

# Antibacterial, haematological parameters and phytochemical analysis of the leaf extracts of *Moringa oleifera*

## Abstract

Antimicrobial resistance has become a global problem and Strategies to improve the current situation necessitate finding new and innovative antimicrobial agents. Forty apparently healthy albino mice of either sex, weighing between 19kg and 27kg, aged 2 to 3 weeks were purchased and the various concentration of the extracts from *Moringa oleifera* leaves were given orally to the mice, the highest mean PCV was recorded in 800mg (33.0±1.22), White blood cell count 400mg (3,725±923.20), Neutrophil count 100mg (24.8±7±.65) and lymphocytes count 400mg (87.5±1.85), ethanol extracts had PCV to be highest in 200mg, White Blood Cell WBC 100mg (14,000±9453.4), neutrophils count 400mg(12.8±2.02) and lymphocytes count 800mg (91.5±2.22), the antimicrobial activity of the extracts on clinical isolates showed that the water extract 800mg/ml had the highest zone of inhibition of 6mm on the *Escherichia coli*, 19mm against *Staphylococcus aureus*, 18mm against *Proteus mirabilis* and 14mm against *Escherichia coli*, the ethanol extracts 800mg/ml had the highest zone of inhibition of 8mm against *Salmonella typhi*, 6mm against *E.coli* and *S.aureus* and 5mm against *P.mirabilis*, The phytochemical screening showed that both water and ethanol extracts had Flavonoid, Tannin, Glycoside, Terpenoid and Saponin with the absence of alkaloids in water, while reducing sugar was absent in both with Terpenoid having the highest concentrations in ethanol extracts 12.95mg/g and water extracts 10.30mg/g.

**Keywords:** antibacterial, haematological parameters, phytochemical, *Moringa oleifera*

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## Introduction

Antibacterial resistance has become a global problem and Strategies to improve the current situation necessitate finding new and innovative antibacterial (antibiotics) though the chemotherapeutic agents have been of value in controlling many infections but they require on judicious use in order to minimize the incidence of resistant. Approximately 20% of the plants are sources of a substantial number of natural or semi-synthetic antibiotics in the market. In developing countries, due to the cost of large proportion of the population utilize medicinal plants for the treatment of infectious diseases and much research has been done worldwide to identify and study antibacterial compounds found in medicinal plants. According to Fahey.<sup>1</sup>

Increased vegetable utilization and consumption are critical to alleviate world-wide incidence of nutritional deficiencies. Our survey and investigations on some plants indicated that *Moringa oleifera* is one of the promising plants which could contribute to increased intake of some essential nutrients and health-promoting phytochemicals. Phytochemicals are present in virtually all of the fruits, vegetables, legumes (beans and peas), and grains we eat, so it is quite easy for most people to include them in their diet. *Moringa oleifera*, or the horseradish tree, is a pan-tropical species that is known by such regional names as benzolive, drumstick tree, kelor, marango, saijhan, and sajna.<sup>1</sup> It is considered one of the world's most useful trees, as almost every part of the tree can be used for food, or has some other beneficial properties. The leaves, especially young shoots, are eaten as

greens, in salads, in vegetable curries, and as pickles. The leaves can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional value. The leaves are considered to offer great potential for those who are nutritionally at risk and may be regarded as a protein and calcium supplement (Rajangam et al. 2001). The Specific Objectives of this research are to: determine the antibacterial activity of the leaf extract of *Moringa oleifera* against selected medically important bacteria, evaluate the phytochemicals present in the leaf extract and the haematological parameters of the leaf extracts in mice.

## Materials and methods

### Plant collection and extract preparation

The leave stalks of *Moringa oleifera* were collected from Ile-Ife, Osun State, Nigeria and identity confirmed in the department of Botany, Obafemi Awolowo University. Physical selection of the apparently healthy leaves was carried out and the selected leaves were air dried and pounded into a coarse powder using laboratory pestle and mortar. To 50g of the leaf powder was added 300mls of distilled water and ethanol respectively and was thoroughly mixed and allowed to stand for one hour before filtering with the aid of whatman filter paper number 1. The filtrates were taken to the Faculty of Pharmacy, Obafemi Awolowo University in order to get the concentrate of the leave extracts which was administered to the mice and used for the antibacterial activity.

## Experimental animal

Forty apparently healthy albino mice of either sex, weighing between 19kg and 27kg, aged 2 to 3 weeks were purchased from the animal house of the College of Health Sciences Obafemi Awolowo University Ile-Ife, Nigeria. The animals were allowed to acclimatize for one week. The mice were divided into six groups of four mice each, groups I and II serve as negative and positive control respectively, while III to VI were for test doses of 100mg, 200mg, 400mg and 800mg/kg body weight.

## Determination of hematological parameters of the mice

**Counting White cells:** 0.38l of diluting fluid (Turk's solution) was measured and dispensed into a small tube. 0.02ml of well-mixed EDTA anti coagulated venous blood from mice was added and mixed. The counting chamber was assembled and the diluted blood sample using a capillary tube held at an angle of about 45 was used to fill one of the grids of the chamber with the sample taking care not to over fill the area. The chamber was left undisturbed for 2 minutes to allow time for the white cells to settle. The underside of the chamber was wiped dried and place it on the microscope stage and using the X10 objective with the condenser iris closed sufficiently to give good contrast, the cells was focused until they appear as small black dots. The cells in the four large corner squares of the chamber were counted including the cells lying on the lines of two sides of each large square. The readings were done.

**Differential white cell count:** To a well made and correctly stained thin blood film, a drop of immersion oil was dropped on the lower third of the blood film and cover with a clean cover glass. The film was examined microscopically using X10 objective with the condenser iris closed sufficiently to see the cells clearly and to view the part of the film where the red cells are just beginning to overlap and the x40objective was used to focus the blood film. The blood film was systematically examined and different white cell was counted as seen in each field using an automatic differential cell counter.

**Estimation of Packed cell volume (PCV):** The blood from the tail of the animals was drawn and duplicate determinations were done by measuring the relative volume of the blood occupied by erythrocytes in Microhaematocrit tube by using the relation:

$$PCV = \frac{\text{Length of red cell column (mm)}}{\text{Length of total column (mm)}} \times 100$$

## Microbiological analysis

**Extracts preparation:** Extract concentrations were prepared by dissolving known weight of the concentration of crude aqueous and ethanol extracts in known volume of distilled water to give 100mg/ml, 200mg/ml, 400mg/ml and 800mg/ml of the crude extract. 250mg/ml of the standard antibacterial agent (tetracycline) was similarly constituted.

**Microorganisms:** The organisms used were *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella aerogenes*, *Proteus mirabilis* and *Salmonella typhi*. The organisms were obtained from the Department of Medical Microbiology and Parasitology Laboratory, Obafemi Awolowo University Teaching Hospital Complex Ile-Ife Osun State and reconfirmed by performing

biochemical test such as sugar fermentation, citrate, oxidase, urease tests, and production of hydrogen sulphide, gas, and motility on the organisms.

**Determination of antibacterial activity:** The antibacterial activity of the leaf extracts was determined using agar well diffusion method by following the known procedure. Diagnostic sensitivity Test Agar (DST) was inoculated with the given microorganisms by spreading the bacterial inoculums (compared with MacFaland standard) on the media. Wells were punched in the agar and filled with different concentrations of the plant extracts. Control wells containing neat solvents (negative control) were also run parallel in the same plate. The plates were incubated at 37°C for 18hours and the antibacterial activity was assessed by measuring the diameter of the zone of inhibition. The antibacterial potential of the different extracts was evaluated by comparing their zones of inhibition.

**Phytochemical evaluation:** The extracts were analyzed by the following procedures to test for the presence of the alkaloids, saponins, tannins, Terpenoids, flavonoids, glycosides, volatile oils and reducing sugars.

**Saponins:** Saponins were detected using the froth test. 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5minutes. The mixture was filtered and 2.5ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30seconds. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.<sup>2</sup>

**Tannins:** To a portion of the extract diluted with water, 3-4 drops of 10% ferric chloride solution is added. A blue color is observed for Gallic tannins and green color indicates for catecholic tannins.<sup>3</sup>

**Reducing Sugars:** To 0.5ml of plant extracts, 1ml of water and 5-8 drops of Fehling's solution was added and heated over water bath. Brick red precipitate indicates the presence of reducing sugars.<sup>3</sup>

**Glycosides:** 25ml of dilute sulphuric acid was added to 5ml extract in a test tube and boiled for 15minutes, cooled and neutralized with 10% NaOH, then 5ml of Fehling solution added. Glycosides are indicated by a brick red precipitate.

**Alkaloids:** 2ml of extract was measured in a test tube to which picric acid solution was added. An orange coloration indicated the presence of alkaloids.

**Flavonoids:** 4ml of extract solution was treated with 1.5ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones.<sup>2</sup>

**Volatile oils:** 2ml of extract was shaken with 0.1ml dilute NaOH and a small quantity of dilute HCl. A white precipitate is formed if volatile oils are present.

**Terpenoids:** Four milligrams of extract was treated with 0.5ml of acetic anhydride and 0.5ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoids.<sup>4</sup>

## Results

The profile of Haematological parameter such as packed cell volume (PCV), White blood cell count (WBC), Neutrophil and Lymphocyte counts on both ethanol and water extracts on *M. oleifera*. Packed cell volume (PCV) for negative control in water extract had mean PCV of 30.8±3.04 while 800mg had (33.0±1.22), 400mg had (32.8±1.32), and 200mg and 100 mg had 32.3±3.22 and 32.3±0.48 respectively. The negative control for the WBC had mean value of 5,950±670.20 while. The highest White blood cell count among the doses was recorded in 400mg(3,725±923.20) while the least was found in 200mg(3150±262.00). The negative control had mean Neutrophil count of 37.6±7.66 while, the highest Neutrophil count was recorded in dose 100mg(24.8±7.65) followed by 800mg(22.3±1.75) and the least 400mg(13.8±1.70). The lymphocytes count in negative control was 59.3±7.76 while the highest Lymphocytes count was found in 400mg (87.5±1.85) and the least was found with the dose 100mg(72.3±7.63). The ethanol extracts had PCV to be higher in 200mg(43.25±1.44) followed by 400mg(39.0±2.80), 800mg(35.75±4.66) and the least 100mg(24.75±3.82)., the WBC was higher in 100mg(14,000±9453.4), followed by 200mg(6150±556.03) and the least is 400mg(3,150±550.00).400mg had the highest neutrophils count, 400mg(12.8±2.02), followed 100mg(11.3±6.09) and the least 200mg(10.8±1.55). The lymphocytes count was

higher in 800mg(91.5±2.22) followed by 400mg(88.8±2.02) and 100mg(88.8±6.09) and the least 200mg(88.3±1.49) (Table 1 & Table 2).

### The antimicrobial activity of the *Moringa oleifera* leaf extract on some clinical isolates

The antimicrobial activity of the extracts on clinical isolates showed that the water extract 800mg had the highest zone of inhibition of 6mm on the *Escherichia coli*. Followed by 400mg with 4mm zone of inhibition and the least 100mg with 2mm zone of inhibition. As compared with the standard antibiotic Tetracycline 250mg having the highest zone of 21mm against *Klebsiella aerogenes*, 19mm against *Staphylococcus aureus*, 18mm against *Proteus mirabilis* and 14 against *Escherichia coli*. The ethanol extracts 800mg had the highest zone of inhibition of 8mm against *Salmonella typhi*, 6mm against *E. coli* and *S. aureus* and 5mm against *P. mirabilis* (Table 3 & Table 4).

### The phytochemical screening of both ethanol and water extracts of *M. Oleifera* leaves

The phytochemical screening showed that both water and ethanol extracts had Flavonoid, Tannin, Glycoside, Terpenoid, Saponin was absence in ethanol while alkaloids was absent in water. Reducing sugar was absent in both has shown in Table 5.

**Table 1** Profile of the packed cell volume, white blood cell, neutrophil and lymphocyte counts of the water extract of *moringa oleifera* leaves in mice (water extract)

Drug group	PCV±SEM	WBC±SEM	NEUT±SEM	LYMPH±SEM
Control(0.22ml)	30.8±3.04	5,950±670.20	37.6±7.66	59.3±7.76
Extract(100mg)	32.3±0.48	3,325±492.23	24.8±7.63	72.3±7.63
Extract(200mg)	32.3±3.22	3,150±262.00	15.8±2.17	83.8±2.25
Extract(400mg)	32.8±1.32	3,725±923.20	13.8±1.70	87.5±1.85
Extract(800mg)	33.0±1.22	3,675±975.85	22.3±1.75	74.3±1.70

Keywords: PCV, packed cell volume; WBC, white blood cell count; NEUT, neutrophil count; LYMP, lymphocyte count

**Table 2** Profile the packed cell volume, white blood cell, neutrophil and lymphocyte counts of the ethanol extract of *moringa oleifera* leaves in mice (ethanol extract)

Drug group	PCV±SEM	WBC±SEM	NEUT±SEM	LYMPH±SEM
Control(0.22ml)	30.8±3.04	5,950±670.20	37.6±7.66	59.3±7.76
Extract(100mg)	24.75±3.82	14,000±9453.4	11.3±6.09	88.8±6.09
Extract(200mg)	43.25±1.44	6,150±556.03	10.8±1.55	88.3±1.49
Extract(400mg)	39.0±2.80	3,150±550.00	12.8±2.02	88.8±2.02
Extract(800mg)	35.75±4.66	5,600±844.59	8.5±2.220	91.5±2.22

Keywords: PCV, packed cell volume; WBC, white blood cell count; NEUT, neutrophil count; LYMP, lymphocyte count

**Table 3** Antibacterial activities of the *Moringa oleifera* water leaf extracts on some clinical isolates

Organisms	Extract doses (mg)					
	50mg/ml	100mg/ml	200mg/ml	400mg/ml	800mg/ml	Tet (250mg/ml)
<i>S. aureus</i>	R	R	R	R	R	19mm
<i>K. aerogenes</i>	R	R	R	R	R	21mm
<i>E. coli</i>	R	R	2mm	4mm	6mm	14mm
<i>P. mirabilis</i>	R	R	R	R	R	18mm
<i>S. typhii</i>	R	R	R	R	R	13mm

Keywords: *S. aureus*, staphylococcus aureus; *P. mirabilis*, proteus mirabilis; *K. aerogenes*, klesiella aerogenes; *S. typhi*, salmonella typhii; *E. coli*, escherichia coli; R, resistance; Tet, tetracycline

**Table 4** Antibacterial activities of the *Moringa oleifera* ethanol leaf extracts on some clinical isolates

Ethanol extract	50mg/ml	100mg/ml	200mg/ml	400mg/ml	800mg/ml	Tet (250mg/ml)
<i>S. aureus</i>	R	R	R	4mm	6mm	19mm
<i>K. aerogenes</i>	R	R	R	R	R	21mm
<i>E. coli</i>	R	R	3mm	5mm	6mm	14mm
<i>P. mirabilis</i>	R	R	2mm	4mm	5mm	18mm
<i>S. typhii</i>	R	R	4mm	6mm	8mm	13mm

Keywords: *S. aureus*, staphylococcus aureus; *P. mirabilis*, proteus mirabilis; *K. aerogenes*, klesiella aerogenes; *S. typhi*, salmonella typhii; *E. coli*, escherichia coli; R, resistance; Tet, tetracycline

**Table 5** Phytochemical analysis of *Moringa oleifera* leaves

Compounds	Water extracts(mg/g)	Ethanol extracts(mg/g)
Tannins	+(8.41)	+(9.83)
Saponins	+(0.40)	-
Glycosides	+(6.09)	+(7.85)
Reducing sugars	-	-
Terpenoids	+( 10..30)	+(12.95)
Flavonoids	+(6..57)	+(9.41)
Alkanoids	-	+(0.34)

(++) - Moderate abundance of compound.

(+) - Low abundance of compound.

(-) - Absent.

## Discussion

Both extracts demonstrated sensitivity to some of the micro-organisms tested in a dose –dependent manner but were not as active as the positive control. Both were resistant to the entire organism at doses below 200mg/kg. Data from the ethanol was more sensitive at 200mg/kg and above than the aqueous extract as the aqueous was only sensitive to *E. coli* above this dose while *K. aerogenes* was resistant at all doses to the ethanol extract which is in agreement with the work done by Abalaka et al.<sup>5</sup> and Karthic et al.<sup>6</sup> where crude extracts shows a remarkable activity against the growth of *E. coli*, Salmonella and Pseudomonas.. The aqueous extract was resistant to all the organisms tested except *E. coli*. Table 3. Within the sensitivity span of the ethanol extract, *S. typhi* elicited the highest zone of inhibition (ZI) of 8mm at the highest dose followed by *S. aureus* and *E. coli* at 6mm but *S. aureus* was resistant at 200mg compared to *E coli* ZI of 3mm at the

same dose. *P. mirabilis* had the lowest ZI at the highest dose tested; this also corroborated Vinoth<sup>4</sup> data in which the ethanol extract was active against *S. typhi* and *S. aureus*. The result indicates that there was a significant increase in haematological indices, suggesting that *Moringa oleifera* is useful in immune activation in these mice.

The results of the phytochemical analyses showed that terpenoids were more elicited in quantity than the other phytochemicals tested. Terpenoids shows promise as an antioxidant. Its pungent scent is also added to fragrances, used in aromatherapy and as an expectorant, the chemical in eucalyptus stimulates the secretion of mucus. The oil might produce a diuretic effect, and can be taken orally to ease gastrointestinal spasms by increasing gastric juices in the gut. Tannins have being effective ameliorative agent in the kidney.<sup>7</sup> Tannins have been shown to be a potential anti-viral, anti-bacterial and anti-parasitic agent.<sup>8</sup> Flavonoids, which according to<sup>9</sup> may modify allergens including

viruses and carcinogens thereby acting like a biological response modifier. In addition, *in vitro* studies,<sup>10</sup> showed that flavonoids could be anti-allergic (Spencer, 2008) and anti-inflammatory properties.<sup>11</sup> Alkaloids are also known to have pharmacological effects and are also used as local anesthetic and stimulants. Cocaine, caffeine, nicotine, the analgesic morphine, the anti-bacterial berberine and antimalarial drug quinine are all Alkaloids.<sup>12</sup> Saponins however are used as adjuvants in the production of vaccines.<sup>13</sup>

## Conclusion and recommendation

From the study it can be concluded that *Moringa oleifera* leaves has antibacterial effect. Both extracts indicate there was a significant increase in haematological indices, suggesting that *Moringa oleifera* is useful in immune activation in these mice. According to the result from the study the following recommendation is given: due to the large scale consumption of this plant in Africa and the protective roles, more toxicological study especially in the use of special stains to study the detail of the toxicological effect should be embarked on so as to ascertain the safety.

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None.

## Conflict of interest

The author declares no conflict of interest.

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