Evaluation of Antioxidant, Anthelmintic and Antimicrobial activity of *Moringa oleifera* root

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Abstract: Microbial resistance and helminthic infections are the continues serious challenges in the global health. Although synthetic drugs are available there prolonged use often leads to resistance. Medicinal plants are the primary source of drugs in the healthcare system, and they have been used to treat illnesses at a low cost all across the world. The *Moringa oleifera* have long been used in traditional medicine to treat disease, the current study aimed to evaluate antioxidant, anthelmintic and antimicrobial activity *in Moringa oleifera* root. The roots of *the Moringa oleifera* plant were collected and authenticated. These roots were dried and extracted using hydroalcoholic through the maceration extraction method. The phytochemical evaluation was conducted, and evaluated antioxidant activity by using a *in vitro* DPPH assay. The absorbance of each mixture was measured at 520nm using UV-visible spectrophotometer and calculated the % scavenging activity and IC50 Value, The anthelmintic activity performed by using hydroalcoholic extract of *Moringa oleifera* root against earthworm and antimicrobial activity against *E. coli and S. aureus* by using cup plate method. The Phytochemicals like Alkoloids, Flavonoids, tannins, Terpinoids, steroids, phenolic compounds, proteins, amino acids are present. In the DPPH scavenging activity the hydroalcoholic extract of *Moringa oleifera* root shows 56.38% and the standard 85.42% at a concentration of 300µg/ml. The anthelmintic activity of *Moringa oleifera* was performed by measuring death time of earthworm at 400mg/ml and 600mg/ml. The *hydroalcoholic* extract *Moringa oleifera* showed significant zone of inhibition and showed antimicrobial effect hence the research conclude that *Moringa oleifera* shows significant athelmintic and antimicrobial properties.

Keywords: anthelmintic, antioxidant, *moringa oleifera*, root, Hydroalcoholic extract, Flavonoids.

I. INTRODUCTION

Moringa oleifera is one of the most important plant widely cultivated in India. It belongs to family Moringaceae. This plant is widely used as nutritional herb and contains valuable pharmacological action like anti-asthmatic, anti-diabetic, hepatoprotective, anti-inflammatory, anti- fertility, anti-cancer, anti-microbial, anti-oxidant, cardiovascular, anti-ulcer, CNS activity, anti-allergic, wound healing, analgesic, and antipyretic activity[1.]

Moringa oleifera Lam. Drumstick tree and horse radish tree are other names for the plant. Vitamin A, vitamin C, and milk protein are all abundant in it. There are a variety of active phytoconstituents, including protein, alkaloids, quinine, saponins, flavonoids, tannin, steroids, glycosides, fixed oil, and fats[2].

Free radicals are highly reactive molecule that can cause significant cell injury Free radicles are highly reactive molecule that can cause significant cellular injury by damaging lipids, proteins, and DNA. This oxidative stress occurs when there is a imbalance between free radicles and antioxidants in the body leading to cellular dysfunction and contributing to various diseases, including cancer, CVD and neurodegenerative diseases. The MOA of free radicles includes lipid peroxidation, which disrupts cell membranes and mutations in DNA that can lead to cell death.

Free radicals are unstable chemicals that can damage cells and possibly cause a number of diseases and aging processes. Antioxidants are essential for neutralizing these molecules. Bioactive substances with possible antioxidant activity are found in the medical plant *Moringa oleifera*, which is well-known for its pharmacological qualities. Because of its ease of use, speed, and repeatability, the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is frequently used to assess the capacity of plant extracts and other substances to scavenge free radicals[3].

Globally, helminthes infections impact more than 1.5 billion people, primarily in tropical and subtropical countries with low sanitation and water access. Gastrointestinal parasites pose a significant danger to cattle production in poor nations. Despite anthelmintic resistance in economically significant parasites, chemotherapy is a popular method for controlling helminths. Helminthiasis, caused by helminthes infection, is a significant limitation on cattle productivity worldwide. Chemotherapeutics continue to be the primary treatment for helminthiasis, despite challenges such as chemical residues, toxicity, high cost, non-adaptability, and limited availability in remote locations[4].The major mechanism of action for benzimidazoles is microtubule disruption within the parasite. In addition, benzimidazole activity on worms has been linked to enzymatic inhibition of acetylcholinesterase and other metabolic pathways[5].

The roots are traditionally used to cure kidney stones, liver ailments, inflammation, ulcers, and pain in the ear and teeth. The bark from the stem is used to cure wounds and skin infections. However, roots have been shown to play a function in the treatment of toothache, helminthiasis, and paralysis. The flowers are used to cure ulcers, enlarged spleens, and to create aphrodisiacs. The tree is thought to have extraordinary properties for healing malnutrition in newborns and breastfeeding moms[6].

II. REVIEW OF LITERATURE

The *Moringa* is medium-sized tree *Moringa oleifera*, also called drumstick, Sahjan, Moringa, etc., belongs to a family Moringaceae, is grown commercially primarily for its many purposes. *Moringa* is gaining popularity as an affordable and practical source of bio-fortified nutrients[7]

M. oleifera is known as the "Miracle Tree" and "Tree of Life" for its numerous benefits to health, nutrition, water cleanliness, and the environment. Its diversity and potential make it a valuable entity in many ways, which contains 13 species of tree and shrub found in sub-Himalayan regions of India, Sri Lanka, North Eastern and South Western Africa and Madagascar. *M.oleifera* roots, leaves, and flowers are traditionally used to treat diarrhea and hypertension in several countries. The root powder is an aphrodisiac that, when blended with milk, can treat asthma, gout, rheumatism, and enlarged spleen or liver. Indigenous medicine uses various parts of the tree, including leaves, roots, seeds, pods, fruits, and flowers, to treat common ailments like skin infections, anemia, asthma, cough, diarrhea, swelling, headaches, hysteria, cholera, respiratory disorders, scurvy, diabetes, sore throat, and chest congestion[8]

Traditional uses of *Moringa oleifera*[9]

M. oleifera has been used as food or traditional medicine in Oriental nations since antiquity. It has traditionally been utilized in India in the comprehensive system of Ayurveda because of its pharmacological qualities. It is nutritious, and nearly every part of the plant is edible. The large output of *M. oleifera* Lam and Moringa seed oil makes *M. oleifera* seeds suitable for biodiesel production. It is also utilized as animal feed because of its high nutrient content. *M. oleifera's* leaves, fruits, roots, and seeds have long been used medicinally to treat paralysis, helminthiasis, ulcers, and skin problems. In Thailand, the leaves and pods are used as antipyretics and antidotes, whereas in South Asia and India, they are utilized to prevent aging. It functions as a laxative, astringent, diuretic, cardiotonic, abortifacient and rubefacient. Treats toothaches, colds, external sores, inflammations, stomatitis, piles, bronchitis, urinary discharges, obstinate asthma, rheumatism, and epilepsy. Useful for lower back and kidney pain and constipation and as a stimulant for nervous debility, paralytic illnesses, hysteria and epilepsy.

III. MATERIALS AND METHOD

The *Moringa oleifera* root were collected from in and around Davangere district, Karnataka state. It was authenticated by Dr. Halesh C, Assistant Professor, DOS in Botony Davangere University, shivaganagotri, Davangere, Karnataka, India. The root of *Moringa oleifera* collected, washed with fresh water and dried in shade at room temperature. The dried root were grounded into fine powder. the powdered root of *Moringa oleifera* were subjected to maceration with Hydroalcoholic solvent of water and alcohol at ratio of 50:50 for four days. Then it is filtered and evaporated to dryness and extract is concentrated and preserved in desicator.

Fig no:01 Maceration of *Moringa oleifera* root extract

3.1 Phytochemical evaluation[10-12]

The hydroalcoholic *Moringa oleifera* root extract was subjected to phytochemical test in order to detect the presence of the secondary metaboilites standard procedure were followed to carry out various test and results were obtained.

3.2 Antioxidant activity[13]:

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a common method for determining antioxidant activity of substances by assessing their ability to donate electrons or hydrogen atoms to neutralize free radicals. The principle

is based on reducing the DPPH radical, a persistent free radical with a distinctive deep violet color, into a colorless or pale-yellow molecule in the presence of an antioxidant. Spectrophotometry is used to quantify the decrease in absorbance at 517 nm caused by an antioxidant scavenging the DPPH radical.

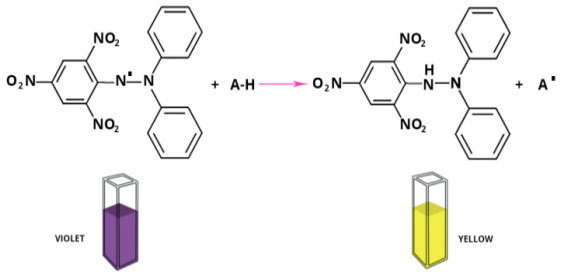


Fig no:02 Principle of DPPH

3.2.2 Procedure for DPPH Activity

Reagent 2, 2-diphenyl 1-picryl hydrazyl solution: 4 mg of DPPH was weighed and dissolved in 100 ml of methanol covered the volumetric flask using aluminium foil and stored in dark place. plant extract was prepared by 20 mg of plant extract was weighed, dissolved in 50 ml methanol, Serial dilutions were prepared by taking 1.25ml, 2.5ml, 3.75ml, 5.0ml, 6.25ml,7.5 ml that gives 50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml, 250µg/ml, 300µg/ml. Standard (Ascorbic acid) was prepared by 24 mg of Ascorbic acid was weighed, dissolved in 30 ml of distilled water, Serial dilutions were prepared by taking 1.25ml, 2.5ml, 3.75ml, 5.0ml, 6.25ml, 7.5ml that gives 50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml, 250µg/ml, 300µg/ml.

$$\text{Serial dilution preparation} = \frac{\text{Concentration} \times \text{Final volume}}{\text{Stock concentration}}$$

As mentioned above, samples were produced in methanol at various concentrations. Three milliliters of DPPH methanol solution were mixed with two milliliters of each concentration of the sample extract or standard. Aluminum foil was used to cover the volumetric flask before it was kept in a dark location. A 30-minute incubation period was given at room temperature in a dark area to finish any necessary reactions. The absorbance was then determined using a UV spectrophotometer at λ max 517 nm in comparison to a blank. The activity of the extract was compared to that of ascorbic acid, which was utilized as a standard free radical scavenger. The absorbance was calculated using the equivalent blank solution as a reference. The formula below was used to get the % inhibition.

$$\text{Inhibition (\%)} = \frac{(A1 - A2)}{A1} \times 100$$

A1:Absorbance of control

A2:Absorbance of Standard /Test

2.3 Anthelmintic Activity[14]:

Herbal medicine has been utilized for millennia to treat a wide range of medical ailments due to its amazing and potent dietary benefits. Helminths, the most prevalent infectious agent, are the major cause of human infections. Poor management strategies have resulted in a global sickness burden in disadvantaged places, with parasite infections associated with pneumonia,

eosinophilia, anemia, and malnutrition. Identifying new medicinal plants with high secondary metabolites and phytomolecules is critical in fighting helminth resistance to conventional antihelmintic treatments.

Procedure[15]: The anthelmintic activity of *Moringa oleifera* root extracts was tested using Indian adult earthworms (Pheretima posthuma) measuring 8±1 cm in length and 0.1±0.2 cm in width. The animals were placed into three groups, each with six earthworms. All *Moringa oleifera* root extracts were diluted in 10 milliliters of distilled water. The extracts and reference medication solution were newly produced prior to beginning the studies. Different concentrations of extract and standard medication solution were placed into separate Petri dishes. All of the earthworms were cleaned in a normal saline solution before being introduced into 10 ml of the following solutions: Control (0.9% normal saline), Standard Albendazole medicine in various concentrations (50 mg/ml, 100 mg/ml, and 150 mg/ml), and Hydroalcoholic extract of *Moringa oleifera* root in various concentrations (200 mg/ml, 400 mg/ml, and 600 mg/ml). Observations were made for the time it took to paralyze (paralysis was defined as the worm failing to revive even in normal saline) and death (death was defined as the worms losing motility). All data were expressed as the average of six animals from each group.

3.4 Antimicrobial assay[16]:

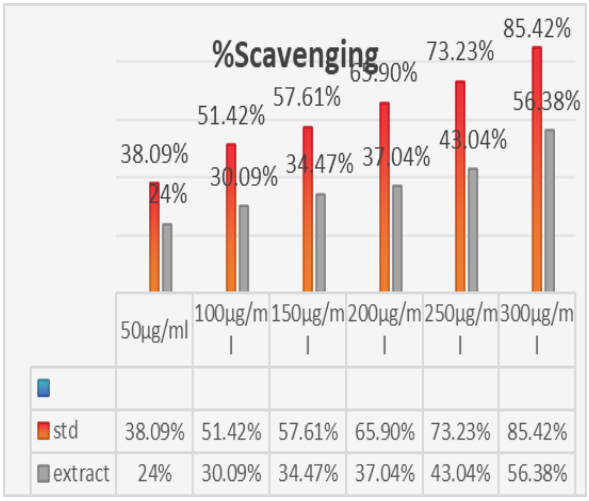
Antimicrobial assay were conducted using the agar well disk diffusion method. *E.coli* and *S.aureus* were used in this experiment. Nutrient agar was used as media. 25ml of sterile media were transferred to petri dishes and allowed to solidify. The media plates were in inoculated with 20µl suspension spread uniformly. A seven mm well were cut using a sterile cork borer 50mg, 100mg, 150mg extract concentration were added to the well. Ciprofloxacin was used as standard with dose 100µg. the plates were incubated for 24hrs. Zones of inhibition around the well is measured in mm.

IV. RESULT AND DISCUSSION:

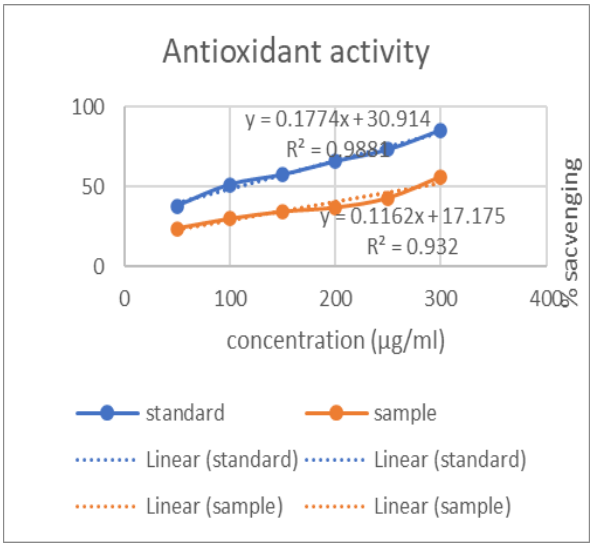
Phytochemical test: The phytochemical evaluation reveals that the presence of Alkaloids, Flavonoids, Steroids, Terpenoids, Phenolic Compouds, proteins and Amino acids These compounds are known for their antioxidant and anthelmintic properties, supporting the therapeutic potential of the extract.

4.1 ANTIOXIDANT ACTIVITY

Numerous diseases can arises from the persistent production of free radicles, which seriously harm biomolecules and tissues. *Moringa oleifera* root extract is substitute medication used to treat disorders associated with oxidative stress. In this work, we assist anti-oxidant of *Moringa oleifera* root extract using DPPH technique. A hydroalcoholic extract of *Moringa oleifera* root demonstrated significant DPPH radical inhibition(56.38%) at concentration 300µg/ml.



Bar graph showing the effect of % DPPH scavenging of test sample and standard compound against the drug concentrations



% Scavening of standard and test compound linear graph

Sample name	R^2 value	Y=mx+c	IC_{50}
Standard (Ascorbic acid)	R^2=0.9881	y = 0.1774x + 30.914	107.58 µg/ml
Test (MO)	R^2=0.932	y = 0.1162x + 17.175	282.48 µg/ml

Table no:01

4.2 ANTHELMINTIC ACTIVITY

Fig no: 03 Paralysis and death of earthworm with test concentration

Table no:02

Treatment	Time taken for paralysis (minutes)	Time taken for death (minutes)
Control		
Normal saline (0.9%Nacl)	Nil	Nil
(Standard Drug) Albendazole-40mg/ml		
50 mg	274 min	Nil
100 mg	32 min	134 min
150 mg	14 min	34 min
Hydroalcoholic *Moringa oleifera* root extract		
200 mg/ml	224 min	Nil
400 mg/ml	183 min	165 min
600 mg/ml	41 min	131 min

The anthelmintic activity resulted that the time taken for death of earthworm was 165min for 400 µg/ml and 131min for 600 µg/ml

4.3ANTIMICROBIAL ACTIVITY

Fig no:04 Zone of inhibition of *Moringa oleifera root* extract

Organism solvent	Gram Positive Bacteria		Gram negative Bacteria	
Standard (Ciprofloxacin)	*S. aureus*		*E.coli*	
	Concentration	Test	Concentration	Test
	100 µg/ml	24 mm	100 µg/ml	22 mm
Moringa oleifera root extract	100 mg/ml	18 mm	100 mg/ml	0 mm
	150 mg/ml	20 mm	150 mg/ml	14 mm
	200 mg/ml	24 mm	200 mg/ml	18 mm

Table no:03

The antimicrobial activity of hydroalcoholic extract of *Moringa oleifera* roots were evaluated by cup plate method. The standard drug Ciprofloxacin had a zone of inhibition of 24 mm and 22 mm for gram positive and gram negative bacteria respectively, and the *Moringa oleifera* extract showed positive results for gram positive bacteria (*S.aureus*) at a concentration of 100 mg/ml,150 mg/ml and 200 mg/ml respectively, and positive results for gram negative bacteria (*E.coli*) at concentraion of 150 mg/ml, 200 mg/ml, and Shows negative results at a concentration of 100 mg/ml.

V. DISCUSSION

Phyto chemical test for hydroalcoholic extract of *Moring oliefera* Root was carried out and Found that alkaloids, flavonoids, phenolic compounds and tannins are present. The DPPH assay demonstrated significant antioxidant activity of *Moringa oliefera* extract.

The Scavenging activity was dose-dependent and comparable to that of the standard antioxidant, Ascorbic acid. The reduction capacity of this radical was determined by decrease in its absorbance at λmax 519 nm. Hydroalcoholic extract of *Moringa oliefera* exhibits potential antioxidant activity at concentration of 300µg/ml hydroalcoholic extract of *Moringa oleifera* root showed significant % scavenging activity (56.38%). The extracts of *Moringa oleifera* root was found to be 282.48 µg /ml by comparing with the standard Ascorbic acid.

In the present investigation, the extraction of *Moringa oleifera* exhibits Anthelmintic activiy compared with standard drug Albendazole. The test extract showed significant Anthelmintic activity by paralyzing the earthworms followed by death causes with different

doses of the extract and also *Moringa oliefera* exhibits Antimicrobial activity compared with standard drug Ciprofloxacin. The test extract showed significant Antimicrobial activity by Minimum inhibitory concentration (MIC) by cup plate method evaluated against selected gram-negative pathogen such as *E.coli* and gram-positive pathogen such as *S. aureus* with different doses of the extract. the anthelmintic activity of Psidium Guajava was found to be due to presence of phenols, taninns, steroids, flavonoids, saponins, terpenoids and the similar phytoconstituents are found in *Moringa oleifera* root extract, hence this study claim to have promising results for anthelmintic property.

VI. CONCLUSION

The goal of the study was to determine the hydroalcoholic extract of *Moringa oleifera* root's Antioxidant, Anthelmintic and Antimicrobial properties. Phytochemical analysis verified that the extract's significant secondary metabolities, including as flavonoids, tannins, steroids, terpenoids and other components, are what give it its action.

The current study was hopeful since hydroalcoholic extract from *Moringa oleifera r*oots have significant Anthelmintic properties due to their flavonoid content, which inhibits parasitic worm growth.

These flavnoinds demonstrate good antioxidant activity by inhibiting free radicals, most likely a result of their capacity for reduction and their ability to bind to proteins and it also acts against microbes by destroying them. result evidence to indicate that, Hydroalcoholic extract of *Moringa oleifera* root demonstrate better therapeutic efficacy.

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