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Evaluation of Hypolipidemic Potential of Aqueous Seed Extract of *Moringa oleifera* –An *In vitro* Study

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Introduction: Various parts of the tree *Moringa oleifera* Lam belonging to the Moringceae family are used extensively by the Indians as a major food constituent and also as herbal medicine. The dried seeds are used as a hypolipidemic agent for patients who suffer from obesity.
Aim: The study was aimed at evaluation of hypolipidemic potential of aqueous seed extract of *Moringa oleifera* - an *In vitro* study.
Materials and Methods: Hypolipidemic potential and antioxidant potential of aqueous seed extract of *Moringa oleifera* was analysed and compared with the standard drug. The data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test and it was used to see the statistical significance among the groups. The results with the $p<0.05$ level were considered to be statistically significant.
Results: The DPPH radical scavenging activity showed that the plant extract possessed a significant *In vitro* antioxidant(Ic50=220µg/ml) and hypolipidemic activity.((Ic50=380µg/ml).
Conclusion: The aqueous seed extract of *Moringa oleifera* exhibited a significant antioxidant and hypolipidemic potential.

Keywords: Moringa oleifera; seed extract; hypolipidemic activity; innovative technology; novel method.

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1. INTRODUCTION

Moringa oleifera, native to India, grows within the tropical and subtropical regions of the planet. It is commonly referred to as 'drumstick tree' or 'horseradish tree'. Moringa oleifera can withstand both severe drought and mild frost conditions and hence it is widely cultivated across the planet. Having a high nutritive value, every part of the tree is suitable for either nutritional or commercial purposes. The leaves are rich in vitamins and other essential phytochemicals. It is used as a potential antioxidant, anticancer, anti-inflammatory, antidiabetic and *M. oleifera* seed, a natural coagulant that is extensively utilized in water treatment [1,2].

Obesity has become a serious risk factor for various disorders worldwide. Additionally to the present attenuation in adipogenesis and over expression of pancreatic lipase enzyme which plays a major role in progression of obesity. Further, obesity has been found to be related to various disorders like osteoarthritis, ischemic heart diseases (IHD), diabetes, and hypertension [1,2]. A streak of evidence indicates that serotonin, histamine, dopamine, and their associated receptor activities are related to obesity regulation. Thus, attempts are made to scale back weight with such pharmacological intervention that possesses minimal side effects. Plants are used as traditional natural medicines for healing many diseases. Especially, various oriental medicinal plants are reported to possess biological activity. Literature review has revealed that various herbal plants like sour orange, Green Tea, and Black Chinese Tea are utilized in the management of obesity.

Moringa oleifera belongs to the Moringaceae family and is usually referred to as the golden shower tree that possesses various nutritional and medicinal values attributed to its leaves, roots, barks, flowers, fruits, and seeds. Recently, hypocholesterolemic activity of crude extract of M. oleifera crude extract was studied but it's thermogenic and antiobesity activity has not been investigated . Lopez et al has investigated the antiobesity property of methanolic extract M. oleifera leaves in experimentally induced obesity in experimental animals [3].

Cardiovascular disease is the leading cause for death in India also as in western countries. Hyperlipidemia is one of the major causes for the development of cardiovascular disorder. In India, the leaves of *Moringa oleifera* Lam. is claimed to possess cholesterol-reducing effect and is employed to treat patients with heart disease and obesity [4]. The aqueous extract of the leaves of M. oleifera was found to have wound healing and anti urolithiasis activity [5]. The methanolic crude extract of *Moringa oleifera* shows antibacterial activity.

In many cultures, herbal remedies are increasingly being employed to realize the medicinal value of the herbal plants. Our team has extensive knowledge and research experience that has translate into high quality publications [6-25]. The current work exhibits the antioxidant and hypolipidemic potential of aqueous seed extracts of *Moringa oleifera.*

2. MATERIALS AND METHODS

2.1 Phytochemical Screening Test

2.1.1 Test for phlobatannin

1ml of the extract was treated with 1ml of 1% HCl and it is boiled for 10 mins. The precipitate formed is red in colour which indicates the presence of phlobatannin.

2.1.2 Test for carbohydrates

Molisch reagent was taken and 3-5 drops of the reagent was added with 1 mL of the extract and then 1 mL of concentrated H2SO4 was added carefully through the sides of the test tube. The mixture was then allowed to stand for 2 minutes and it is diluted with 5 mL of distilled water. The development of red or dull violet rings at the junction of the two liquids showed the presence of carbohydrates in the test tube.

2.1.3 Test for flavonoids

Few drops of 1% liquid ammonia were taken in a test tube and along with it 1ml of the extract was added resulting in the formation of yellow color thereby indicating the presence of flavonoids.

2.1.4 Test for alkaloids

2ml of sample was mixed with 2ml of HCl. Then 6 drops of HCN was added and further 2 drops of picric acid was added that resulted in a creamish pale yellow ppt indicating the presence of alkaloids.

2.1.5 Test for terpenoids

2 ml of sample along with 2ml of chloroform and 3ml of con. H2SO4 was added. Red color ppt obtained indicates the presence of terpenoids.

2.1.6 Test for proteins

One mL of ninhydrin was dissolved in 1 mL of acetone and then a few drops of extract was added with ninhydrin. The formation of purple colour revealed the presence of protein.

2.2 Detection of Saponins

2.2.1 Foam test

A fraction of the extract was vigorously shaken with water and observed for persistent foam.

2.2.2 Test for steroids

1ml of chloroform was mixed with 1 mL of extract and then few drops of acetic anhydride and 5 drops of concentrated H2SO4 were added and mixed. The formation of dark red colour or dark pink colour indicates the presence of steroids.

2.3 DPPH Free Radical Scavenging Activity of Aqueous Seed Extract of *Moringa oleifera*

Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radicals was assessed by the method of Hatano et al, (1989). DPPH solution (1.0 ml) was added to 1.0 ml of extract at different concentrations (0.1 to 0. 5mg/ml). The mixture was kept at room temperature for 50 minutes and the activity was measured at 517 nm. Ascorbic acid at the same concentrations was used as standard. The capability to scavenge the DPPH radical was calculated and expressed in percentage (%) using following formula:

$$\text{DPPH radical scavenging (\%)} = \frac{\text{Control OD-Sample OD X100}}{\text{Control OD}}$$

2.4 *In vitro* Anti-Cholesterol Activity of Aqueous Seed Extract of *Moringa oleifera*

The anti-cholesterol assay was carried out as described as per the kit method (Spinreact, S.A.U-Ctra Santa Coloma, Girona, Spain). Cholesterol was dissolved in chloroform at a concentration of 2.5 mg mL/ml. Ten microliter of the extract was pipetted into a microtiter plate followed by the addition of 2000 µL of R1 reagent and 10 µL of cholesterol as a sample. Twenty microliters of distilled water and 2000 µL of R1 reagents were used as blank. Negative control consisted of 20 µL cholesterol and 2ml R1; standard consisted of 20 µL simvastatin and 2000 mL R1 reagent. The contents were incubated between 0-30 min at room temperature and the absorbance was read at 500 nm in a UV-Vis spectrophotometer against reagent blank. Anti-cholesterol assay of the extract was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{\text{Negative control-Sample} \times 100}{\text{Negative control}}$$

2.5 Statistical Analysis

The data were subjected to statistical analysis using one – way analysis of variance (ANOVA) and Duncan's multiple range test to assess the significance of individual variations between the groups. In Duncan's test, significance was considered at the level of $p<0.05$.

3. RESULTS AND DISCUSSION

Table 1. Phytochemical analysis of Moringa oleifera seed extract

Phytochemicals	Extract
Protein	+
Amino Acids	+++
Flavonoids	+
Alkaloids	-
Terpenoids	++
Steroids	+
Saponins	+

Fig. 1. Bar graph depicts the In vitro antioxidant activity of aqueous seed extract of *Moringa oleifera.* The X axis represents the different concentrations of *Moringa oleifera* seed extract taken and the Y axis represents the percentage of inhibition. Green colour denotes Vitamin C and the Yellow colour denotes *Moringa oleifera* seed extract. The difference was statistically significant. Each line represents Mean ± SEM of 3 independent observations. Significance at $p \leq 0.05$.

Fig. 2. Bar graph depicts the In vitro anti cholesterol activity of aqueous seed extract of *Moringa oleifera.* The X axis represents the different concentrations of *Moringa oleifera* seed extract and the Y axis represents the percentage of inhibition. Blue colour denotes the concentration of the standard drug Simvastatin and Orange colour denotes the *Moringa oleifera* seed extract. The difference was statistically significant. Each line represents Mean ± SEM of 3 independent observations. Significance at $p< 0.05$.

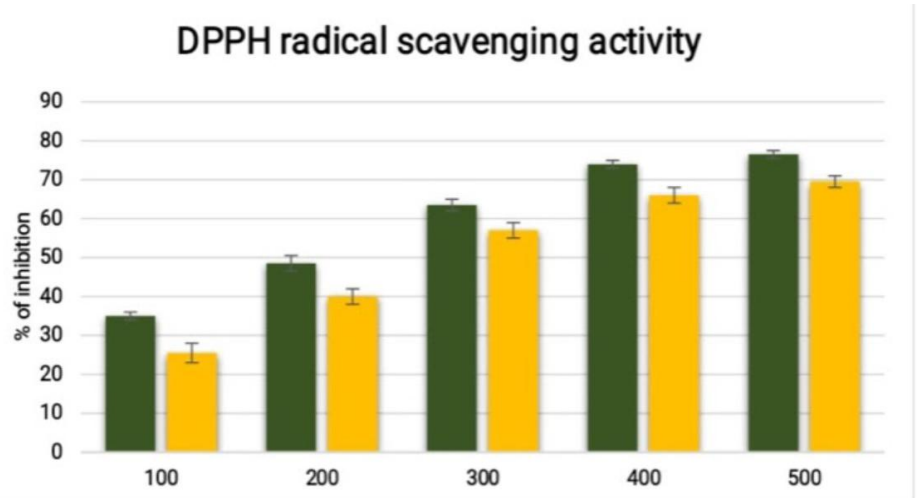

Fig. 1. *In vitro* antioxidant activity of aqueous seed extract of *Moringa oleifera*

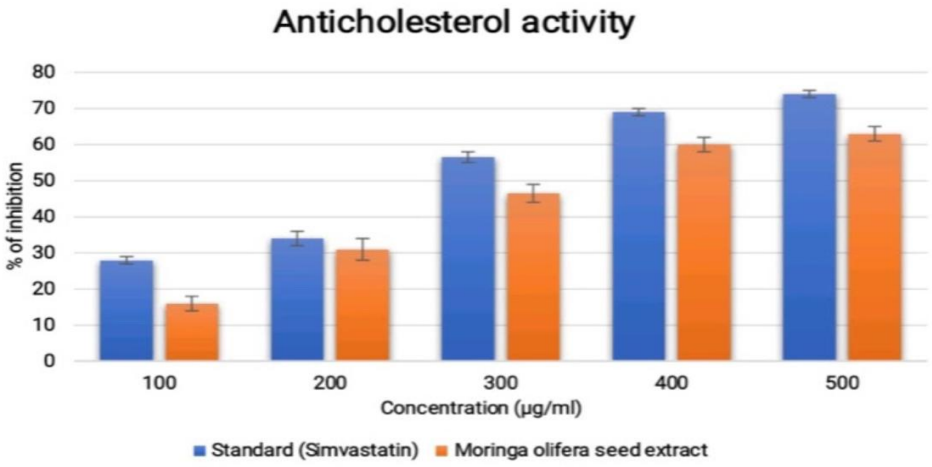

Fig. 2. *In vitro* anti-cholesterol activity of aqueous seed extract of *Moringa oleifera*

The qualitative phytochemical analysis of *Moringa oleifera* seed extract strongly showed the presence of proteins, amino acids, flavonoids, terpenoids, steroids, and saponins. (Table 1). Phytochemical screening refers to the identification of medicinally active substances that are found abundant in plants [26]. Aqueous seed extract of *Moringa oleifera* showed in vitro antioxidant activity in a concentration dependent manner. Vitamin C is used as the standard drug for checking the antioxidant activity. Free radicals are reactive particles in numerous physiological cycles and are associated with many diseases such as cancer. Therefore there is a need to investigate substances with free extremist searching and cell reinforcement activity [26,27]. Every piece of M. oleifera might be a storage facility of significant supplements and antioxidants [28]. The leaves of Moringa oleifera are very rich in minerals like calcium, zinc, iron and copper. Beta-carotene of vitamin A, B-complex nutrients like nutrient Bc, pyridoxine and niacin, L-ascorbic acid, vitamin D and E are also additionally present in Moringa oleifera. Phytochemicals like tannins, steroids, terpenoids, flavonoids, anthraquinones, alkaloids and reducing sugar present alongside anticancer agents like glucosinolate, glycoside compounds and glycerol-1-9-octadecanoate [29]. Moringa leaves have a low calorific value and can be utilized in the diet for a obese person. The pods are very fibrous and can be utilized to treat stomach related issues and treat carcinoma. A study done on Moringa shows that immature pods contain 46.78% fiber and around 20.66% protein content. Another study showed that pods

have 30% of amino alkanoic acid content, the leaves have 44% and flowers have 31%. The immature flowers and pods showed similar amounts of palmitic, linoleic and oleic acids [30].

Moringa oleifera has a lot of minerals that are required for growth and development among which calcium is taken into account together with the vital minerals needed for human growth. While 8 ounces of spinach can provide 300–400 mg, moringa leaves can provide 1000 mg and moringa powder can provide quite 4000 mg. Moringa powder is often used as a substitute for iron tablets, hence as a treatment for anemia. A study showed that moringa contains more iron than spinach. An honest dietary intake of zinc is important for correct growth of sperms and is additionally necessary for the synthesis of RNA and DNA. M. oleifera leaves show around 25.5–31.03 mg of zinc/kg, which is the daily requirement of zinc within the diet [28,31,32].

Antioxidant activity of the seed extract was found to (Ic 50= 280µg/ml) increase in a dose dependent manner as compared to the standard (Vitamin C). The strong antioxidant property can be due to the rich phytoconstituents the seed possesses (Fig.1).

The seed extract also exhibited a strong and significant hypolipidemic potential (Ic 50= 350µg/ml) as compared to the standard drug statin (Fig. 2).

Hypolipidemia might be a typical problem influencing around 2-3% of healthy individuals. It would be a marker for a fundamental, huge issue. Unexplained hyperlipidemia ought to be examined for a potential reason. A few clinical conditions additionally as lipid bringing down medications might end in clinically huge hypolipidemia. Past investigations recommend that low cholesterol levels might work as a prognostic pointer in malignant growth patients [33]. Hypocholesterolemia is moreover an inclining factor for contamination in specific conditions additionally as a prognostic marker during sepsis. There's a positive connection between low total serum cholesterol levels, and expanded mortality from all causes especially in critically sick patients. Hyperlipidemia might incline the critically sick patient toward sepsis and adrenal failure should carry a significantly increased risk of mortality. Presently, as we work in forceful administration of hyperlipidemia we ought to consistently focus an eye on the possible complications of drug-induced hyperlipidemia [34].

4. CONCLUSION

Thus, from the present study it can be concluded that aqueous seed extract of *Moringa oleifera* showed potent in vitro antioxidant activity which was evident from the DPPH radical scavenging assay. A dose dependent anti cholesterol activity was observed for the extract and the standard drug statin. In the present study, the standard drug statin showed greater activity compared to the extract in all the tested concentrations.

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CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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