Phytochemical Screening, Nutritional Composition and Antimicrobial Activity of Moringa Oleifera Seed and Leaf Extract against Selected Gastrointestinal Pathogens

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Abstract: In recent times, the use of plants as a source of novel compounds to combat microbial infections has gained prominence. The necessity to search for plant-based antimicrobials is increasing due to high cost, reduced efficacy and increased resistance to conventional medicine. Moringa oleifera is one of the plants that is rapidly gaining popularity because of the numerous benefits associated with it. This study aims to determine the phytochemical content, nutritional composition and antimicrobial activity of Moringa oleifera seed and leaf. The phytochemical and nutritional composition was determined using standard methods. The antagonistic activity of the aqueous and ethanol extract of the leaf and seed of Moringa oleifera was tested against selected gastrointestinal pathogens (Escherichia coli, Proteus mirabilis, Staphylococcus aureus, Enterobacter aerogenes and Bacillus cereus) using the agar well diffusion assay. The qualitative screening of phytochemicals in both the aqueous and ethanol leaf extracts of Moringa oleifera showed the presence of alkaloids, flavonoids, phenols, tannin, terpenoids, cardiac glycosides, coumarin, steroids and saponin while both aqueous and ethanol seed extract showed the presence of terpenoids, cardiac glycosides, steroids and saponin. Proximate composition of Moringa oleifera showed that the leaf had higher percentage composition of moisture (6.39%), ash (13.16%), and fibre (18.11%) while the seed had higher percentage of lipid (41.79%), protein (8.31%) and carbohydrate (39.41%). The result for antimicrobial activity showed that the aqueous leaf and seed extract had high activity against P. mirabilis (14 - 24mm), S. aureus (12 - 16mm), E. aerogenes (6 - 16mm) and E. coli (8 - 18mm), while the ethanol leaf and seed extracts showed inhibition for E. coli (6 - 24 mm), P. mirabilis (8 – 12 mm), S. aureus (10 mm), B. cereus (12 mm) and E. aerogenes (12 - 16 mm). Moringa oleifera may be used in treating gastrointestinal infections particularly those caused by the organisms under study. It is therefore concluded that the leaf of Moringa oleifera has a high potential for use as an antimicrobial agent against gastrointestinal pathogens than the seed. However, the seed can be used as a food supplement to increase the nutritional composition of foods lacking protein, carbohydrate and lipid.

Keywords: Moringa oleifera, Phytochemicals, nutrient content, antimicrobial activity, gastrointestinal pathogens

I. Introduction

Moringa oleifera is one of the most widely cultivated species of monogenic family in recent times [1]. It is a drought-tolerant plant that thrives best under the tropical climate and tolerates different soil types [2]. Moringa oleifera is highly valued since almost every part of the plant i.e. the leaves, seeds, roots, bark, fruit and flower is directly or indirectly used as food with high nutritional value [3].

Moringa oleifera is known by various names; "Drumstick tree" and "Horseradish tree" in English, "Soaanjna" in Hindi, "Sajna" in Bengali, "Nugge" in Kannada, etc. Moringa oleifera is an edible plant from ancient times; it has been a regular component of conventional eatables in India [4]. Moringa oleifera is a fast growing tree with about 10 - 12 m in height and a diameter of 2.04 m at chest height. It has a soft trunk, white cork and a gummy bark bearing branches. The flowers are pleasantly fragrant, white in colour; and the three wings seeds are scattered by the winds. Moringa oleifera flowers, tender leaves and pods are eaten as vegetables [5].

In Nigeria, Moringa oleifera leaf is increasingly gaining popularity, consumed by almost everyone and thus referred to as "miracle tree" because of its versatile therapeutic applications and outstanding nutritive value, though it has different local names among the three major ethnic groups, amidst the Yoruba it is either called "Ewe igbale" or "Idagbo monoye", amidst the Igbos it is either called "Odudu oyibo" or "Okwe oyibo" and amidst the Hausas it is called "Zogalli"[6]. It has been reported that Moringa oleifera in Nigeria is gaining ground because of its outstanding indigenous nutritive and medicinal value. This study aims at analyzing the phytochemical content, nutritional composition and antimicrobial activity of Moringa oleifera seed and leaf.

II. Materials And Methods

Collection of samples: Leaves and seeds from an uninfected and healthy Moringa oleifera tree was collected from Talba farm at Minna, Niger state. The leaves and seeds were dried for one week at room temperature after which the dried leaves and seeds were blended into powdery form.

Aqueous and ethanol extraction of Moringa oleifera leaf and seed

The method of [7] was employed. The aqueous and ethanol extract of Moringa oleifera leaf and seed was prepared by soaking 10 g of each powdered sample in different conical flasks containing 100 ml of distilled water and 100 ml of ethanol respectively placed on a shaker for 24 h. The extract was then filtered using sterile Whatman filter paper. The extract was then concentrated and stored in an airtight container.

Phytochemical analysis of Moringa oleifera leaf and seed

Test for tannins: The method as described by [7] was employed. About 0.5 g of the sample was mixed with 10 ml of distilled water and filtered. Few drops of 1% ferric chloride solution was 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 [7] was employed. The crude extract was mixed with chloroform and a few drops of concentrated H_2SO_4 were added. The mixture was agitated vigorously and allowed to stand for 5 min. A red colouration at the lower layer indicates the presence of steroid.

Test for cardiac glycosides: The method as described by [7] was employed. 0.5% (w/v) extract, 2 ml of glacial acetic acid and few drops of 5% ferric chloride were mixed together. This was under layered with 1 ml of concentrated sulphuric acid. The formation of a brown ring at the interface indicates the presence of cardiac glycosides.

Tests for phytosteroids: The method as described by [7] was employed. 1% (w/v) of the plant extract was mixed with 1 ml of chloroform. Few drops of concentrated sulphuric acid was added to the mixture, the appearance of a bluish brown ring indicates the presence of phytosteroids.

Test for saponins: The method as described by [7] was employed. 1 g of each sample extract was boiled with 5 ml of distilled water and filtered. About 3ml of distilled water was added to the filtrate and shaken vigorously for about 5 min. Persistent frothing indicates the presence of saponin.

Test for phenol: The method as described by [7] was employed. 1% (w/ v) of the extract was 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 [7] was employed; 0.5% (w/v) of the extract was 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 was each pipetted individually into 3 test tubes. To each 1 ml in each test tube; Mayer, Wenger and Dragendroffs reagents were 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 was used to ascertain the presence of alkaloids.

Test for terpenoides: The method as described by [7] was employed; 5% (w/v) of each sample extract was mixed with 2 ml of chloroform (CHCl₃) in a test tube. 3 ml of concentrated H₂SO₄ was carefully added to the mixture to form a layer. An interface with reddish brown colouration indicates a positive result.

Test for flavonoids: The method as described by [7] was employed; a small quantity of each test extract was dissolved separately in dilute NaOH. A yellow solution that turns colourless on addition of concentrated HCL indicates the presence of flavonoids.

Test for quinones: The method as described by [7] was employed; 1% (w/v) of extract was mixed with 1 ml of concentrated H₂SO₄. The formation of a red color indicates the presence of quinones.

Test for coumarins: The method as described by [7] was employed. 3 ml of 10% NaOH was added to 2% (w/v) of the extract. A yellow colour indicates the presence of coumarins.

Test for antraquinones: Borntrager's test was used as described by [7]. 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 colour indicates the presence of free anthraquinones. **Proximate analysis of** moringa oleifera **leaf and seed.**

Determination of moisture content

The method of [8] was employed. 2 g of the powdered sample was weighed (W_1) into pre-weighed crucible (W_0) and placed into a hot drying oven at 105° C for 3 h. The crucible were removed, cooled in desiccators and weighed. The process of drying, cooling and weighing were repeated until a constant weight (W_2) was obtained. The weight loss due to moisture was obtained thus; Moisture $(\%) = -W_1 - W_2 \times 100$ $\mathbf{W}_1 - \mathbf{W}_0$

Where:

 W_0 = weight of the empty crucible (g) W_1 = weight of the powder sample + empty crucible (g) W_2 = weight of dried sample + empty crucible.

Determination of ash content

The method of [9] was used. 2 g of the dried powdered leaves samples was weighed (W_1) into preweighed empty crucible (W_0) and placed into a lenton muffle furnace at 550^oC for 5 h. The ash was cooled in a desiccator and weighed (W_2) . The weight of the ash was determined by the difference the dry powdered leaves sample, pre-weighed and the ash in the crucible. Percentage ash was obtained by equation;

Ash (%) = $W_2 - W_0 \times 100$

$$\mathbf{W}_1 - \mathbf{W}_0$$

Where:

 W_0 = weight of empty crucible (g), W_1 = Weight of crucible + powdered sample (g), W_2 = weight of crucible + ash sample (g).

Determination of Crude Lipids

The crude lipid content in the sample was extracted using soxhlet extraction procedure, described by [8]. The ground sample was weighed (W_0) into a porous thimble and covered with a clean white cotton wool. Petroleum ether (200 cm³) was poured into a 250 cm³ extraction flask, which was previously dried in the oven at 105^oC and weighed (W_2). The porous thimble was placed into the soxhlet and the rest of the apparatus was assembled. Extraction was done for 5 h. the thimble was removed carefully and the extraction flask placed in a water bath so as to evaporate the petroleum ether and then dried in the oven at a temperature of 105^oC to completely free the solvent and moisture. It was cooled in a desiccator and weighed (W_1). The percentage crude lipid was calculated using the equation below:

Crude lipid (%) =
$$W_1 - W_2 \times 100$$

 $-W_0$

Where:

 W_0 = weight of sample, W_1 = weight of flask + oil, W_2 = weight of flask

Determination of crude fiber content

Percentage of crude fiber was determined by the method of [8] in which 2 g of ground sample was weighed (W_0) into a 1dm³ conical flask. Water (100 cm³) and 20% H₂SO₄ (20 cm³) were mixed and boiled gently for 30 min. The content was filtered through a Whatman No.1 filter paper. The residue was scrapped back into the flask with a spatula. Water (100 cm³) and 20 cm³ of 10% NaOH were added and allowed to boil gently for 30 min. The content was filtered and the residue was washed thoroughly with hot distilled water, and then rinsed once with 10% HCl and twice with ethanol and finally three times with petroleum ether. It was allowed to dry and scrapped into the crucible and dried overnight at 105°C in an hot air oven. It was then removed and cooled in a dessicator. The sample was weighed (W_1) and ashed at 55°C for 90 min in a lenton muffled furnace. It was finally cooled in a dessicator and weighed at again (W_1). The percentage crude fiber was calculated using this equation:

Crude fibre $\binom{1}{W_0} = \frac{W_1 - W_2 \times 100}{W_0}$ Where: Wo = weight of sample, W_1 = weight of dried sample, W_2 = weight of ash sample.

Determination of crude protein

The crude protein of the sample was determined by using the microKjedahl method described by [10]. The sample (2 g) was weighed along with 20 cm³ of distilled water into a micro-Kjedahi digestion flask. It was shaken and allowed to stand for some time. One tablet of selenium catalyst was added followed by the addition of 20 cm³ concentrated sulphuric acid. The flask was heated on the digestion block at 100°C for 4 h until the digestion became clear. The flask was removed from the block and allowed to cool. The content was transferred into 50 cm³ volumetric flask and diluted to the mark with water. An aliquot of the digest (10 cm³) was transferred into another micro-Kjedahi flask along with 20 cm³ of distilled water and placed in the distilling outlet of the micro-Kjedahi distillation unit. A conical flask containing 20 cm³ of boric acid indicator was placed under the condenser outlet. 40% sodium hydroxide solution (20 cm3) was added to the content in the Kjeldahi flask by opening the funnel stopcock. The distillation process was started and heat was supplied and regulated to avoid sucking back. When all the available distillate was collected in 20 cm³ of boric acid, the distillate was stopped. The nitrogen in the distillate was determined by titrating with 0.01M of H₂SO₄; the end point was

obtained when the colour of the distillate changed from green to pink. Protein content was calculated by multiplying the total nitrogen content by a constant, 6.60 which is based on the assumption that protein contain about 16% N which include both true protein and non-protein N and does not make a distinction between available or unavailable protein. The crude protein was calculated using the formula:

Crude protein (%) = % N x 6.60

The nitrogen content of the sample is given by the formula below:

$$N(\%) = \frac{T_v X Na X 0.014 X V_1}{G X V_1} X 100$$

Where:

 T_v = titre value of acid, Na= concentration or normality of acid, V_1 = volume of distilled water used for distilling the digest (50 cm³) V_2 = volume of aliquot used for distillation (10 cm³), G= original weight of sample used.

Determination of carbohydrate

The method of [9] was adopted where the total proportion of carbohydrate in the leaves sample was obtained by calculation using the percentage dry method. That is by subtracting the % sum of other food nutrients from 100%. This is done by using the equation below:

CHO (%) =100% - (% crude protein + % crude lipids + % crude fiber + % ash + % moisture)

Antimicrobial activity of ethanol extract of moringa oleifera leaf and seed against selected gastrointestinal pathogens

Agar well diffusion assay as described by [11] was employed.

Collection and Standardization of Clinical Isolates

Clinical isolates of gastrointestinal pathogen was collected from University College hospital (UCH), Ibadan, Oyo state. Two Gram positive isolates namely; Staphylococcus aureus and Bacillus cereus and three Gram negative isolates namely; Escherichia coli, Proteus mirabilis and Enterobacter aerogenes were collected. Confirmatory distinctive tests like, coagulase test for Staphylococcus aureus, spore staining for Bacillus cereus, while Escherichia coli, Proteus mirabilis and Enterobacter aerogenes were grown on EMB and SSA media to observe the color of their colony. Each isolate was inoculated into Mueller Hilton broth and incubated at 37° C for 4 - 6 h. The inoculum was adjusted to 0.5 MacFarland standard.

Preparation of different concentration of ethanol and aqueous moringa oleifera leaf and seed extract

Different concentrations (50 mg/ml, 100 mg/ml, 150 mg/ml and 200 mg/ml) of the ethanolic and aqueous Moringa oleifera leaf and seed extracts were prepared. Preparation was done by weighing 50 mg, 100mg, 150 mg and 200 mg of the respective extracts and dissolving it in 1 ml of DMSO in separate test tubes.

Agar well diffusion assay

Mueller Hinton agar plates were streaked with each of the test isolates separately using sterile cotton swab sticks. Wells were bored using 6 mm cork borer. 50 μ l of the ethanol extract, aqueous extract, antibiotic (positive control), sterile distilled water (negative control) were dispensed into different wells. The plates were allowed to stand for 1-2 h prior to incubation so as to allow the diffusion of extracts. The plates were incubated at 37°C for 24 h. The diameter of the zone of inhibition was measured using a ruler and recorded in mm.

III. Results

The phytochemicals indicated in the ethanol and aqueous Moringa oleifera seed and leaf extracts is shown in Table 1. Flavonoids, tannin, terpenoids, cardiac glycoside, coumarin, saponin and steroids were present in the ethanol extract of Moringa oleifera leaf while terpenoids, saponin, cardiac glycoside and steroids were present in the ethanolic extract of Moringa oleifera seed. In the aqueous extract of the leaf and seed of moringa, alkaloids, flavonoids, saponin, phenols, tannins, terpenoids cardiac glycoside, coumarin and steroids were present.

The proximate analysis of Moringa oleifera leaf and seed is shown in Fig. 2. The leaf had more moisture content (6.39%), ash content (13.16%) and fibre (18.11%) than the seed which contained 4.59% moisture, 4.00% ash and 3.87% fibre while the seed had more carbohydrate (39.41%) and protein (8.31%) than the leaf which contained 35.64% of carbohydrate and 6.35% protein.

The antimicrobial activity of the ethanol and aqueous Moringa oleifera seed and leaf extracts against selected gastrointestinal pathogens is shown in Fig. 3, 4, 5 and 6. All the concentrations of the ethanol leaf extract of Moringa oleifera demonstrated high antagonistic activity against Escherichia coli. Proteus mirabilis was inhibited by 150mg/ml and 200mg/ml of the leaf extract and Enterobacter aerogenes was inhibited by the 200mg/ml ethanol leaf extract. The aqueous leaf extract also showed inhibitory activity against Escherichia coli. Enterobacter aerogenes was only inhibited by 150mg/ml and 200mg/ml and 200mg/ml and 200mg/ml ethanol leaf extract.

inhibited by only the 200mg/ml concentration. The aqueous and ethanol extract of Moringa oleifera showed no inhibitory activity against Staphylococcus aureus.

The ethanol seed extract of Moringa oleifera showed high antagonistic activity against Enterobacter aerogenes at 150mg/ml and 200mg/ml followed by Bacillus cereus, Staphylococcus aureus and Escherichia coli but had no activity against Proteus mirabilis. The aqueous seed extract showed maximum activity against Proteus mirabilis and Staphylococcus aureus at all concentrations followed by Escherichia coli and Enterobacter aerogenes at 100mg/ml, 150mg/ml and 200mg/ml concentrations but had no activity against Bacillus cereus.

IV. Figures And Tables Table 1: Qualitative screening of phytochemicals in moringa leaf and seed extracts.							
Parameters extract				5		Ethanolic seed extract	Aqueous seed
Alkaloids	_	+	_	_			
Flavonoids	+	+	_	_			
Phenol	_	+	_	_			
Tannins	+	+	_	_			
Terpenoids	+	+	+	_			
Cardiac glycosid	e	+	+	+	+		
Quinones	_	_	_	_			
Coumarin	+	+		_			
Steroids	+	+	+	+			
Phytosterol	_	_	_	_			
Saponin	+	+	+	+			
Anthocyanin		_	_	_	_		

Key:

+ indicates presence

- indicates absence

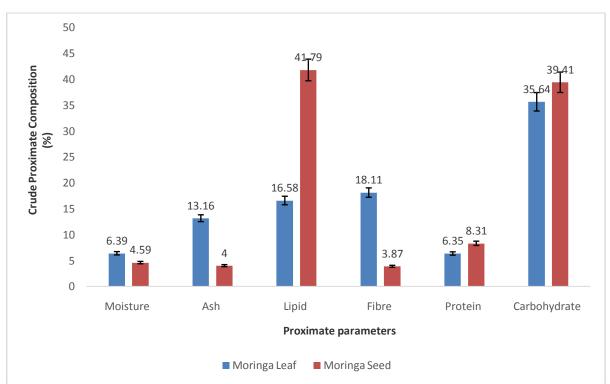


Fig. 2: Proximate Composition of Moringa oleifera leaf and seed

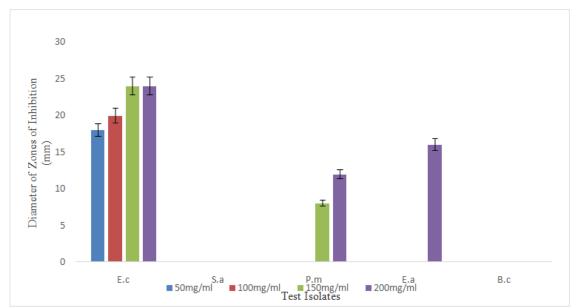


Fig 3: Effect of ethanolic moringa oleifera leaf extract against selected test isolates

Keys: E.c = Escherichia coli, S.a = Staphylococcus aureus, P.m = Proteus mirabilis E.a =Enterobacter aerogenes, B.c= Bacillus cereus

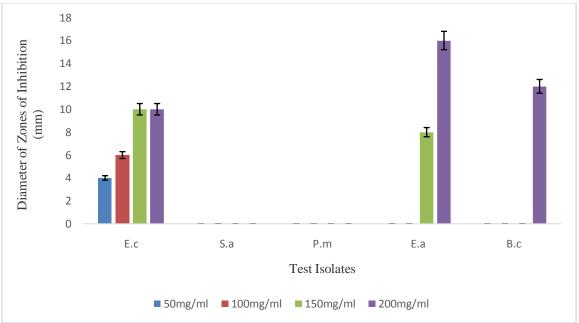


Fig 4: Effect of Aqueous Moringa oleifera Leaf Extract against selected Test Isolates

Keys: E.c = Escherichia coli, S.a = Staphylococcus aureus, P.m = Proteus mirabilis E.a = Enterobacter aerogenes, B.c= Bacillus cereus

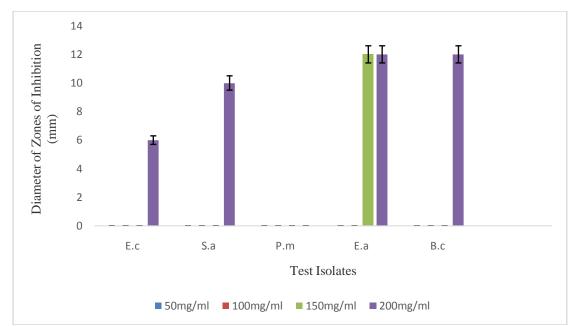


Fig 5: Effect of Ethanolic Moringa oleifera **Seed Extract against selected Test Isolates** Keys: E.c = Escherichia coli, S.a = Staphylococcus aureus, P.m = Proteus mirabilis E.a =Enterobacter aerogenes, B.c= Bacillus cereus

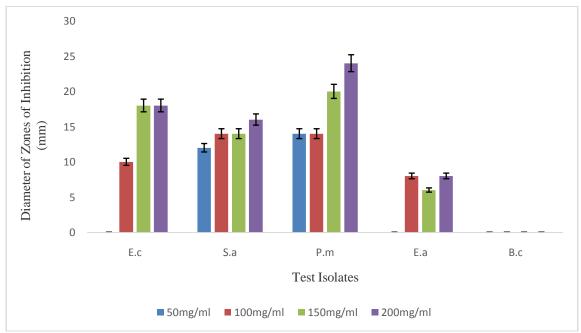


Fig 6: Effect of aqueous moringa oleifera **seed extract against selected test isolates** Keys: E.c = Escherichia coli, S.a = Staphylococcus aureus, P.m = Proteus mirabilis E.a =Enterobacter aerogenes, B.c= Bacillus cereus

V. Discussion

The qualitative screening of phytochemical constituents of both ethanol and aqueous leaf extracts of Moringa oleifera indicated the presence of flavonoids, phenols, alkaloids, saponins, steroids, terpenoids, cardiac glycosides, coumarin, tannins and quinones and this is in accordance with the report by [12] and [13] while the aqueous and ethanol seed extracts contained terpenoids, cardiac glycosides, saponin and steroids similar to the report by [14, 15] however terpenoids, tannin and alkaloids were detected from their analysis. [16] reported that tannins are polyphenols with pronounced ability to suppress bacterial cell proliferation by blocking essential enzymes of microbial metabolism such as the proteolytic macerating enzymes. Furthermore, [17] stated that tannin-containing plant extracts are used as astringents, against diarrhea, as diuretics, against stomach and duodenal tumours, and as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals. Other

phytochemicals with antimicrobial activities are alkaloids which are significant for the protecting and survival of plant because they ensure their survival against micro-organisms (antibacterial and antifungal activities), insects and herbivores and also against other plants by means of allelopathically active chemicals [18] and saponin which are known to be antimicrobial, that inhibit mould growth, and protect plants from insect attack, thus, making saponins a part of plants' defence systems, and as such have been included in a large group of protective molecules found in plants named phytoanticipins or phytoprotectants [19].

Proximate analysis of Moringa oleifera showed that the leaf had higher percentage composition of moisture, ash and fibre while the seed had higher percentage of lipid, protein and carbohydrate. This variation may be as a result of difference in climatic condition, time of the year and different soil types from which the samples were collected.

The high moisture content, ash, lipid and protein content of Moringa oleifera leaf and seed suggests that Moringa oleifera leaf and seed maybe useful for body building, prevention of ageing while the high dietary crude fibre content will help in bowel movement and high carbohydrate content may be useful in making both Moringa oleifera leaf and seed a good source of energy for the body. The result from the proximate analysis showed that Moringa oleifera leaves and seeds can be used as food supplement and are essential for infants and nursing mothers.

All the concentrations of the aqueous seed extract inhibited S. aureus while only the highest concentration of the ethanol extract inhibited the same pathogen which was similar to the work by [20, 21]. All concentrations of both the aqueous and ethanol leaf extract inhibited E. coli.. All concentrations of the aqueous seed extract (50mg/ml, 100mg/ml, 150mg/ml and 200mg/ml) inhibited while P. mirabilis while at 150mg/ml and 200mg/ml inhibited same pathogen for ethanol leaf extract. E. aerogenes was inhibited by aqueous seed extract at the concentrations of 100mg/ml, 150mg/ml and 200mg/ml while aqueous leaf extract and ethanol seed extract inhibited same pathogen at the concentration of 150mg/ml and 200mg/ml and 200mg/ml and ethanol leaf extract inhibited same pathogen at the concentration of 200mg/ml only. B. cereus was inhibited at 200mg/ml concentration only by ethanol seed and aqueous leaf extracts. The antimicrobial activity of moringa leaf against the gastrointestinal pathogen could be attributed to the high amounts of alkaloids, phenols tannin, terpenoids and saponin.

VI. Conclusion

Moringa leaf and seed contain high amounts of glycosides, phenols, steroids, flavonoids, which are medicinally important because of the antimicrobial activity. Moringa seed has high nutritional value as reflected in the appreciable amount of nutrients determined thus it can be included in diets to supplement human's daily nutritional needs. The high inhibitory activity of moringa leaf against some selected gastrointestinal pathogens implies that it may be useful in treating gastrointestinal infections particularly those caused by the organisms under study. The leaf of Moringa oleifera has a high potential for use as an antimicrobial agent against gastrointestinal pathogens than the seed. However, the seed can be used as a food supplement to increase the nutritional composition of foods lacking protein, carbohydrate and lipid.

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