VOL. 20 NO. 1
ISSN: 2877-1990
MARCH, 2021

African Scholar Publications & Research International

African Scholar Journal of Agriculture and Agricultural Tech. (JAAT-1)

Proximate Analysis and Anti-Ulcer Activity of Methanolic Extract of *Moringa Oleifera*

Hamisu Abdu[1], Ashiru Garba A.[2]

[1]Department of Polymer Technology, Hussaini Adamu Federal Polytechnic, Kazaure, Jigawa State, Nigeria. [2] Federal College of Education Gusau, Zamfara State, Nigeria

Abstract

Moringa oleifera is a native tree which has been reported to have medicinal properties. The leaves and the seeds were traditionally reported for the treatment of peltic ulcers. The objectives of this study was to evaluate the proximate analysis and anti-ulcer activity of the methanolic extracts of *Moringa oleifera (*Lam) leaves. The results of proximate analysis in methanolic *Moringa oleifera* leaves extract shows relatively low moisture content (7.36%) and low protein content (6.99%), however, it shows high carbohydrate content (45.60%) and high fibre content (21.28%). The preliminary phytochemical screening revealed the presence of flavonoids, Saponin, Alkaloids, Steroids and terpenoids. The anti-ulcer activity of the methanolic extract of *Moringa oleifera* leaves was evaluated in swiss albino mice against aspirin induced peptic ulcer. After the administration of the methanolic extract of *Moringa oleifera*, the acute toxicity was determined by oral administering a single dose of 2,000 mg/kg orally to swiss albino mice which was observed within an hour post-dosing and once daily for a period of 2 weeks. Methanolic extract of *Moringa oleifera* leaves shows a significant ($p<0.01$) anti-ulcer activity in a dose dependent manner as well as significant ($p<0.01$) reduction in the ulcer index when compared to control group, the anti-ulcer activity results was relatively comparable to the positive control.. This dose did not produce mortality or acute signs of toxicity throughout the

observation period. Our study shows that *Moringa oleifera* leaves has considerable anti-ulcer activity**.** These findings suggest that *Moringa oleifera* leaves possess anti-ulcer potential which may contribute to its ethno-medicinal uses.

Keywords; Proximate analysis, Phytochemical screening, Anti-ulcer activity, *Moringa oleifera.*

Introduction

Moringa oleifera (MO) is native to the western and sub-Himalayan region, India, Pakistan, Asia, Africa and Arabs. The Moringa tree is cultivated and used as a vegetable (leaves, pods flowers, roasted seeds), for spice (mainly roots), cooking and cosmetics oil (seeds) and as a medicinal plant (all plant organs) (Rebecca *et al*., 2006). Important medicinal properties of the plant include antipyretic, antiepileptic, anti-inflammatory, anti-ulcerative, anti-hypertensive, cholesterol lowering, anti-oxidant, anti-diabetic, hepatoprotective, anti-bacterial and anti-fungal activities (Nickon, *et al*., 2003). In addition, *M. oleifera* seeds possesses water purifying powers (Ruckmani *et al*., 1998). They are known to be anti-helminthic, antibiotic, detoxifiers, immune builders and have been used to treat malaria and it can also be used as a less expensive bio-absorbent for the removal of heavy metals (Sharma *et al*., 2006). *Moringa oleifera* is a highly valued plant, distributed in many countries of the tropics and subtropics. It has impressive range of medicinal uses with high nutritional value. Different parts of this plan contain a profile of important minerals, and a good source of protein, vitamin, â carotene, amino acids and various phenolics (Farooq *et al*., 2007). The Moringa plant provides a rich and rare combination of zeatin, quercetin, kaempferom and many other phytochemicals. However, a very important step in the screening of the sanitizing and preservative activity of a plant material is to evaluate its antimicrobial properties. It is important to evaluate the antimicrobial properties of *M. oleifera* leaves on some selected microorganisms and also to verify its phytochemical constituent.

In northern Nigeria, *M. oleifera* is highly a sourced as food vegetables, particularly because of their health-

promoting and disease-preventing properties strongly suspected to be due to the presence of many phytochemicals in them. Phytochemicals are a group of non-nutrient bioactive compounds found naturally in plant parts such as flowers, buds, leaves, fruits, roots, barks, spices and medicinal plants; and work in conjunction with other plant components as a defensive mechanism for the plants against diseases and many external attacks.

Phytochemicals also provide characteristic color, aroma and flavour in plants. They are plant metabolites. In humans, many phytochemicals have been found to be protective and preventive against many degenerative diseases and pathological processes such as in ageing, coronary heart disease, Alzheimer's disease, neuro-degenerative disorders, atherosclerosis cataracts, and inflammation. Both epidemiological and clinical studies provided evidence that most of these phytochemicals exhibit their protective and disease-preventing functions through their antioxidant activities. Typical phytochemical compounds that possess antioxidant activity include phenols, phenolic acids and their derivatives, ascorbic acid, flavonoids, phytic acid and many sterols. As antioxidants, these species are capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes and inhibit oxidases.

Peptic ulcer is a disease characterized by mucosal damage that usually occurs in the stomach and proximal duodenum. It is a serious injury occurring by spicy food, stress, alcohol, gastric surgery and Helicobacter pylori. Aspirin (ASP), one of the most widely used NSAIDs, damages gastrointestinal mucosa by irritant action, causing alterations in mucosal permeability and/or suppression of prostaglandin synthesis. Aspirin induced ulcer has been used as a model for the evaluation of anti-ulcerogenic agents. Moringa *oleifera* is rich in fairly unique group of phytochemicals, glucosinolates and isothiocyanates. Moringa *oleifera* leaves contain more vitamin A than carrots, more calcium than milk, more iron than spinach, more vitamin C than orange and more potassium than bananas[and protein quality is more than milk and eggs. In many cultures through the tropics, different parts of this plant are being used as foods as well as medicinal purpose. Leaves of this plant have been reported to possess hypotensive, antispasmodic, diuretic, abortifacient, wound healing, analgesic, hepatoprotective, anti-tumor agent and radio-protective effect (Fahey, 2005; Faizi *et al.,* 1995; Hukkeri *et al.,* 2006; Murakami *et al.,* 1998; Nadro *et al.,* 2006; Rao *et al.,* 2003).

Moringa oleifera, commonly called the drum stick, is a tree native to India, but has been planted and domesticated in many other countries, including Nigeria. It is the most known and widely cultivated variety of the genus *Moringa*, family *Morigaceae* . *Moringa oleifera* is also known by other common names such as Mallungay (Philippines), Benzolive tree (Haiti), Horse raddish tree (Florida) and Nebeday (Senegal). In Nigeria, it is known as Zogale in Hausa, Okwe Oyibo in Igbo, Ewe Ile in Yoruba and Jeghel-agede in Tiv. The leaves, seeds and flowers all have good nutritional and therapeutic value. The flowers are eaten cooked in soups and resemble mushrooms while the leaves are eaten cooked as vegetables. The flowers and leaves are good sources of vitamins A, B group and C when raw and are among the best sources of minerals. The plant has been linked to the treatment or at least suppression of many degenerative diseases among many rural consumers.

Plant-derived substances have recently received a great interest owing to their versatile applications. Medicinal plants are the richest bio-resource for drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Ncube *et al*., 2008). Plants are an integral part of human diet as they supply vitamins and minerals which are the important constituents essential for human health (Mumzuroglu *et al.,* 2003).

Extraction (as the term is pharmaceutically used) is the separation of medicinally active portions of plant (and animal) tissues using selective solvents through standard procedures (Nonita *et al.,* 2010). The products so obtained from plants are relatively complex mixtures of metabolites, in liquid or semi-solid state or (after removing the solvent) in dry powder form, and are intended for oral or external use. Several epidemiological studies established a link between phytochemicals and the range of biological activities that impart health benefits in human beings.

Phytochemicals are chemical compounds that occur naturally in plants (phyto means "plant" in Greek). Some are responsible for color and other organoleptic properties, such as the deep purple of blueberries and the smell of garlic. The term is generally used to refer to those chemicals that may have biological significance, for example antioxidants, but are not established as essential nutrients (Ncube *et al.,* 2008). These phytochemicals often secondary metabolites present in smaller quantities in higher plants, include the

alkaloids, steroids, flavonoids, terpenoids, tannins, and many others (Nonita *et al.*, 2010).

Oluduro (2012) reported the presence the following minerals in the leaves: sodium (11.86), potassium (25.83), calcium (98.67), magnesium (107.56), zinc (148.54), iron (103.75), manganese (13.55) among others in parts per million and nutrients such as carbohydrate (45.43%), protein (16.15%), fat (9.68%), crude fibre (9.68%), moisture (11.76%) and ash (10.64%). The leaves are edible and are commonly cooked and eaten like spinach or used to make soups and salads. The composition of the amino acids in the leaf protein is well balanced (Ogbe and Afikku, 2011). The leaves and pods are helpful in increasing breast milk in nursing mothers during breastfeeding (Oluduro, 2012) and the seeds have been found to contain a non-toxic natural polypeptide that sediments mineral particles and organics in the purification of drinking water, for cleaning vegetable oil, and for sedimenting fibers in the juice and beer industries.

Nutritional analysis indicates that *Moringa* leaves contain a wealth of essential disease preventing nutrients which make it suitable to be included in diets as food supplement (Krishnaiah *et al*., 2009). *Moringa* leaves have been used to combat malnutrition, especially among infants and nursing mothers and hasten uterine contraction during child birth in pregnant women (Oluduro, 2012). It has also been found that extract obtained from the leaves of *Moringa* in 80 % ethanol contains growth enhancing principles for higher plants (Makkar and Becker, 1996).

In ethno-medicine, *Moringa oleifera* leaves have been used by local traditional healers in treatment of various ailments such as gastric discomfort, stomach ulcers, diarrhea, dysentery and skin infections. In certain case of diabetes, *Moringa* can also be used to stabilize sugar levels and can stabilize arterial tension. The leaves have also been found to possess antitumour, antipyretic, antiepileptic, anti-inflammatory, antiulcer, anti-spasmodic, diuretic, anti-hypertensive and antioxidant properties.

However, It has been reported that climatic factors and stages of maturity could cause variation in distribution of these phytochemicals in leaves of *M. oleifera* as well as the choice of solvent as different solvents have different extraction capabilities and spectrum of solubility for phytoconstituents. In this view, the experiment was to evaluate the phytochemical constituents of the aqueous and ethanolic extracts and determine the nutritional values of the whole leaf of *Moringa oleifera* in Nsukka, South-Eastern Nigeria.

The most recent studies on *M. olefiera* are using the crude protein from the dried and green pod in animal feeding, while no brillient studies have been achieved to *M. olefiera* seed in human nutrition so far. Although its leaves represent an important source of proteins, the nutritional quality depends on the absolute and relative contents of essential amino acids and its bioavailability after digestion and absorption. Pinto *et al.* (2015) demonstrated that vegetable proteins are less susceptible to *in vivo* digestion than animal proteins. The low content of sulfur amino acids, compact structure, presence of non-protein components (dietary fiber, tannins, phytic acid) and anti-physiological proteins (protease inhibitors, lectins) can affect digestion. Teixeira *et al.* (2014) found that whole leaf flour contained 28.7% crude protein, 7.1% fat, 10.9% ashes, 44.4% carbohydrate and 3.0 mg 100 g^{-1} calcium and 103.1 mg 100 g^{-1} iron. The protein profile revealed levels of 3.1% albumin, 0.3% globulins, 2.2% prolamin, 3.5% glutelin and 70.1% insoluble proteins. Otherwise, the most recent investigations reported that a flocculating protein (6.5 kDa, IEP pH 10) from the seeds of *M. oleifera* was isolated and purified. Amino acid analysis and sequencing showed high contents of glutamine, arginine and proline, and a total of 60 residues. Moreover, Mo-CBP3 is an anti-fungal protein produced by *M. oleifera* which has been investigated as potential candidate for developing transgenic crops (Pinto *et al*., 2015). Freire *et al.* (2015) found that Mo-CBP3 is a chitin-binding protein that inhibits the germination and mycelial growth of phytopathogenic fungi. This protein is highly thermo stable and resistant to pH changes, and therefore may be useful in the development of new anti-fungal drugs.

The oil concentration in *moringa* seed was ranged from 25.8% to 31.2%. The physicochemical properties and oxidative stability of extracted oil from seeds of *M. oleifera* recorded that cold pressed oil contains high levels of *β*-sitosterol (up to 50.07%), stigmasterol (up to 17.27%), and campesterol (up to 15.13%).

Materials and Methods

Sample collection

Leaves from an uninfected and healthy *Moringa oleifera* tree will be collected from Ringim Local Government Area, Jigawa State. The leaves will be air dried for one week at room temperature after which the dried leaves will be blended into powdery form and stored in a sealed container prior to use. Swiss albino mice of both sexes weighing 120 - 150 g were housed in cages and they were maintained at a temperature of approximately 25^{o}C. They were fed with

standard dry pellets and tap water *ad libitum*. The mice were allowed to acclimatize to the environmental conditions for 14 days before the experiments commenced.

Methanolic extraction of *Moringa oleifera* leaves

The method of Debela (2002) will be employed. The aqueous and ethanol extract of *Moringa oleifera* leaves will be prepared by soaking 50g of the powdered sample in a conical flask containing 100 ml of methanol respectively placed on a shaker for 24 h. The extract will then be filtered using sterile Whattman filter paper. The extract will be concentrated using rotavapor and stored in an airtight container.

Phytochemical analysis of *Moringa oleifera* leaf and seed

Test for tannins: The method as described by Debela (2002) will be employed. About 0.5 g of the sample will be mixed with 10 ml of distilled water and filtered. Few drops of 1% ferric chloride solution will be added to 2 ml of the filtrate. The occurrence of blue-black, green or blue green precipitate indicates the presence of tannins.

Test for steroids: Salkowski test as described by Debela (2002) will be employed. The crude extract was mixed with chloroform and a few drops of concentrated H_2SO_4 will be added. The mixture will be agitated vigorously and allowed to stand for 5 mins. A red coloration at the lower layer indicates the presence of steroid.

Test for cardiac glycosides: The method as described by Debela (2002) will be employed. 0.5% (w/v) extract, 2 ml of glacial acetic acid and few drops of 5% ferric chloride will be mixed together followed by the addition of 1 ml of concentrated sulphuric acid. The formation of a brown ring at the interface indicates the presence of cardiac glycosides.

Test for saponins: The method as described by Debela (2002) will be employed. 1 g of each sample extract will be boiled with 5 ml of distilled water and filtered. About 3ml of distilled water will then be added to the filtrate and shaken vigorously for about 5 mins. Persistent frothing indicates the presence of saponins.

Test for phenol: The method as described by Debela (2002) will be employed. 1% (w/ v) of the extract will be mixed with 2 ml of distilled water followed by

the addition of few drops of 10% ferric chloride. The formation of a blue or green color indicates the presence of phenols.

Test for alkaloids: The method as described by Debela (2002) will be employed. 0.5% (w/v) of the extract will be mixed with 5 ml of 1% aqueous HCl on water bath with continuous stirring for few minutes and then filtered. 1 ml of the filtrate will be pipetted individually into 3 test tubes. To each 1 ml in each test tube; Mayer, Wenger and Dragendroffs reagents will be added respectively. The formation of precipitate indicated the presence of alkaloids. Mayer's gives a white precipitate, Wenger's gives a reddish brown precipitate while Dragendroff's gives orange brown precipitate the three reagents can be used to ascertain the presence of alkaloids.

Test for terpenoides: The method as described by Debela (2002) will be employed. 5% (w/v) of each sample extract will be mixed with 2 ml of chloroform ($CHCl_3$) in a test tube. 3 ml of concentrated H_2SO_4 will be carefully added to the mixture to form a layer. An interface with reddish brown coloration indicates a positive result.

Test for flavonoids: The method as described by Debela (2002) will be employed. A small quantity of each test extract will be dissolved separately in dilute NaOH. A yellow solution that turns colorless on addition of concentrated HCl indicates the presence of flavonoids.

Test for quinones: The method as described by Debela (2002) will be employed. 1% (w/v) of extract will be mixed with 1 ml of concentrated H_2SO_4. The formation of a red color indicates the presence of quinones.

Test for antraquinones: Borntrager's test was used as described by Debela (2002) will be employed. About 0.2% (w/v) of the sample extract was shaken with 10 ml of benzene and then filtered. 0.5 ml of 1% ammonia solution was added to the filtrate and thereafter shaken. Appearance of pink, red or violet color indicates the presence of free anthraquinones.

Proximate analysis of *Moringa oleifera* leaf and seed.

Proximate analysis of the powdered *Moringa oleifera* will be carried out using standard procedure (Pearson, 1976). The parameters to be determined were ash content, moisture content, protein content, lipid content, fibre content and carbohydrate.

Mean ulcer index

Treatment with MO methanolic extract for consecutive fourteen days (14) reduced the severity of ulcer intensity. In this study significant increase in the extent of ulceration mainly in glandular part of gastric mucosa was observed after aspirin treatment, as evidenced by increased mean ulcer index ($p < 0.01$).

Mucosal Thickness

MO methanolic extract treatment for 14 days increased mucosal thickness in group 4 while indomethacin treated group was not found to have increasedthe thickness as evidenced by the results ($P < 0.01$)

Measurement of Ulcer Index (UI)

The dissected rats were examined for ulceration under a 3-fold magnifier. The number of ulcers was recorded and the severity scored as follows:

0 = no ulcer; 1 = superficial ulcers; 2 = deep ulcers; and 3 = perforated or penetrated ulcer. Ulcer index (UI) was calculated using the formula:

$$\mathbf{UI = UN + US + UP \times 10\text{-}1}$$

Where UN = average number of ulcers per animal, US = average of severity score, and UP = percentage of animals with ulcer (Parmar, 1993). The percentage protection was calculated using the following formula:

$$\% \text{ Protection} = \frac{\text{Mean ulcer index of control} - \text{Mean ulcer index of test}}{\text{Mean ulcer index of control}} \times 100$$

Determination of Total Acidity

Gastric juice was collected from the ligated-rats, the volume and pH were measured. For the determination of total acidity, 0.5 mL of the supernatant fluid was pipetted into a 100 mL beaker. Two drops of phenolphthalein solution were added and the solution titrated with 0.1N NaOH until a pink colour appears (Vogel, 2002). The titration was repeated where the volume of gastric juice was adequate. The total volume of alkali added was noted for each titration. Total/titratable acidity was calculated and expressed as micro Eq/L per 100 g of body weight. Acidity was calculated using the formula:

$$\text{Acidity} = \frac{\text{volume of NaOH} \times \text{Normality of NaOH} \times 100 \text{ mEq}}{0.1 \text{ L}}$$

Indomethacin Induced Ulcers in Swiss Albino Mice

Swiss albino mice of either sex were fasted overnight. The animals were divided into 5 groups of 6 animals each. Group I was administered distilled water in 0.1% Tween 80 orally. The animals in Groups II, III and IV were pre-treated orally with 200-800 mg/kg of MO extract in 0.1% Tween 80 respectively while Group V was pre-treated with the standard drug cimetidine also dissolved in 0.1% Tween 80. After 30 min, 20 mg/kg indomethacin dissolved in 0.1% Tween 80 solution was administered orally to the animals. Six hours later, the mice were sacrificed in chloroform chamber and their stomachs removed. Formol-saline (2% v/v) was injected into the totally ligated stomachs and stored in plain tubes filled with formol-saline overnight. The next day, the stomachs were opened along the greater curvature, washed in warm water and examined under a 3-fold magnifier. The lengths of the longest diameters of the lesions were measured and summated to give a total lesion score (in mm) for each animal. The mean count for each group was then calculated. Inhibition (protection) of the lesion production is expressed as percentage value (Vogel, 2002).

Results and Discussion

The results of the phytochemical screening was presented in **Table 1**. This study has confirmed the presence of secondary metabolites such as flavonoids, phenols, tannins, saponins, alkaloid and glycosides which are claimed to be found in *Moringa oleifera*. The results obtained in this was in good agreement with the work reported by Fadila *et al.,* 2018. The proximate analysis of *Moringa oleifera* leaves provides an information that its consumption is safe and beneficial to human health. The results of the nutrional content in *Moringa oleifera* leaves (**Table 2)** shows relatively low moisture content (7.36%) and low protein content (6.99%), however, it shows high carbohydrate content (45.60%) and high fibre content (21.28%). The low moisture content indicates that the powered sample in less liable spoilage by microbial contamination of properly stored. The ash value indicate that the powdered sample contain more of organic components. It is a good source of protein, carbohydrate and fat as these are present in large amount and within the dietary recommended allowance. According to the work conducted by Oluduro (2012) it was revealed that *Moringa oleifera* contains carbohydrate (45.43%), protein (16.15%), fat

(9.68%), crude fibre (9.68%), moisture (11.76%) and ash (10.64%), which was in good agreement with our findings.

Table 1: Results of phytochemical of *Moringa oleifera* leaves

PHYTOCHEMICALS	INFERENCE
Flavonoids	Positive
Alkaloids	Positive
Phenols	Positive
Tannins	Positive
Saponins	Positive
Glycosides	Positive

Table 2: Results of phytochemical of *Moringa oleifera* leaves

COMPOUNDS	% COMPOSITION
Fibre	21.28
Moisture	7.36
Ash content	12.63
Carbohydrate	45.60
Protein	6.99
Lipids	15.4

Table 3: Effect of methanolic seed extract of *M. oleifera* on indomethacin-induced ulcers in rats

Experiment	Ulcer Index	PH gastric Juice	Acidity (Eq/L)	Mucosal thickness (µm)	Protection (%)
Distilled water 10 mL/kg	6.66±1.13	5.66	6.02	24.88	**…….**
MO extract 200mg/kg	4.98±2.02	9.22	5.66	43.44	45.67
MO extract 400mg/kg	8.73±2.24	4.01	3.01	58.97	67.89
MO extract 600mg/kg	7..77±1.09	4.62	8.00	73.33	58.89

MO extract 800mg/kg	9.77±3.11	3.04	8.22	84.21	98.45

Ulcer index and gastric juice volume are expressed as mean ± SEM; (n = 6); ns (not statistically significant) when compared with distilled water (control) alone using one-way ANOVA, MO = Moringa *oleifera*

Conclusion

The present study of proximate and phytochemical screening revealed that *Moringa oleifera* leaves are safer to be used. The study showed that administration of the aqueous seed extract of *M. oleifera* at 200-800 mg/kg decreases gastric volume, total acidity while gastric pH was increased. In indomethacin-induced ulcer model, the extract also produced a significant ($p < 0.01$) and dose-dependent reduction in ulcer index. This dose did not produce mortality or acute signs of toxicity throughout the observation period. Our study shows that *Moringa oleifera* leaves has considerable anti-ulcer activity**.** These findings suggest that *Moringa oleifera* leaves possess anti-ulcer potential which may contribute to its ethno-medicinal uses

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