.6%) were found in ethanol extract was proven experimentally by Deepak V *et al.*, 2018. So, Ethanol was used as a solvent for the extraction of extract from *Moringa Oleifera* flower by Soxhlet extraction.

The results of preliminary phytochemical screening confirmed the presence of various classes of secondary metabolites in the *M. Oleifera* flower extract including poly phenols (tannins and flavonoids). Plant polyphenols, produced either from phenylalanine or from its precursor shikmic acid, are important dietary antioxidants because they possess an ideal structural chemistry for free radical scavenging activity.

DPPH free radical scavenging activity is an easy and widely used method for testing in-vitro antioxidant activity of natural compounds or plant extracts (Santha S, *et al.*, 2010). Its reduction capability to accept an electron or a hydrogen radical from antioxidants is determined by measuring decrease in its absorbance values at 517 nm.

DPPH radical scavenging activity of *M. Oleifera* flower extract was compared with standard ascorbic acid in this study. Although standard antioxidant had higher scavenging activity at all tested concentrations than the extract, the extract still showed good free radical scavenging activity.

Inflammation is a very common symptom of many chronic diseases. It is a normal protective response to tissue injury caused by physical trauma, noxious chemical or microbial agents. Inflammation is a protective attempt by the body to remove injurious stimuli as well as initiate the healing process for the tissue (Ashley NT *et al.*, 2012). *M. Oleifera* flower extract showed a moderate free radical scavenging activity, its effect on inhibition of protein denaturation was found to be comparable with the standard. Thus, it can be concluded that anti-inflammatory activity of

M. Oleifera flowers could be due to their high phenolic content.

In the present study, anthelmintic activity of ethanol flower extracts of *M. Oleifera* was tested against *P. posthuma* which resembles intestinal worms in their reaction to anthelmintic agents and are easily available. The ethanol extract showed the highest activity than the standard drug. The presence of alkaloids, phenols, flavonoids and tannins in ethanol extract may be accountable for the highest anthelmintic activity.

The anthelmintic activity of ethanol extracts increased with increase in the concentrations. Already Vijay Bhutda *et al.*, 2009 has reported That *Moringa Oleifera* leaves shown potent anthelmintic activity while the *Vitexnegundo* has taken long time for death of worms. *Moringa Oleifera* leaves is showing paralysis within 6-15 min while death is comparable with that of piperazine citrate as death of worms was observed at 64 min. Phytochemicals such as alkaloids, tannins, phenols etc. are reported to have significant anthelmintic activity (Bate - Smith EC., 1962) and alkaloids were reported to act on the central nervous system and cause paralysis of the earthworm. Tannins were reported to interfere with energy generation of worms by uncoupling oxidative phosphorylation or they bind to the free protein of the gastrointestinal tract and leads to the death of the worms (Jain Det *et al.*, 2011).

Phytochemicals together or separately may act by inhibition of tubulin polymerization and block glucose uptake (Roy Het *et al.*, 2010) and damage the mucopolysaccharide membrane of worms and this will expose the outer layer and restrict their movement of earthworms which finally may cause paralysis and ultimately death (Chandrasekhar CHet *et al.*, 2008). In future, work will be needed to isolate the active constituents of fresh *Moringa Oleifera* flower extract to locate potential anthelmintic phytochemical.

5. CONCLUSION:

The present research work described the in-vitro antioxidant, anti-inflammatory activity and anthelmintic activity of *M. Oleifera* flowers. The study was well performed by the authors and explanation of the medicinal uses of *M. Oleifera* is based on the phytochemicals, especially the phenolic compounds Tannin and Flavonoids. The Phytochemical screening showed the presence of Alkaloids, Flavonoids, Terpenoids, Glycosides, Steroids, Carbohydrates and Phenols.

The Antioxidant Activity of *M. Oleifera* shows % H₂O₂ inhibition high at 68.39. The Anti-

inflammatory Activity of the *M.Oleifera* flower extract shows high at the concentration of 1mg/ml which shows 79.545 % inhibition . *M. Oleifera* flower extract showed a moderate free radicalscavenging activity, its effect on inhibition of protein denaturation was found to be comparable with the standard.Thus ,it can be concluded that anti-inflammatory activity of *M. Oleifera* flowers could be due to their high phenolic content.

Anthelmintic activity of Ethanolic extract *M.Oleifera* flower shows paralysis at 12 minutes and Death at 27 minutes at 80% concentration of ethanolic extract of *M.Oleifera*. The Standard drug Albendazole shows paralysis at 8 minutes and Death at 14 minutes. The anthelmintic activity of ethanol extracts increased with increase in the concentrations .

6.ACKNOWLEDGEMENT:

I would like to thank Dr.P.Dhasarathan and Mr.V.Saravana Perumal for helping me to complete this project.

7.REFERENCES:

1. . Chumark, P., P. Khunawat, Y. Sanvarinda, S. Phornchirasilp, N.P. Morales, L. Phivthong-ngam, P. Ratanachamnong, S. Srisawat and K.S. Pongrapeeporn. 2008. The *in -vitro* and *ex-vivo* antioxidant properties, hypolipidaemic and antiatherosclerotic activities of water extract of *Moringa Oleifera* Lam. leaves. J. Ethnopharmacol. 116:439-446.
2. Delfin E. Cabardo Jr. & Harvie P. Portugaliza (2017) Anthelmintic activity of *Moringa Oleifera* seed aqueous and ethanolic extracts against *Haemonchus contortus* eggs and third stage larvae, International Journal of Veterinary Science and Medicine, 5:1, 30-34.
3. Ghasi, S., E. Nwobodo and J.O. Ofili. 2000. Hypocholesterolemic effects of crude extract of leaf of *Moringa Oleifera* Lam. in high-fat diet fed wistar rats. J. Ethnopharmacol. 69:21-25.
- 4.Moyo B, Masika PJ, Hugo A, Muchenje V. Nutritional characterization of Moringa (*Moringa Oleifera* Lam.) .Afr J Biotech 2011; 10(60): 12925-12933.
5. Pontual EV, Carvalho BEA, Bezerra RS, Coelho LCB, Napoleao TH, Paiva PMG. Caseinolytic and milk-clotting activities from *Moringa Oleifera* flowers. Food Chem 2012; 135(3): 1848-1854.

6. Pratap Chandran, Deepak V, Sai Krishna, Saniya Fathima, Ameena Thaha, Jeenuja Raj . Analysis of Phytochemical Constituents and Anthelmintic Activity of Leaf Extracts of *Mimosa pudica* L. Asian Journal of Biomedical and Pharmaceutical Sciences 2018; Volume 8 Issue 65

7.Udupa, S.L., Udupa, A.L., Kulkarni, D.L. (1994) Studies on the anti-inflammatory and wound healing properties of *Moringa Oleifera* and *Aegle marmelos*, Fitoterapia, 65, 119–12.