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**Research Article**

# EVALUATION OF PHYTOCHEMICAL CONSTITUENTS OF *MORINGA OLEIFERA* (LAM.) LEAVES COLLECTED FROM PUDUCHERRY REGION, SOUTH INDIA

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## ABSTRACT

*Moringa oleifera* (Lam.) is a plant that possesses high nutritional value and has been used in folklore medicine to treat various and socio-economic benefits. In the present study, we have investigated the phytochemicals constituents of *M. oleifera* leaves extracts by biochemical method and Fourier transform infrared spectrophotometer (FTIR). The qualitative phytochemical screening indicated presence of alkaloids, triterpenoids, flavonoids, tannins, saponins, glycosides and carbohydrates in the leaves extract. The quantitative analysis of the *M. oleifera* leaves aqueous extract showed high content of Vitamin C and active compounds such as Polyphenols and Flavonoids which has a strong antioxidant activity. The infrared spectral data revealed the following bonds: O-H, N-H, C=O, N-O, C-N, C-H and C-Br that are diagnostic markers of aliphatic as well as aromatic compounds. The methanolic extract of *M. oleifera* leaves showed the presence of characteristic functional groups like alcohol, hydroxyl, alkane, aldehydes, alkenes groups, nitro compounds, aromatic amines, aliphatic amines and alkyl halides etc. Presence of phytochemicals indicates nutritional and medicinal properties of *M. oleifera* leaves.

**Keywords:** *Moringa oleifera,* FTIR spectroscopy, Nutritional plant, Medicinal plant.

## INTRODUCTION

The plants have been used for medicinal purpose as old as the history of mankind. Herbal medicine is an achievement of popular therapeutic diversity since they may possess hundreds of medicinal materials and produce their curative effects. Thus extraction and characterization of several active phytocompounds from the medicinal plants is the basis for the formation of some high activity profile drugs (Mandal *et al*., 2007).

*Moringa oleifera* (Family: Moringaceae) is an aboriginal of Indian subcontinent. It has been widely distributed and naturalized in the tropical and subtropical areas around the world, where it is known by various vernacular names; In Tamil it is called as Murungai (Ramachandran *et al.*, 1980). It is also an important food commodity which has enormous attention as the natural nutrition of the tropics. These rapidly-growing trees are utilized by the ancient Romans, Greeks and Egyptians. It has been reported that Indians might have been using *Moringa oleifera* as a regular component of conventional eatables for nearly 5000 years (Anwar and Bhanger, 2003).

Further it has been states that *M. oleifera* as an outstanding source of nutritional components. The leaves, fruit, flowers and immature pods of this tree are used as a highly nutritive vegetable in many countries. *M. oleifera* leaves has been reported to be a rich source of β-carotene, protein, vitamin C, calcium and potassium and act as a good source of natural antioxidants (Anwar *et al.*, 2005; Mahmood *et al.*, 2010). Its leaves (weight per weight) possess calcium which is equivalent of four times as that of milk, the vitamin C content is seven times that of oranges, while its potassium content is three times that of bananas, three times the iron content of spinach, four times the amount of vitamin A in carrots, and two times the protein content than that of milk (Kamal, 2008).

Preventive medicine has been immensely improved by the used of these natural plant antioxidants. Plants contain lots of free radical scavenging molecules some of which include alkaloids, phenolic acids, amines, betalains, terpenoids, lignins, stilbenes, tannins and vitamins as well as other secondary metabolites with high level of antioxidant activity (Manjula and Ammani, 2012).

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*M. oleifera* has enormous medicinal potential, which has long been recognized in the Ayurvedic and Unani system (Mughal *et al.,* 1999). An examination of the phytochemicals of *M. oleifera* leaves affords the opportunity to examine a range of fairly unique compounds. *Moringa* has been found to be a good source of polyphenols and antioxidants which essentially reduce the damages caused in tissue during physiological processes. It has been reported that, the leaves of *M. oleifera* have various biological activities including anticancer activities, prevention of cardiovascular diseases, Liver disease (Kumar and Pari, 2003) antitumor, nervous disorder inflammation digestive disorders, skin disorders, anti-microbial, immunomodulatory and regulation of thyroid status (Bernett *et al.,* 2003).

*M. oleifera* leaves juice is known to have a stabilizing effect on blood pressure (Dahot, 1988). The fresh leaves juice was found to inhibit the growth of pathogenic microorganisms (Caceres *et al.*, 1991). The antispasmodic activity of the ethanol extract of *M. oleifera* leaves, is exhibited by different constituents such as 4-[α-(L-rhamnosyloxy) benzyl]- O-methyl thiocarbamate (*trans*), which forms the pharmacological basis for the traditional uses of this plant in treating diarrhea and gastrointestinal motility disorder (Gilani *et al*., 1992; Gilani *et al.*, 1994). The methanol fraction of *M. oleifera* leaves extract showed antiulcerogenic and hepatoprotective effects in rats (Pal *et al.*, 1995). Makonnen *et al.* (1997) found *M. oleifera* leaves to be a potential source for antitumor activity. The crude extract of *M. oleifera* leaves has a significant cholesterol lowering action in the serum of high fat diet fed rats (Ghasi *et al*., 2000). *M. oleifera* leaves are effective for the regulation of thyroid hormone status (Tahiliani and Kar, 2000). A methanol extract of *M. oleifera* leaves conferred significant radiation protection to the bone marrow chromosomes in mice (Rao *et al*., 2001). Anjorin *et al* (2010) reported that nutritional content in the leaves of *Moringa* varies with location.

In recent years, although technology and medicine have developed extensively, some countries have made it obligatory to use natural products for many purposes, India is one amongst them. For this reason we have chosen *M. oleifera* an important native plant, which is a table food with incredible nutritional and medicinal properties. This study is to evaluate the phytochemical composition of *M. oleifera* leaves and the objective was to highlight its nutritional and medicinal benefits.

## MATERIALS AND METHODS

### Sample collection

Fresh leaves of *M. oleifera* were collected around the premises of Kanchi Mamunivar Centre for Post Graduate Studies (KMCPGS), Lawspet, Puducherry and taxonomically identified with the help of the Department of Botany, KMCPGS. The fresh leaves were air dried at room temperature in the Department of Zoology, KMCPGS until constant weight was attained. The dried leaves were pulverized into fine powder using electric blender and stored in air-tight container for further use.

### Preparation of leaves extract for qualitative analysis

50 g of the dried leaves powder was soaked in 200 ml methanol (Analytical grade) in a conical flask and wrapped with aluminum foil for 72 hours with occasional shaking. After 72 hours, the extract was filtered using Whatman filter paper No: 1. then the solvent was removed from the extract by vacuum distillation. The concentrated leaves extract (110 mg) was dried and stored at 4$^{o}$C for further study.

### Phytochemical screening

Phytochemical screening of leaves extract for qualitative detection of tannins, alkaloids, phytosterols, triterpenoids, flavonoids, glycosides, saponins, carbohydrates, proteins, amino acids and fixed oils & fats was performed by following standard methods of Harborne (1998) and Kokate (2005).

### Alkaloids

Approximately 50 mg of leaves powder was dissolved in 5 ml of distilled water, followed by 2M hydrochloric acid was added until an acid reaction occurred and filtered. The filtrate was tested for the presence of alkaloids by Wagner test.

**Wagner Test:** Two drops of Wagner's reagent was added to 1ml of the test solution along the sides of the test tube. The formation of yellow or brown precipitate indicates presence of alkaloids.

### Phytosterols

**Liebermann-Burchard's Test**: The leaves extract (2 mg) was dissolved in 2 ml of acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulfuric acid was added along the side of the test tube. A brown ring formation at the junction and the turning of the upper layer to dark green color is positive for phytosterols.

### Triterpenoids

**Salkowski Test:** Approximately 2 mg of dry leaves extract was shaken with 1 ml of chloroform and a few drops of concentrated sulfuric acid were added along the side of the test tube. A red brown color formed at the interface indicated triterpenoids.

### Flavonoids

**Lead acetate test:** A few drops of 10% lead acetate added to 1ml of the test solution resulted in the formation of yellow precipitate confirmed the presence of flavonoids.

### Tannins

**Ferric chloride Test:** Added a few drops of 5% ferric chloride solution to 2 ml of the test solution. Formation of blue color indicated the presence of hydrolysable tannins.

**Saponins**

**Olive oil test:** A few drops of olive oil was added to 2 ml of the test solution and shaken well. Formation of a soluble emulsion was considered positive.

**Glycosides**

To 5 ml extract 25 ml of dilute sulphuric acid was added in a test tube and boiled for 15 minutes, cooled and neutralized with 10% NaOH, and then 5 ml of Fehling solution was added. The appearance of red color indicates positive reaction.

**Test for carbohydrates**

**Fehlings test:** Dissolved 2 mg dry extract in 1 ml of distilled water and added 1ml of Fehling's (A+B) solution, mixed well and heated in a water bath for 10 minutes. The brick red precipitate indicates carbohydrates.

**Test for proteins**

**Biuret test:** To 2 ml of the test solution added 5 drops of 1% copper sulphate solution and 2 ml of 10% NaOH and mix thoroughly. Formation of purple or violet color indicates presence of protein.

**Fats and fixed oils**

To 5 drops of the sample were added to 1 ml of 1% copper sulphate solution and a few drops of 10% sodium hydroxide. Formation of a clear blue solution indicates presence of fats and fixed oils.

**Preparation of leaves extract for quantitative analysis**

The slightly modified method of Anwar *et al*, (2013) was used to prepare the extracts from the shadow dried *Moringa oleifera* leaves.

**Aqueous Extract:** One hundred (100) grams of shadow dried *M. oleifera* leaves powder was accurately weighed on an analytical balance and poured into a 1 litre conical flask wrapped in aluminium foil. Eight hundred (800) ml of distilled water was then gradually added to the powder and the contents shaken until slurry of uniform consistency was formed. Phytochemicals present in the leaves powder were extracted using the stirring technique. For this purpose, magnetic bar and magnetic stirrers were used. The magnetic stirrer was set to operate at 200 revolutions per minute (RPM) for 48 hours. This process was repeated again for another batch of 100 grams of leaves powder. The resultant slurry was then centrifuged at 3000 revolutions per minute (RPM) for 5 minutes, the supernatant collected into light-resistant bottles and stored at -4°C. After 24 hours, the mouth of the bottles was covered with muslin cloth and attached to a freeze drier. The set up was left overnight to obtain a freeze dried product. The percentage yield of the freeze dried product was then calculated as percentage weight by weight (%w/w). Calculation of percentage yield; % of crude extract yield= (M2-M1/M0) ×100, Where; M2=mass of container + extract, M1=mass of empty container, M0=mass of the initial leaves powder sample. This product was then stored under refrigeration in well closed, light resistant bottles awaiting analysis.

**Determination of Phenolic compounds**

The extraction of phenolic compounds of the extracts was performed according Anwar *et al*, (2007), with some modifications. The values were added in a 10.0 mL flask, following the order: first, 5.0 mL of distilled water, then 100 µL of the specific gallic acid diluted solution, then 0.5 mL of Folin-Ciocalteu was added. After 3 minutes was added 2.0 mL of sodium carbonate 15% (m/v), which was prepared 24 hours before and kept under refrigeration, was pipetted. At last, the total volume of the flask was filled using 10.0 mL of distilled water. The flasks were shaken for homogenization. Immediately, all the flasks were placed in a dark place, and the reaction happened during the next 2 hours. All analysis was performed in triplicate. After the 2 hours rest, the absorbance of the samples was read using a spectrophotometer, on a wave-length of 765 nm. The total phenol content was determined through a calibration curve, which was built following the gallic acid pattern and expressed as mg GAE (gallic acid equivalents) per 100 g of extract.

**Determination of Total flavonoids content**

Total flavonoids content of air dried leaves were determined calorimetrically using aluminum chloride as described by Chang *et al*, (2002). About of 0.1g of air dried Leaves were dissolved in 1ml of distilled water. Resulting solution (0.5 ml) was mixed with 1.5 ml of 95% ethyl alcohol, 0.1 ml of 10% aluminum chloride (AlCl3), 0.1ml of 1M potassium acetate (CH3COOK) and 2.8 ml of distilled water. After incubation at room temperature for 40 min, the reaction mixture absorbance was measured at 415nm against distilled water as blank, using spectrophotometer. Quercetin was chosen as a standard of flavonoids for making the standard curve (0–50 mg/l). The concentration of total flavonoids contents was expressed as milligram quercetin equivalent (QE)/g based on dry weight.

**Determination of ascorbic acid contents**

The method of Benderritter *et al.* (1998) was adopted with slight modifications. This assay involved the use of ascorbic acid as a standard substance. This substance also served as a representation of vitamin C in the *Moringa* plant. Ten (10) mg of this standard was dissolved in one hundred (100) ml distilled water to give a stock solution of 100 µg/ml concentration.

**Preparation of standard calibration curve of ascorbic acid**

From the above stock solution aliquots ranging between 0.05-1.5 ml were taken in six (6) different ten (10) ml standard flasks. A two hundred (200) µl mixture of

trichloroacetic acid (13.3% w/v) and distilled water were then added followed by seventy-five (75) µl of dinitrophenyl hydrazine. The resulting mixtures were then incubated at 37°C for three (3) hours and thereafter 65% v/v of sulphuric acid (0.5 ml) was added. The intensity of the developed colour was then measured at 520 nm using a Spectronic 21D Milton Roy UV-VIS spectrophotometer (USA). Distilled water was used as reagent blank (Benderritter *et al.*, 1998). The calibration curve was prepared by plotting a graph of absorbance (y) against concentration (x).

**Table 1.** Qualitative phytochemical screening of *M. oleifera* leaves methanolic extract.

| Sl. No | Chemical test | Methanolic extract of *M. oleifera* leaves |
|---|---|---|
| 1 | Alkaloids | Present |
| 2 | Phytosterols | Absent |
| 3 | Triterpenoids | Present |
| 4 | Flavonoids | Present |
| 5 | Tannins | Present |
| 6 | Saponins | Present |
| 7 | Glycosides | Present |
| 8 | Carbohydrates | Present |
| 9 | Proteins | Present |
| 10 | Fats and fixed oils | Absent |

**Quantitative analysis of *M. oleifera* leaves aqueous extract**

The leaves was found to be rich in Vitamin- C, it contained 2.18 ± 0.89 mg.AAE/g. Phytochemical analysis revealed high concentrations of Phenol and Flavonoids, it contained 627 ± 12.26 mgGAE/100 g of phenolic compound and it contained 22.16 ± 1.54mgQE/g of Flavonoid respectively.

**Table 2.** Quantification of Vitamin-C, total phenolic content and total flavonoid contents in *M. oleifera* leaves.

| Parameters | Mean±SEM |
|---|---|
| Vitamin-C (mg.AAE/g) | 2.18 ± 0.89 |
| Total Phenolic compounds (mgGAE/100 g) | 627 ± 12.26 |
| Total flavonoid contents (mgQE/g) | 22.16 ± 1.54 |

Data referring to the average of three results ± SEM: standard error of the mean.

**FTIR spectrum of *M. oleifera* leaves methanolic extract**

The FTIR spectrum of *M. oleifera* leaves methanolic extract exhibited absorption in the range from 3387.33 $cm^{-1}$ to 593.50 $cm^{-1}$ (Figure 1). The spectrum exhibited a broad band around 3387.33 $cm^{-1}$ assigned to Alcohol and hydroxyl group (N-H, O-H) stretching. This indicates the presence of phenol and flavonoid. The peak observed at 2931.66 $cm^{-1}$ indicates the presence of alkanes group (C-H). Another sharp peak at 2360.33 $cm^{-1}$ assigned to C=O stretching vibrations in carbonyl groups which clearly confirms the presence of aldehydes. A long and sharp peak at 1632.94 $cm^{-1}$ attributed to tertiary amines (N-H). The peak at 1510.22 $cm^{-1}$ indicated the presence of Nitro compound (N-O). The peak at 1384.44 $cm^{-1}$ shows the presence of aromatic amines (C-N). Sharp peak observed at 1235.99 and 1056.89 $cm^{-1}$ indicates the presence of aliphatic amines (C-N). The sharp peak observed at 926.80 and 798.24 $cm^{-1}$ represent C-H stretch of alkenes. Alkyl halides C-Br stretching was observed at 593.50 $cm^{-1}$. FTIR spectrum analysis of *M. oleifera* leaves methanolic extract shows the presence of characteristic functional groups of alcohol and hydroxyl groups, alkane groups, aldehydes, alkenes groups nitro compound, aromatic amines, aliphatic amines and alkyl halides.

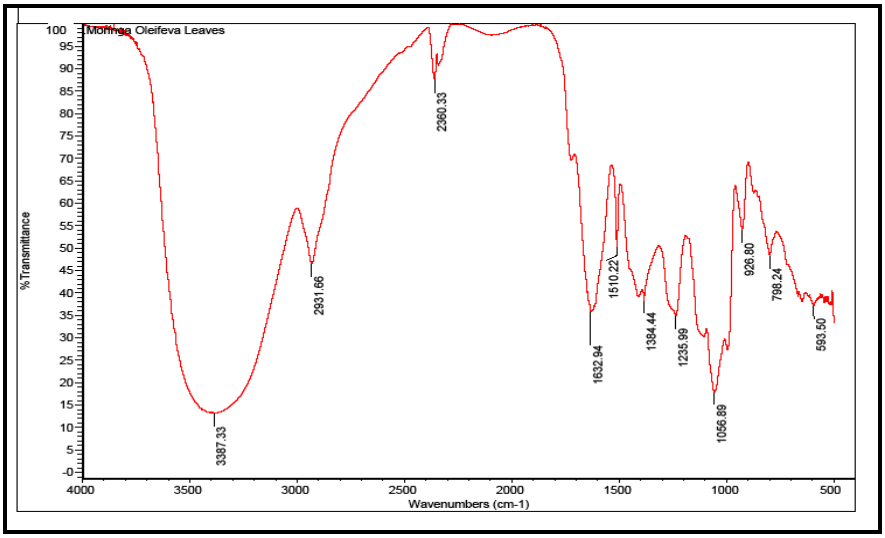


**Figure 1.** FTIR Spectroscopic analysis of *M. oleifera* leaves methanolic extract.

## DISCUSSION

*M. oleifera* is an important food commodity which has enormous attention as the 'natural nutrition of the tropics'. In the present study presence of alkaloids, triterpenoids, flavonoids were confirmed in the leaves of *M. oleifera* similar observation were observed in the previous studies, *M. oleifera* leaves act as a good source of natural antioxidant due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics and carotenoids (Anwar *et al.,* 2013). In the present study the presence of Alkaloids has been identified in the leaves extract of *M. oleifera*. Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. They often have pharmacological effects and are used as medications and recreational drugs (Rhoades and David, 1979). Further, in the present study we have determined the amount of vitamin C in the leaves of *M. oleifera* which are in agreement with the previous reports. Vitamin C is an excellent antioxidant and free radical scavenger, capable of protecting the cells from oxidative damage by oxidants. It required for connective tissue metabolism especially the scar tissues, bone and teeth. Added to this, it prevents scurvy and enhances iron absorption from the intestine (Gafar and Itodo, 2011).

Flavonoids enhance the effects of Vitamin C and function as antioxidants. They are also known to be biologically active against liver toxins, tumors, viruses and other microbes (Korkina and Afanasev, 1997). In the present study we have estimated the amount of Flavonoids in the leaves of *M. oleifera,* which is in line with the earlier reports of Pakade *et al,* (2012) and Erian *et al,* (2016).Flavonoids and tannins are phenolic compounds and plant phenol is a major group of compounds that act as primary antioxidant or free radical scavengers (Polterait, 1997). The biological function of flavonoids include protection against allergies, inflammation, free radicals scavenging platelets aggregation, microbes, ulcers, hepatoxins, virus and tumors (Ayoola *et al.,* 2008). Reduction of coronary heart disease has been reported to be associated with consumption of flavonoid (Hertog *et al.*, 1993).

Plant terpenoids are used extensively for their aromatic qualities. They play a role traditional herbal medicine and are under investigation for Antibacterial, Antineoplastic and other Pharmaceutical fun (Yamunadevi *et al.*, 2011). In the present study tannins and saponins are identified in the leaves of *M. oleifera.* Tannins have shown potential Antiviral, Antibacterial and Antiparasitic effects. Saponins are known for their antihypertension properties and involves in hemolysis of red blood cells (Winter *et al.*, 1993). Further, Saponins causes hypocholestrolaemia because it binds cholesterol making it unavailable for absorption (Soetan and Oyewole, 2009).

*M. oleifera* leaves contains essential amino acids such as methionine, cystine, tryptophan and lysine which make it a virtually ideal dietary supplement (Makkar and Becker, 1996). In the Philippines, it is known as 'mother's best friend' because of its utilization to increase lactation in mother and is also prescribed for anemia (Estrella *et al.*, 2000; Siddhuraju and Becker, 2003). In developing countries, Young children, women of reproductive age and pregnant women are most vulnerable to micronutrient deficiency and anemia, thus consumption of *M. oleifera* by these children and women could prevent anemia. In the present study presence of protein has been identified in the leaves of *M. oleifera.* Our observation are in line with earlier studies of Oduro *et al.,* (2008) who reported that *M. oleifera* leaves contain higher levels of proteins, further they observed the presence of iron and calcium making it a very rich source of dietary nutrient. Therefore, *M. oleifera* is highly recommended for expected mothers. Ingesting *M. oleifera* leaves with oils can improve vitamin A nutrition and can delay the development of cataract (Pullakhandam and Failla, 2007). Thus, consumption of *M. oleifera* leaves powder contains high proportion of vitamin-A can help to prevent night blindness and eye problems in children.

Gilani *et al.*, (1994) isolated four pure compounds, niazinin A, niazinin B, niazimicin and niazinin A+B from the leaves of *M. oleifera*, which showed a blood pressure lowering effect in rats mediated possibly through a calcium antagonist effect. Further, Niazimicin has been proposed to be a potent chemopreventive agent in chemical carcinogenesis and also showed the inhibition of tumor promoter teleocidin B-4-induced Epstein-Barr virus (EBV) activation (Murakami *et al.,* 1998; Guevara *et al.,* 1999). Niazimicin, Nitrile, mustard oil glycosides and thiocarbamate glycosides have been isolated from *M. oleifera* leaves, which were found to be responsible for the blood pressure lowering effect (Faizi *et al.,* 1995). The crude extract of *M. oleifera* leaves has a significant cholesterol lowering action in the serum of high fatdiet fed rats which might be attributed to the presence of a bioactive phytoconstituent, i.e. β-sitosterol (Ghasi *et al.*, 2000).

*M. oleifera* leaves significantly decrease blood glucose concentration in Wistar rats and Goto-Kakizaki (GK) rats, modeled type 2 diabetes (Ndong *et al.,* 2007). Mittal *et al* (2007) stated that *M. oleifera* leaves extract was effective in lowering blood sugar levels within 3 h after ingestion. As a mechanistic model for antidiabetic activity of *M. oleifera*, it has been indicated that dark chocolate polyphenols (Grassi *et al.,* 2005) and other polyphenols (Moharram *et al.,* 2003; Al-Awwadi *et al.,* 2004) are responsible for hypoglycemic activity. Further, in the present study we have determined the amount of Phenol present in the leaves of *M. oleifera.* Phenols comprise the largest group of plants secondary metabolite and it has been reported to have multiple biological effects, including antioxidant property. Ara *et al.* (2008) performed a comparison study of *M. oleifera* leaves extract with antenolol (a selective β1 receptor antagonist drug, used for cardiovascular diseases) on serum cholesterol level, serum triglyceride level, blood glucose level, heart weight and body weight of adrenaline induced rats, it was found that *M. oleifera* leaves extract cause significant changes in cardiovascular parameters. Thisstudy reported *M. oleifera* leaves extract as hypolipidimic, lowering body weight, heart weight, serum

triglyceride level and serum cholesterol level in experimental animals.

It has been reported that the methanol and ethanol extracts of Indian origin *M. oleifera* leaves have the highest antioxidant activity with 65.1 and 66.8%, respectively (Lalas and Tsaknis, 2002; Siddhuraju and Becker, 2003). It was also reported that the major bioactive compounds of phenolics, such as quercetin and kaempferol are responsible for antioxidant activity of *M. oleifera* (Bajpai *et al.,* 2005). *M. oleifera* leaves ethanolic extracts showed significant protection against liver damage induced by antitubercular drugs [isoniazid (INH), rifampicin (RMP) and pyrazinamide (PZA)] in rats. Moreover, methanolic and chloroform extracts of *M. oleifera* leaves also showed significant protection against CCl4 induced liver damage in albino rats (Pari and Kumar, 2002).Thus, the present study showed that different compounds were identified from *Moringa oleifera* leaves has nutritional and medicinal properties could attribute to such compounds.

## CONCLUSION

The present study concludes that *M. oleifera* leaves possesses phytochemicals, such as Alkaloids, Triterpenoids, Flavonoids, Tannins, Saponins, Glycosides and Carbohydrates etc., which are of high therapeutic value. The results of our study suggest that *M. oleifera* leaves are rich in phenolic compounds, flavonoids and Vitamin C which have strong antioxidant activity. The infrared characterization revealed the presence of C=O, C=C, C-O, N-O etc. bond stretching's. The present study suggest that the presence of phytochemical in *Moringa oleifera* leaves acts as a good source of nutrient which can be used as a food and it also has potent medicinal property to improve the health status in humans by consuming it. Further, synthesis of the active principle can lead to development of promising drugs in treating various diseases.

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