Antioxidative and antimicrobial activities of different solvent extracts of *Moringa oleifera*: an *in vitro* evaluation

Esayas Welday Tekle[1,2], N. P. Sahu[1], Makesh M.[3]

[1] Fish Nutrition, Biochemistry and Physiology Division, Central Institute of Fisheries Education, Mumbai-India
[2] College of Marine Sciences and Technology, Massawa, Eritrea
[3]Aquatic Environment and Health Management Division, Central Institute of Fisheries Education, Mumbai-India

Abstract- Aqueous, methanolic (80%) and ethanolic (70%) extracts of the leaves, flower, stem bark and pod/fruit (denoted as AL, AF, AB, AP, ML, MF, MB, MP, EL, EF, EB and EP for aqueous leaf, aqueous flower, aqueous bark, aqueous pod, methanolic leaf, methanolic flower, methanolic bark, methanolic pod, ethanolic leaf, ethanolic flower, ethanolic bark and ethanolic pod extracts, respectively) of *Moringa oleifera* were evaluated for their antioxidative activities and antimicrobial properties. 2,2-Diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity (%), total phenolic contents (TPC) and ferric reducing antioxidant power (FRAP) were used as a means of evaluating the *in vitro* anti-oxidative activities of the extracts. All the extracts showed a dose-dependent activity with each method. There was a significant positive correlation ($P<0.01$) between DPPH values and TPC ($r^2 = 0.76$) as well as between TPC and FRAP value ($r^2 = 0.93$). With the DPPH and FRAP methods, EL and AB showed highest and lowest ($P<0.05$) overall activity, respectively. Moreover, EL exhibited highest activity with all the three methods starting from the lowest concentrations. At the higher two concentrations (7.5 and 10 mg/ml), EF showed highest activity ($P<0.05$) as compared to all the other extracts and a standard antioxidant, BHT. Anti-microbial activity of the extracts using the disc diffusion method and minimum inhibitory concentration (MIC) assay revealed that ethanolic extracts from all parts of moringa showed highest activity against *Aeromonas hydrophila* and *Micrococcus luteus* on concentration dependent manner than the aqueous extract counterpart. Although all the extracts exhibited considerable anti-oxidant activities with either one or both of the assays, EL and EF showed the best results and can be recommended as natural anti-oxidants in food/feed preparation and formulation.

Index Terms- antimicrobial property, antioxidative activity, DPPH, MIC, *Moringa oleifera*, total phenolic content,

I. INTRODUCTION

Reactive oxygen species (ROS) ⁻ commonly known as "free radicals" – such as superoxide ion (O_2^-), hydroxyl radical (·OH), peroxynitrite ($OONO^-$), hypochlorous acid (HOCl) etc. are generated in the body of animals in response to a number of stress factors, [1] The ROS can also be produced as the result of the respiratory burst of the cells in the mitochondria during the normal metabolic functioning of the body cells. Pertinent to these ROS, different cellular and extracellular components, primarily the nucleic acids, are damaged causing or enhancing a number of degenerative and carcinogenic diseases [2] These ROS are primarily responsible for the occurrence of cardiovascular diseases (CVD), hypertension, anemia, diabetes, inflammation, liver disease, Alzheimer's, Parkinson's and HIV disease and also accelerate the aging process [3]. Synthetic antioxidants such as the butylated hydroxytoluene (BHT) and butylated hydroxyanisol (BHA) have been widely used (since 1947) as anti-oxidants and preservatives in the food industry as well in animal feed production (mainly aquafeeds) to alleviate the common occurrence of lipid peroxidation where fats, specifically unsaturated fatty acids, are in considerable composition in the food/feed item. However, synthetic antioxidants are suspected for their health impairments in animals and humans as well. Butylated hydroxytoluene (BHT) was identified as a lung tumor promoter by causing chronic inflammation in mice [4].

Plant and herbal extracts are considered to be powerful and healthy natural anti-oxidant and anti-microbial agents. The use of plant extracts as antioxidants to mitigate stress as well as to combat pathogenic microorganisms such as bacteria, virus, fungi and parasites are some of the latest advances in the nutrition and nutraceuticals research. Some co-authors in their previous study to evaluate the antioxidant and antimicrobial properties of ten Indian medicinal plant extracts found that all the plant extracts showed strong radical scavenging and reducing power as well as antimicrobial properties in a dose dependent manner, suggesting their use as nutraceuticals in the food/feed formulation [5]. Plant extracts act as antioxidants by scavenging and inactivating the damaging free radicals that are produced through normal cellular activity and from various stress factors [6]. It has been suggested that the antioxidant function of plant extracts which are rich in micronutrients could enhance immunity by preserving the functional and structural integrity of the immune cells [7]

Moringa oleifera Lam., commonly known as "drumstick tree", "horseradish tree" or "ben oil tree" is the best known and widely distributed species of the Moringaceae family and is having an impressive range of medicinal uses apart from its high nutritional value. Native to Western and sub-Himalayan tracts, India, Pakistan, Asia and Africa, this plant is distributed throughout the world [8] because of its resistance to wide range of environmental conditions including arid and semiarid climatic zones with dry, sandy soils [9]. Almost every part of the plant – the roots, stem, the barks, leaves, branches, flower, fruit/pods, etc. are useable either by animals or humans, making it to be reputable as "the multipurpose tree".

The roots, leaves, gum, flowers, and infusion of seeds have active chemical constituents such as nitrile, glycosides and thiocarbamate glycosides, which are suggested to be responsible for the diuretic, cholesterol lowering, antiulcer, hepatoprotective, and cardiovascular protective property [10]. The roots have been reported to possess antispasmodic activity through calcium channel blockade, which forms the basis for justifying its traditional use in diarrhoea treatment [11,12]. It also possesses antimicrobial activity due to its principle component pterygospermin. The leaves have been reported to be valuable source of both macro- and micronutrients, rich source of *β*-carotene, protein, vitamin C, calcium, and potassium and act as a good source of natural antioxidants; thus enhancing the shelf-life of fat-containing foods [13]. The fresh leaf juice was found to inhibit the growth of human pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* [14,15]. The flowers showed effective hepatoprotective effect due to the presence of quercetin [16]. Methanolic fruit extract showed wide spectrum antimicrobial and antifungal activities when tested against eleven species of bacteria and one species of fungus with highest susceptibility against *Pseudomonas aeruginosa* as assayed by the disc diffusion method [17]. The seeds are also considered to be antipyretic, and for their acrid characteristics are reported to show antimicrobial activity [18].

Moringa contains specific plant pigments such as the carotenoids lutein, alpha-carotene and beta-carotene, xanthins and chlorophyll with demonstrated potent antioxidant properties; it is well known for other phytochemicals with known powerful antioxidant ability – kaempferol, quercetin, rutin and caffeoylquinic acids; powerful antioxidant vitamins – C, E, and A [13, 19]. The antioxidant activity of all the phytochemical components of the tree makes it an important nutritional component in the body's defences against free radical oxidative damage. Saalu and co-authors [20] concluded that leaf extract of *Moringa oleifera* has a modulating role on hydroxyurea-induced testicular toxicity in Sprague –Dawley rats which is caused due to oxidative stress.

Both water and ethanolic extracts of *M. oleifera* leaves have similar antioxidant activity (about 95% inhibition of peroxidation) to that of the well-known synthetic antioxidants, BHA and BHT and by far greater than that of vitamin C[21]. Several co-authors in their study observed that ethanolic extract of moringa fruit showed highest phenolic contents along with strong reducing power and free radical scavenging capacity. Besides, their results proved the potent antioxidant activity of aqueous and ethanolic extract of moringa which adds one more positive attribute to its known pharmacological properties and hence its use in traditional system of medicine [22]. In conclusion, the active principles from the different parts of the plant provide tremendous medicinal and health benefits for human as well as other animals including anti-inflammatory, antimicrobial, antioxidant, anticancer, cardiovascular, hepatoprotective, anti-ulcer, diuretic, antihelmintic, cholesterol lowering [23, 10], antiurolithiatic [24]and antispasmodic activity [12].

Although a number of research works have indicated the medicinal benefits of the extracts from the different parts of moringa, few or none of them made a comprehensive and comparative study of the extracts from the various parts of the plant. In the current study, *in vitro* antioxidant and antimicrobial activity assays of aqueous, methanolic and ethanolic extracts from four different parts of *M. oleifera* (leaves, fruit, flower and bark) were carried out to evaluate the medicinal and health benefits of the plant.

II. MATERIALS AND METHODS

Collection of plant material

Leaves, flowers, fruit and stem bark of *Moringa oleifera* were collected from Kutmia area of the port city of Massawa, Eritrea. All the parts of the tree were collected fresh and allowed to air-dry in a shaded place thereafter were transported to the Central Institute of Fisheries Education, Mumbai, India for the required research work.

Preparation of plant extracts

Each plant material was blended into fine powder using an electrical grinder and passed through 60 mesh. Three solvents, namely aqueous (distilled water), 80% methanol and 70% ethanol were used for the extraction. Forty grams of the leaf, fruit and stem bark powder and 30 grams of the flower were weighed and transferred into four conical flasks of one liter. Eight hundred mililitre of each of the solvents was added to dissolve the dry powder of each plant material in the flasks. The aqueous dispersion was heated on a magnetic stirrer cum hotplate (SPINOT®, Tarsons, India) at a temperature of 70^{o}C for 1 hour. After allowing cooling down, the dispersion was filtered using a fine mesh cheese cloth and the residue was re-extracted in the similar manner using 200 ml distilled water. The methanolic and ethanolic dispersions of each part were divided into four 200 ml conical flasks and were set on a shaker (ORBITEK®, Scigenics, India) at 160 rpm for 24 hrs. Dispersions were filter through cheese cloth and residues re-extracted using 200 ml of the respective solvent. All the filtrates were centrifuged (REMI-CPR24, Mumbai, India) at 5000 rpm and 4^{o}C for 10 min. The supernatant was carefully collected into labeled bottles for each extract. The filtrates were concentrated using rotary evaporator (IKA® RV 10 basic) at a temperature of 40-65^{o}C (depending on the boiling point of the solvents) and a rotor speed of 40 rpm. The remaining were collected and further concentrated to form a thick semi-solid (tar) using a water bath set at 70-80^{o}C after which all extracts were properly labeled and kept at 4^{o}C until use. Aliquots of known weight from each extract were freeze-dried for the estimation of yield on dry weight basis.

Determination of antioxidantive activity

The antioxidant activity of the various extracts was evaluated through three antioxidant activity assays: 2,2-dipheny-1-dipycryl hydrazyl (DPPH) radical scavenging assay, the ferric reducing antioxidant power (FRAP) assay and total phenolic contents (TPC).

DPPH radical scavenging activity

The DPPH assay was conducted following the methods adapted by previous researchers [25] with some modifications. Aliquots 30µl of each extract at concentrations 0.5-10mg/ml were pipetted and returned into test tubes set in triplicate for each sample. Two milliliter of 0.06mM DPPH in methanol was mixed

diluting the extracts to a final concentration of 7.5-150µg/ml. The reaction mixture was incubated in a dark place for 30 min. After 30 min incubation period, absorbance was measured at 515 nm using Shimadzu UV-1800 spectrophotometer. A blank reading was also taken using 2ml of DPPH without the extract. Ascorbic acid and butylated hydroxytoluene (BHT) were also used as positive controls for the comparison of the antioxidant activities. Each test was performed in triplicate and the percentage radical scavenging activity was calculated according to the following equation:

$$\textbf{Radical Scavenging (\%)} = (A_b - A_s)/A_b * 100$$

where A_b is the absorbance of the blank (DPPH in methanol) and A_s is the absorbance reading of the sample (extract) at 515nm.

Total Phenolic Contents (TPC)

The Folin Ciocalteu (FC) colorimetry method [26] with slight modifications was used to determine the total phenolic contents of the extracts. Briefly, 0.5 ml of Folin Ciocalteu reagent (FCR) was added into aliquot of the various extracts at various concentrations. After adding 7 ml of distilled water, the mixture was well vortexed and 1.5 ml, 20% Na_2CO_3 was added after 10 min of incubation period at room temperature. The mixture was heated in water bath at 40^{o}C for 20 min. Absorbance readings at 765 nm were taken after cooling in ice. A blank with all the reagents but without the extracts was used as a reference for the absorbance readings. All tests were carried out in triplicates. A standard curve was established using gallic acid at concentrations 0.05-0.5 mg/ml and the total phenolic contents of the extracts was expressed as gallic acid equivalent (GAE) in mg/100g dry weight extract.

Ferric Reducing Antioxidant Power (FRAP)

Previously adopted method [22] with some modifications was followed to determine the FRAP for each extract. Aliquot (50µl) of the sample extracts (concentrations 0.5-10mg/ml) was mixed with 2ml FRAP reagent composed of 300mM acetate buffer (pH 3.6), 20 mM ferric chloride ($FeCl_3.6H_2O$) and 10 mM 2,3,6-tripycridyl-s-triazine (TPTZ) in 40 mM HCl in the ratio of 10:1:1. The mixture was warmed at 37^{o}C in water bath for 10 min. Absorbance reading was taken at 593 nm. Ferrous sulphate, Fe(II) sulphate, standard curve was established by preparing aqueous solutions of known concentrations between 0.1-1.0 mM and mixing with the FRAP working solution. All samples were carried out in triplicates. FRAP was expressed in mM of extract as Fe(II)-sulphate equivalent.

Antimicrobial properties of Moringa extracts

The antimicrobial activity of the various extracts was tested using the disc diffusion method and minimum inhibitory concentration (MIC) determination.

Preparation of standard bacterial working suspension

Muller Hinton broth, 21g in 1000ml distilled water was prepared in a sterile condition. About 10ml of the broth was transferred into each of 3 centrifuge tubes. Following aseptic microbial techniques, a loop-full of pure culture of three bacterial species namely *Aeromonas hydrophila*, *Micrococcus luteus* and *Staphylococcus aureus* was inoculated into each of the prepared broth medium separately and the tubes were incubated in a shaker-incubator at 37^{o}C for 24 hrs. The bacterial suspensions with a visible turbidity were centrifuged at 4000 rpm, 4^{o}C for 10 min. The supernatant was discarded and the bacterial pellets were washed using PBS and re-centrifuged following the same procedure. After discarding the supernatant, the pellets were dissolved in newly added PBS and the turbidity was adjusted to 1McFarland standard to obtain a bacterial suspension with an approximate density of $3x10^8$CFU/ml.

Disc diffusion method

This method assesses the antimicrobial activity of a bioactive compound by culturing bacteria in the presence of the compound and measuring the zone of inhibition which corresponds to the area where no bacterial growth is observed at an optimum bacterial growth conditions. The higher the diameter (zone of inhibition), the more the bacteria are susceptible to the bioactive compound/extract. The method was carried out according to previous works [27] with some modifications. Ninety millimeter sterile culture plates were used to prepare a Muller Hinton agar growth media. Aseptic techniques were followed to inoculate the test bacteria from the standardized bacterial suspension ($3x10^8$CFU/ml) in PBS. Using sterile cotton buds, aliquots were drawn from the suspension and evenly swabbed on the prepared Muller Hinton agar growth medium. Sterile filter paper discs approximately of 6 mm in diameter were placed on the plates which were properly labeled according to the bacterial species and type of extract along with a series of four concentrations. From each extract, 10 µl was carefully pipetted onto the discs and the plates were left at room temperature for about 10 min so as to allow the extracts to be diffused before incubation at 37^{o}C for 24 hrs. thereafter zone of inhibition (in millimeters) was measured using a ruler. Amikacin (30µg) was used as a positive control. Each test was carried out in triplicates.

Minimum inhibitory concentration (MIC)

The MIC for each extract against the three bacterial species was determined according to previously adopted methods [28] with some modifications. A 150µl of Muller Hinton broth was transferred into each wells of a 96-wells flat-bottomed microtitre plate. Equal volume of each extract was added into each of the first row of the wells which were properly labeled to indicate the extracts and their respective concentrations. Using a multichannel transfer micropipette, the extracts were serially diluted to obtain concentrations between 75 and 0.59mg/ml. A 15µl of the bacterial suspension was pipetted into each of the wells with the broth and extract. This brings about the dilution of the bacteria 10 fold making the concentration $3x10^7$CFU/ml. A positive growth controls (broth and bacteria), negative control (broth and the diluent of each extract) and a sterility control (broth only) were also prepared in three rows of the plates. After an incubation period of 18 hrs. at 37^{o}C, 10 µl, 0.1% tetrazolium salt was added to each well and incubated for additional 1 hr. A colour change from colourless or faint colour to brightly coloured red – the formazans – indicates bacterial growth and no change in colour indicates inhibition of growth. The lowest sample concentration showing no color change (clear) and exhibited complete inhibition of bacterial growth is considered to

be the MIC. Each test was conducted in triplicates and the means were used for comparison.

III. DATA ANALYSIS

All data were subject to one way ANOVA and univariate ANOVA in the SPSS version 20 and means were compared for significant differences using the Tukey HSD pairwise comparison. Correlation and linear regression analysis were also carried out to reveal the relationship between the various antioxidant activity assays.

IV. RESULTS

Antioxidant activity

The antioxidant activity of the various extracts were evaluated through various antioxidant activity assays including 2,2-diphenyl-1-picryl hydrazine (DPPH) radical scavenging activity, ferric reducing antioxidant power (FAP) and total phenolic contents (TPC).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical is scavenging the most widely used antioxidant activity assays to evaluate the activity of bioactive components of plant extracts. The percentage DPPH scavenging activity of the various moringa extracts is shown in Table 1. While the activity is concentration dependent, univariate ANOVA ranked the various extracts in the order EL>EF>ML>AL>MF>MB>EP>AF>AP>EB>MP>AB.
Ethanolic leaf extract (EL) and aqueous stem bark extract (AB) showed the highest and lowest activities, respectively with respect to overall concentrations. However, ethanolic flower extract showed highest activity at the higher concentrations (94.30±1.07 and 96.33±0.20 at 7.5mg/ml and 10mg/ml, respectively. The ethanolic leaf extract showed higher activity starting from the lowest concentration. Both EL and EF extracts were comparable to or even better than the synthetic antioxidant BHT or ascorbic acid. At the higher concentrations, the activity of EF extract was significantly higher ($P<0.05$) than the BHT or AA.

Total phenolic contents (TPC)

The result for the total phenolic contents of the various extracts is presented in Table 2. TPC is concentrations dependent with all the extracts having a positive correlation (overall $r^2 = 0.64$). Overall, the EF extract showed the highest TPC though there was no significant difference ($P>0.05$) as compared to the EL extract but significantly higher ($P<0.05$) than all the other extracts. Both EF and EL extracts revealed higher activity starting from the lowest concentration as compared to all other extracts. Aqueous flower (AF) extract was also showing considerably higher activity as compared to the other extracts except the EF and EL. In general, EF extract and AP extract showed the highest and the lowest TPC, respectively. TPC for the various extracts was in the order EF>EL>AF>EB>MF>ML>AL>MB>EP>AB>MP>AP.

Ferric reducing antioxidant power (FRAP)

The FRAP having a positive correlation with the TPC, which is also a good measure of the antioxidant activity of plant extracts. All the extracts of moringa showed good activity on a dose dependent manner. The ethanolic leaf extract (EL) showed an overall higher activity with significant difference ($P<0.05$) while the ethanolic flower (EF) extract showed its maximum activity at the highest concentrations (7.5mg/ml and 10mg/ml) surpassing all the other extracts with a significant difference ($P<0.05$). The FRAP for the moringa extracts was in the order EL>EF>AL>ML>EB>MF>AF>MB>EP>AP>MP>AB.

In general, the EL and AB showed the highest and the lowest FRAP values, respectively.

Correlation between the various antioxidant assays

The regression analysis shows that there is a significant ($P<0.01$) positive correlation between total phenolic contents and DPPH radical scavenging ($r^2 = 0.76$; $y= 0.28x + 16.89$) as well as between ferric reducing antioxidant power and total phenolic contents ($r^2 = 0.93$; $y = 1.384x + 4.794$) as shown in figure 1 and figure 2, respectively.

Table 1: Antoxidative activity of the various moringa extracts at various concentrations (15-300µg/2ml) as evaluated by the DPPH radical scavenging (%) assay

	DPPH radical scavenging activity (%)*						
Extract* **	**15**	**45**	**90**	**150**	**225**	**300**	**Overall mean**
AB	10.47±2.16^{aABC}	15.07±1.81^{aA}	22.39±2.30^{bA}	27.29±0.14^{bcA}	32.14±3.02^{cdA}	35.85±0.58^{dA}	23.87±9.37^{A}
MB	21.00±1.07^{aE}	28.94±1.99^{bBC}	50.18±1.10^{cCD}	64.61±2.67^{dD}	69.54±3.40d^{eCD}	75.67±2.79^{eB}	51.66±21.19^{D}
EB	9.72±0.54^{aAB}	19.70±2.01^{bAB}	32.39±1.65^{cB}	46.06±3.02^{dBC}	57.39±0.87^{eB}	76.89±3.20^{fB}	40.36±23.41^{B}
AP	12.24±0.28^{aB}	24.44±0.41bB	36.49±0.63^{cB}	51.53±1.33^{dC}	65.60±2.75^{eC}	75.83±0.89^{fB}	44.36±22.97^{C}
MP	7.38±0.56^{aA}	16.52±2.04^{bA}	31.99±0.48^{cB}	42.56±1.39^{dB}	58.88±2.40^{eB}	74.85±2.64^{fB}	38.70±23.98^{B}
EP	13.98±0.79^{aC}	28.00±1.74^{bBC}	45.56±3.47^{cC}	59.13±6.57^{dD}	74.65±1.90^{eD}	82.93±1.58^{eC}	50.71±25.27^{D}
AL	16.38±0.97^{aD}	36.73±0.67^{bCD}	60.09±1.57^{cE}	87.74±1.02^{dF}	89.81±0.38^{dE}	88.40±0.10^{dE}	63.19±29.26^{F}
ML	15.66±1.24^{a}	41.04±2.89^{bD}	69.50±2.55^{cF}	90.03±3.14^{dFG}	93.11±0.43^{dEF}	93.68±0.68^{dEF}	67.17±30.46^{G}
EL	38.80±2.61^{aF}	75.30±3.53^{bE}	93.59±0.64^{cG}	94.00±0.14^{cFG}	94.00±0.07^{cEF}	93.01±0.19^{cEF}	81.45±20.86^{H}
AF	6.81±0.55^{aA}	32.29±0.96^{bC}	47.75±0.77^{cC}	58.08±1.87^{dCD}	71.61±2.78^{eD}	86.48±0.23^{fCD}	50.50±26.75^{D}
MF	9.83±1.69^{aA}	14.77±0.83^{bA}	52.74±1.27^{cD}	75.80±1.13^{dE}	91.72±0.88e^{EF}	93.11±0.26^{eEF}	56.33±34.87^{E}
EF	16.11±2.55^{a}	41.67±0.43^{bD}	68.16±2.10^{cF}	92.74±1.31^{dFG}	94.30±1.07^{dEF}	96.33±0.20^{dF}	68.22±31.16^{G}
AA	42.03±0.38^{aF}	94.32±0.19^{bF}	95.12±0.13^{bcG}	96.26±0.20^{dG}	95.95±0.13^{cdF}	95.70±0.51^{cEF}	86.56±20.50^{I}
BHT**	91.61±0.99^{G}	91.61±0.99^{F}	91.61±0.99^{G}	91.61±0.99^{FG}	91.61±0.99^{EF}	91.61±0.99^{DE}	91.61±0.83^{J}

* Values with different lower case superscripts across a row and upper case superscripts down a column indicate significant differences (P<0.05). Values are mean±SD of triplicate samples.

** Concentration was at 0.02mg/ml

*** AB = aqueous bark extract, MB = methanolic bark extract, EB = ethanolic bark, AP = aqueous pod/fruit, MP = methanolic pod, EP = ethanolic pod, AL = aqueous leaf, ML = methanolic leaf, EL = ethanolic leaf, AF = aqueous flower, MF = methanolic flower, EF = ethanolic flower

Aqueous extract = moringa powder material solubilized in distilled water

Ethanolic extract = moringa powder material solubilized in 70% ethanol

Methanolic extract = moringa powder material solubilized in 80% methanol

Table 2: Total phenolic contents (in mg/100g dry extract as GAE) of the various moringa extracts at varying concentrations (0.5-10mg/ml)

Extract**	Total phenolic content (mg/100g)*						
	0.5	1.5	3	5	7.5	10	Overall mean
AB	16.92±0.69^{a}	44.77±0.95^{b}	57.16±0.18^{c}	69.03±0.87^{d}	99.61±9.35^{e}	116.94±0.36^{f}	67.41±34.44^{A}
MB	21.86±0.31^{a}	60.01±3.93^{b}	104.45±3.34^{c}	144.68±3.12^{d}	203.57±4.85^{e}	228.14±6.66^{f}	127.12±75.76^{B}
EB	29.57±2.46^{a}	76.82±4.69^{b}	125.01±4.41^{c}	168.35±7.62^{d}	222.61±5.57^{e}	282.54±8.42^{f}	150.82±87.91^{C}
AP	12.94±0.80^{a}	35.47±0.57^{b}	54.25±0.40^{c}	62.13±0.84^{d}	103.45±1.08^{e}	114.05±0.14^{f}	63.72±36.58^{A}
MP	8.93±0.69^{a}	34.02±3.19^{b}	53.31±2.57^{c}	68.30±3.67^{d}	98.96±0.95^{e}	127.28±2.91^{f}	65.13±40.53^{A}
EP	7.04±0.35^{a}	32.84±1.92^{b}	55.08±3.68^{c}	74.13±4.09^{d}	103.54±3.17^{e}	136.73±6.79^{f}	68.23±44.47^{A}
AL	8.83±0.09^{a}	70.55±29.08^{b}	139.16±1.77^{c}	186.18±2.94^{d}	224.60±3.56^{e}	253.70±1.64^{e}	147.17±88.63^{C}
ML	22.46±1.54^{a}	78.15±1.72^{b}	133.35±2.09^{c}	169.58±3.27^{d}	229.63±4.62^{e}	264.12±6.05^{f}	149.55±85.58^{C}
EL	37.83±1.36^{a}	102.62±3.37^{b}	167.78±5.00^{c}	218.38±2.99^{d}	287.26±1.8^{e}	326.87±7.16^{f}	190.12±103.2^{E}
AF	32.84±1.12^{a}	96.74±2.33^{b}	151.28±4.94^{c}	179.54±1.09^{d}	245.22±6.49^{e}	276.21±4.63^{f}	163.64±85.45^{D}
MF	28.53±0.87^{a}	40.29±1.64^{b}	135.79±3.86^{c}	181.74±1.34^{d}	246.14±4.14^{e}	271.51±3.95^{f}	150.67±95.83^{C}
EF	35.69±2.19^{a}	95.02±2.09^{b}	156.31±1.84^{c}	200.42±3.33^{d}	303.68±12.37^{e}	349.80±3.45^{f}	190.15±113.24^{E}
Mean	21.95±10.73^{a}	63.94±26.48^{b}	111.08±43.13^{c}	143.54±56.66^{d}	197.35±73.86^{e}	228.99±81.64^{f}	127.81±90.02

* Values with different lower case superscripts across a row and upper case superscripts down a column indicate significant differences (P<0.05). Values are mean±SD of triplicate samples.

** AB = aqueous bark, MB = methanolic bark, EB = ethanolic bark, AP = aqueous pod/fruit, MP = methanolic pod, EP = ethanolic pod, AL = aqueous leaf, ML = methanolic leaf, EL = ethanolic leaf, AF = aqueous flower, MF = methanolic flower, EF = ethanolic flower.

Table 3: Concentration-dependent antioxidative activity of the various moringa extracts by the ferric reducing antioxidant power – FRAP assay expressed in millimole/100g dry extract as Fe(II)-sulphate equivalent, (extract concentrations 0.5-10mg/ml)

	FRAP (mM/100mg)*						
Extract**	**0.5**	**1.5**	**3**	**5**	**7.5**	**10**	**Overall mean**
AB	10.15±0.78^{a}	42.62±2.16^{b}	63.26±3.70^{c}	76.35±3.95^{d}	110.32±2.05^{e}	148.58±9.12^{f}	75.21±46.28^{A}
MB	9.4±0.48a	58.11±5.43^{b}	115.6±5.37^{c}	182.05±6.03^{d}	265.15±16.95^{e}	312.61±19.26^{f}	157.15±111.4^{D}
EB	19.23±4.02^{a}	90.76±8.65^{b}	162.21±2.58^{c}	249.2±4.01^{d}	330.39±25.46^{e}	445.25±2.69^{f}	216.17±148.25^{F}
AP	16.38±2.45^{a}	56.54±4.23^{b}	89.08±4.67b^{c}	103.23±11.75^{c}	181.70±25.13^{d}	204.99±10.10^{d}	108.65±68.86^{B}
EP	17.96±1.99^{a}	56.38±7.26^{b}	104.79±9.83^{c}	148.75±7.11^{d}	219.96±26.88^{e}	281.25±10.79^{f}	138.18±94.14^{C}
MP	9.28±1.75^{a}	52.24±4.12^{b}	93.29±13.95^{c}	97.37±5.12^{c}	164.24±6.94^{d}	190.74±6.76^{e}	101.19±63.90^{B}
AL	46.54±0.5^{a}	142.39±5.39^{b}	243.03±8.51^{c}	321.83±6.85^{d}	352.08±1.85^{e}	356.09±1.37^{e}	243.66±118.61^{G}
ML	25.87±2.44^{a}	114.96±2.36^{b}	213.58±8.96^{c}	295.16±12.23^{d}	325.98±4.16^{e}	329.20±1.25^{e}	217.46±117.1^{F}
EL	47.34±0.65a	182.35±16.88^{b}	308.47±16.10^{c}	359.27±3.87^{d}	367.96±1.44^{d}	364.50±1.71^{d}	271.65±122.9^{H}
AF	12.76±0.91^{a}	82.69±4.12^{b}	159.84±2.66^{c}	206.75±11.04^{d}	310.36±5.04^{e}	362.82±3.18^{f}	189.20±125.03^{E}
MF	21.79±1.46^{a}	35.67±1.13^{a}	171.66±3.90^{b}	242.83±41.8^{c}	391.94±10.56^{d}	414.81±18.45^{d}	213.12±159.87^{F}
EF	24.42±1.88^{a}	107.31±3.10^{b}	207.1±7.36^{c}	289.86±14.99^{d}	392.61±25.79^{e}	472.92±16.22^{f}	249.04±160.25^{G}
Mean	21.76±12.74^{aA}	85.17±43.64^{bB}	160.99±70.86^{cC}	214.39±92.13^{dD}	284.39±93.17^{eE}	323.65±99.23^{fF}	181.72±129.56

*Values with different lower case superscripts across a row and upper case superscripts down a column indicate significant differences (P<0.05). Values are mean±SD of triplicate samples.

**AB = aqueous bark, MB = methanolic bark, EB = ethanolic bark, AP = aqueous pod/fruit, MP = methanolic pod, EP = ethanolic pod, AL = aqueous leaf, ML = methanolic leaf, EL = ethanolic leaf, AF = aqueous flower, MF = methanolic flower, EF = ethanolic flower

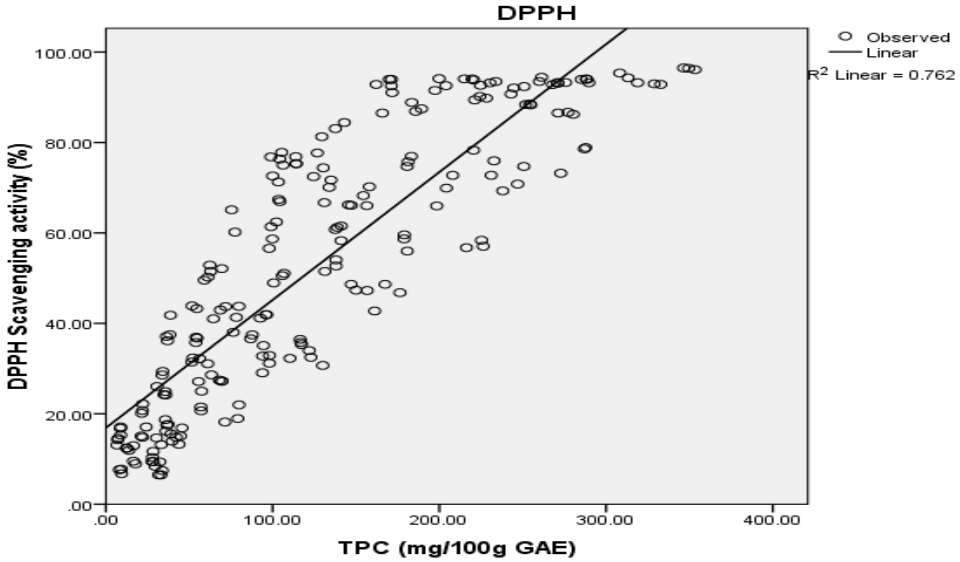

Figure 1: Correlation between total phenolic content and DPPH radical scavenging activity for the various Moringa extracts tested on six concentrations (r^2 = 0.76; y= 0.28*x* + 16.89)

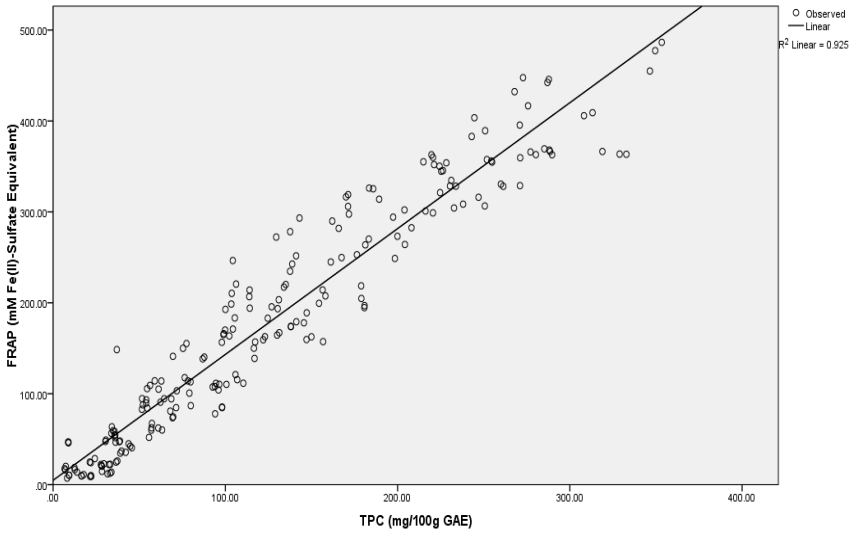

Figure 2: Correlation between total phenolic content and ferric reducing antioxidant power for the various Moringa extracts tested on six concentrations (r^2 = 0.93; y = 1.384*x* + 4.794)

Antimicrobial properties of moringa extracts

Results for the antimicrobial activity by the MIC and zone of inhibition method are presented in Table 4 and Table 5, respectively. With both the MIC and zone of inhibition assays, all the extracts exhibited antimicrobial activities at varying degrees.

The MIC assay against the three bacteria, *Aeromonas hydrophila*, *Micrococcus luteus* and *Staphylococcus aureus* showed significant difference (P<0.050) among the various extracts. In general, the ethanolic extracts of all the moringa parts showed higher antimicrobial activity against the three species of bacteria. With all the three species, AB extract showed the least activity, while EL against *M. luteus* and EF extract against *A. hydrophila* and *S. aureus* exhibited highest activity (P<0.05). Overall, EF extract followed by EL extract revealed the lowest MIC (5.33±1.12 and 7.95±3.82, respectively) with significant difference (P<0.05). Comparison between the three species over all the extracts also revealed significant difference (P<0.05), whereby *S. aureus* and *A. hydrophila* exhibited lowest and highest MIC, respectively.

With the disc diffusion assay, ethanolic bark and pod extracts were more efficient with significantly higher zone of inhibition, followed by ethanolic flower and leaf extracts for *A. hydrophila*. For *M. luteus*, ethanolic bark extract resulted in significantly higher (P<0.05) inhibition zone followed by ethanolic flower and leaf extracts.

Table 4: Antimicrobial susceptibility of moringa extracts against *Aeromonas hydrophila* and *Micrococcus luteus* by the minimum inhibitory concentration (MIC) assay

Extracts	MIC (mg/ml)			
	Aeromonas hydrophila	***Micrococcus luteus***	***Staphylococcus aureus***	**Overall mean**
AB	45.66 ± 1.89^{fA}	$47.54 \pm .00^{gA}$	36.88 ± 1.22^{eB}	43.36 ± 5.05^{h}
MB	12.20 ± 1.20^{bc}	$11.00 \pm .00^{cd}$	$11.00 \pm .00^{bc}$	$11.40 \pm .85^{cde}$
EB	$11.00 \pm .00^{bcB}$	$9.47 \pm .74^{cA}$	$10.50 \pm .00b^{cBC}$	$10.32 \pm .77^{cd}$
AP	22.83 ± 2.08^{eB}	$33.20 \pm .00^{fC}$	$15.80 \pm .80^{dA}$	23.94 ± 7.66^{g}
MP	12.20 ± 1.00^{bc}	$10.55 \pm .65^{cd}$	11.80 ± 2.42^{bc}	11.52 ± 1.54^{de}
EP	$10.00 \pm .00^{bB}$	$10.17 \pm .23^{cdB}$	$8.70 \pm .00^{bA}$	$9.62 \pm .71^{bc}$
AL	$14.80 \pm .00^{cd}$	$14.80 \pm .00^{e}$	15.80 ± 1.00^{d}	$15.13 \pm .71^{f}$
ML	12.77 ± 1.33^{bc}	$11.90 \pm .00^{d}$	14.03 ± 2.81^{cd}	12.90 ± 1.81^{e}
EL	12.05 ± 1.34^{bcC}	$3.40 \pm .00^{aA}$	$8.40 \pm .00^{bB}$	7.95 ± 3.82^{b}
AF	16.75 ± 1.55^{dC}	$11.55 \pm .00^{dB}$	10.29 ± 1.14^{bcA}	12.86 ± 3.12^{e}
MF	12.63 ± 1.79^{bc}	11.18 ± 1.93^{cd}	10.76 ± 1.69^{bc}	$11.52 \pm 1.78d^{e}$
EF	$5.96 \pm .80^{aB}$	$5.96 \pm .80^{bB}$	$4.06 \pm .419^{aA}$	5.33 ± 1.12^{a}
Overall mean	15.74 ± 10.09^{C}	15.06 ± 12.20^{B}	13.19 ± 7.99^{A}	14.66 ± 10.17

Lower case superscripts down a column and uppercase superscripts across a row indicate significant differences ($P<0.05$). Values are mean ± SEM for triplicate samples.

Table 5: Concentration-dependent antimicrobial susceptibility of *Moringa oleifera* extracts against *Aeromonas hydrophila* and *Micrococcus luteus* at varying concentrations (20, 35, 50 and 65 mg/ml) assayed by the agar plate disc diffusion method.

Bacteria species	Extract	Zone of inhibition (diameter in mm)				
		20	**35**	**50**	**65**	**Overall mean**
Aeromonas hydrophila	AB	$10.00 \pm .00^{bc}$	$10.00 \pm .00^{bcd}$	$12.00 \pm .00^{bcd}$	$11.00 \pm .00^{bcd}$	$10.75 \pm .87^{e}$
	EB	$13.50 \pm .50^{d}$	14.00 ± 1.00^{g}	15.00 ± 1.00^{d}	13.50 ± 1.5^{defg}	14.00 ± 1.11^{g}
	MB	$9.00 \pm .00^{bcd}$	$7.00 \pm .00^{a}$	$10.00 \pm .00^{ab}$	$10.00 \pm .00^{abc}$	$9.00 \pm 1.28b^{c}$
	AF	$8.500 \pm .50^{abcA}$	$10.00 \pm .00^{bcdB}$	$11.50 \pm .50^{bcC}$	$13.00 \pm .00d^{efgD}$	10.75 ± 1.78^{e}
	EF	10.50 ± 1.50^{bc}	$12.00 \pm .00^{ef}$	12.50 ± 2.50^{bcd}	14.50 ± 2.50^{fg}	12.38 ± 2.22^{f}
	MF	$9.00 \pm .00^{bcdA}$	$10.50 \pm .50^{cdeB}$	$10.00 \pm .00^{abB}$	$14.00 \pm .00^{efgC}$	10.87 ± 1.98^{e}
	AL	$7.50 \pm .50^{abA}$	$9.00 \pm .00^{bcB}$	$10.50 \pm .50^{abC}$	$11.50 \pm .50^{cdeC}$	9.62 ± 1.63^{cd}
	EL	11.00 ± 1.00^{cA}	11.00 ± 1.00^{deA}	11.50 ± 1.50^{bA}	$15.50 \pm .50^{gB}$	12.25 ± 2.17^{f}
	ML	$8.50 \pm .50^{abcA}$	9.00 ± 1.00^{bcA}	10.50 ± 1.50^{abAB}	$12.50 \pm .50^{cdeB}$	10.13 ± 1.82^{de}
	AP	$7.50 \pm .50^{ab}$	$8.50 \pm .50^{ab}$	8.00 ± 1.00^{a}	$8.50 \pm .50^{ab}$	$8.13 \pm .71^{ab}$
	EP	$12.00 \pm .00^{cdA}$	$13.00 \pm .00^{fgB}$	$14.50 \pm .50^{cC}$	$15.00 \pm .00^{fgC}$	13.62 ± 1.26^{g}
	MP	$7.00 \pm .00^{a}$	$7.00 \pm .00^{a}$	$7.50 \pm .50^{a}$	$7.50 \pm .50^{a}$	$7.25 \pm .4^{a}$
	Amikacin*	20				
Micrococcus luteus	AB	$8.00 \pm .00^{abA}$	$8.00 \pm .00^{abcA}$	$9.50 \pm .50^{bcdB}$	$10.50 \pm .50^{bC}$	9.00 ± 1.15^{b}
	EB	16.50 ± 1.5^{d}	17.00 ± 1.00^{g}	16.50 ± 1.50^{f}	$18.50 \pm .50^{f}$	17.13 ± 1.33^{e}
	MB	12.00 ± 2.0^{bc}	10.50 ± 50^{e}	12.50 ± 1.50^{e}	11.00 ± 1.0^{bc}	11.50 ± 1.43^{c}
	AF	$7.00 \pm .00^{aA}$	$8.50 \pm .50b^{cB}$	$10.00 \pm .00^{cdC}$	$11.50 \pm .50^{bcD}$	9.25 ± 1.78^{b}
	EF	$10.00 \pm .00^{b}$	$13.00 \pm .00^{f}$	$15.00 \pm .00^{f}$	$17.00 \pm .00^{e}$	13.75 ± 2.70^{d}
	MF	$7.00 \pm .00^{a}$	$7.00 \pm .00^{a}$	$11.00 \pm .00^{de}$	$12.00 \pm .00^{cd}$	9.25 ± 2.39^{b}
	AL	$8.00 \pm .00^{abA}$	$8.50 \pm .50^{bcA}$	$9.50 \pm .50^{bcdB}$	$11.00 \pm .00^{bcC}$	9.25 ± 1.23^{b}
	EL	$13.00 \pm .00^{c}$	$11.00 \pm .00^{e}$	$16.00 \pm .00^{f}$	$13.00 \pm .00^{d}$	13.25 ± 1.86^{d}
	ML	$8.00 \pm .00^{abA}$	$9.00 \pm .00^{cdB}$	$9.00 \pm .00^{abcB}$	$11.50 \pm .50^{bcC}$	9.37 ± 1.37^{b}
	AP	$7.00 \pm .00^{aA}$	7.50 ± 50^{abAB}	$8.00 \pm .00^{abBC}$	$8.50 \pm .50^{aC}$	$7.75 \pm .66^{a}$
	EP	$9.00 \pm .00^{ab}$	$10.00 \pm .00^{de}$	$12.00 \pm .00^{e}$	$13.00 \pm .00^{d}$	11.00 ± 1.65^{c}
	MP	$7.00 \pm .00^{a}$	$7.00 \pm .00^{a}$	$7.50 \pm .50^{a}$	$7.50 \pm .50^{a}$	$7.25 \pm .40^{a}$
	Amikacin*	20				

Lower case superscripts down a column and uppercase superscripts across a row for each group of bacteria indicate significant differences ($P<0.05$). Values are mean ± SD for triplicate samples.

* Amikacin at 30µg/disc (HIMEDIA, India)

V. DISCUSSION

The growing concern of adverse health impacts of synthetic antioxidants and drugs has led to the discovery of natural herbal and plant bioactive principles that are believed to have high activity against damaging reactive oxygen species and various microbial infections. *Moringa oleifera* is among the most common and widely used plants for their extracts to be used as antioxidant and antimicrobial agents. However, no work has shown the concurrent evaluation and comparison of solvent-specific extracts from the various parts of the plant.

In the current study, although all the extracts were showing considerable activity, the 70% ethanolic extract of leaf and flower exhibited highest antioxidant activity ($P<0.05$). Antioxidant activity is highly correlated to the polyphenolic contents of the particular extract. These bioactive compounds are maximally extracted in solvents that have moderate polarity, such as ethanol and methanol [29, 30] which is supportive for the findings of this study. Moreover, polyphenolic bioactive compounds are maximally extracted at 80% and 70% methanolic and ethanolic solvents, respectively [13], which is in agreement with the findings of our study. At 10mg/ml, EF, EL and ML revealed significantly higher activity ($P<0.05$) as compared to the synthetic antioxidant, BHT. At the same concentration, the EF extract showed higher activity than ascorbic acid. The strong positive correlation ($r = 0.93$) between TPC and FRAP which is similar to the findings for gold mohar flower, leaves and bark extracts [31] as well as between TCP and DPPH ($r = 0.76$) which is similar to that of some Malaysian plants [32] suggests that phenolic compounds in plant extracts might have acted as powerful radical scavenging and reducing agents. The results of all the antioxidative assays in the current study suggest that ethanolic *M. oleifera* extracts can be good substitutes for the synthetic antioxidants, which are suspected for their negative impact on the health of human and animals. Although the antioxidative efficacy of ethanolic and methanolic extracts is comparable, the ethanolic extract is more suitable for nutraceutical purposes for the reason that methanolic extraction may leave residues that lead to toxicity even at low concentrations [33].

Various researches on phytochemical screening of *M. oleifera* extracts indicate the presence of alkaloids, tannins, flavonoids, polyphenols, saponins, essential oils, etc., which contribute to the antimicrobial properties of the extracts [34]. These phytochemicals are present in almost all tissues of the plant including the leaves, roots, stem, fruits [35] and flowers. Specifically, ethanolic extracts are rich in alkaloids, terpenoids and propolis [36] that are believed to have high antimicrobial properties. In the current study, ethanolic extract of all the tested moringa tree parts (leaves, flowers, bark and fruit) showed highest antimicrobial activity with both the MIC and disc diffusion assays. With the disc diffusion method, aqueous and methanolic extracts of stem bark also showed higher antimicrobial activities as compared to their counterpart extracts of leaf, flower and fruit. The antimicrobial activities of the bioactive phytochemicals may involve multiple mechanisms of action. For instance, oil acts by degrading the cell wall and interacting with the composition resulting in the ultimate disruption of the cytoplasmic membrane[37]. Moreover, oils damage membrane protein, interfere with membrane integrated enzymes, cause leakage of cellular components, coagulate cytoplasm, deplete the proton motive force, change fatty acid and phospholipid constituents, alter nutrient uptake and electron transport and impair enzymatic mechanism for energy ([38]. Findings of the current study indicated that the microbial inhibition potency of the extracts is related to their TPC. This is in agreement with the work of some co-authors who suggested that the inhibition of microorganisms by phenolic compounds may be due to iron deprivation or hydrogen bonding with vital proteins such as microbial enzymes [39]. Phenolic compounds show generally a good antimicrobial effectiveness against Gram-positive bacteria with their effect dependent on their amount: at low concentrations they are able to interfere with enzymes involved in the production of energy; at higher concentrations, they can induce the denaturation of proteins until an irreversible modification of the cell and death [40]. Antimicrobial activity may also be due to the presence of short peptides that may act directly on microorganisms and result in growth inhibition by disrupting cell membrane synthesis or synthesis of essential enzymes [41].

In the current study, all the extracts of moringa exhibited antimicrobial effect at varying degrees, a number of them with significant difference ($P<0.05$) in susceptibility between the Gram-negative bacterium (*Aeromonas hydrophila*) and Gram-positive bacteria (*Micrococcus luteus* and *Staphylococcus aureus*). The variation in susceptibility between Gram-negative and Gram-positive bacteria may be attributable to the structural difference in the cell membrane of the two groups of bacteria (the presence of outer membrane – the lipopolysaccharide – in the Gram-negative bacteria makes access to the gram-negative bacteria more restrictive) [42]. The EF extract did not show significant difference between *A. hydrophila* and *M. luteus*. This may be indicative that moringa extracts, specifically the ethanolic extract of flower can be used as a wide spectrum antimicrobial agent to prevent both the Gram-positive and Gram-negative bacteria.

VI. CONCLUSION

Moringa oleifera extracts from all the tested parts of the plant exhibited high potency of antioxidative activities and antimicrobial properties against both Gram-negative and Gram-positive bacteria in a concentration dependent manner. Ethanolic extracts of all the moringa parts showed highest activity as compared to the aqueous or methanolic extracts. This is probably attributable to the high phenolic contents (which are positively correlated to the antioxidative and antimicrobial activities) of this extract suggesting the choice of extraction solvent is a critical factor for obtaining the desired bioactive compounds in the plant extracts. With the growing concern of the health impairments of the synthetic antioxidants that are commonly used as food/feed preservatives in the processing industry, moringa extracts can be alternatives to substitute these noxious synthetic chemicals. The powerful antimicrobial properties of the ethanolic extracts of moringa against both the Gram-positive and Gram-negative bacteria also suggests a promising natural alternative for the synthetic drugs that are also not immunized from the blame for their negative health impacts on human and animals. Among the

ethanolic extracts of the different parts of *M. oleifera*, the extracts of flower and leaf signified best activities and can be recommended as prophylactic agents against oxidative stress and infectious diseases. However, characterization and detailed studies on the specific components of the bioactive crude extracts responsible for the antioxidative and antimicrobial attributes and their mechanism of action is required for the better understanding of their application as immunomodulators.

ACKNOWLEDGMENT

The first author would like to acknowledge the National Board for Higher Education, Government of Eritrea for providing the financial support to carry out the research project. We also greatly acknowledge ICAR and Dr. W. S. Lakra, Director, Central Institute of Fisheries Education, Mumbai, for providing all the laboratory and research facilities.

REFERENCES

[1] Bayr, H. MD, Reactive oxygen species. Critical Care Medicine: 2005: 33(12): S498-S501

[2] Krushynska, O. A., Olga A. Zaporozhets, Natalia A. Lipkovska and Valentina N. Barvinchenko, A New Test Method for the Evaluation of Total Antioxidant Activity of Herbal Products. J. Agric. Food Chem. 2004: 52(21). p. 25.

[3] Alina A., Cristea V., Iulia G., Lorena D., Angela D., Mirela C. M, Săndiţa I. P. and Coadă M.T., The influence of some phytobiotics on oxidative stress at Oreochromis niloticus grown in an intensive recirculating aquaculture system. Lucrări Ştiinţifice-Seria Zootehnie, 2013: 59.

[4] Bauer A. K., Lori D. Dwyer-Nield, Joseph A. H., Robert C. M. and Alvin M. M. The lung tumor promoter, butylated hydroxytoluene (BHT), causes chronic inflammation in promotion-sensitive BALB/cByJ mice but not in promotion-resistant CXB4 mice. Toxicology, 2001: 169, pp. 1–15

[5] Fawole, F. J., Sahu, N. P., Pal, A. K. and Lakra, W. S., Evaluation of antioxidant and antimicrobial properties of selected Indian medicinal plants. Int. J. Med. Arom. Plants, 2013: 3(1),pp. 69-77.

[6] Chew B. P., . Antioxidant vitamins affect food animal immunity and health, J. Nutri. 1995: 125, pp. 18045-18085.

[7] Innocent, B. Xavier, M. Syed Ali Fathima and A. Sivagurunathan, Haematology of Cirrhinus mrigala fed with Vitamin C supplemented diet and post challenged by Aphanomyces invadens; Journal of Applied Pharmaceutical Science, 2011: 1 (9), pp. 141-144.

[8] Makkar, H. P. S. and Becker, k. Nutrients and antiquality factors in different morphological parts of the Moringa oleifera tree. J. Ag. Sci. 1997: 128, pp. 311-322.

[9] Okechukwu U., Nwodo P. C., Okwesili F. C., Joshua P. E., Bawa Abubakar, Ossai Emmanuel and Odo Christian E. Phytochemical and acute toxicity studies of Moringa oleifera ethanol leaf extract. Int. J. LifeSc. Bt & Pharm. Res. 2013: 2(2), pp. 67-71.

[10] Anwar F, Latif S, Ashraf M and Gilani AH. Moringa oleifera: a food plant with multiple medicinal uses. Phytotherapy Research, 2007: 21(1), pp.17–25.

[11] Caceres, A., Saravia, A., Rizzo, S., Zabala, L., De Leon, E. andNave, F. Pharmacologic properties of Moringa oleifera. 2: screening for antispasmodic, antiinflammatory and diuretic activity. Journal of Ethnopharmacology, 1992: 36(3), pp.233–237.

[12] Gilani, A.H., Aftab, K. and Suria, A. Pharmacological studies on hypotensive and spasmolytic activities of pure compounds from Moringa oleifera. Phytotherapy Research, 1994: 8(2) pp.87–91.

[13] Siddhuraju P. and Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (Moringa oleifera Lam) leaves. J. Agric Food Chem. 2003: 51(8) pp. 2144-2155.

[14] Das, B.R., Kurup, P.A. and Rao, P.L. Antibiotic principle from Moringa pterygosperma. VII. Antibacterial activity and chemical structure of compounds related to pterygospermin. The Indian Journal of Medical Research. 1957: 45(2) pp.191–196.

[15] Eilert, U., Wolters, B. and Nahrstedt, A. The antibiotic principle of seeds of Moringa oleifera and Moringa Stenopetala. Planta Medica, 1981: 42(1), pp.55–61.

[16] Gilani, A.H., Janbaz, K.H. and Shah, B.H. Quercetin exhibits hepatoprotective activity in rats. Biochemical Society Transactions, 1997: 25, article 85.

[17] Sayeed, A. M., Hossain, S. M., Chowdhury, M. E. H. and Haque, M. In vitro Antimicrobial Activity of Methanolic Extract of Moringa oleifera Lam. Fruits. Journal of Pharmacognosy and Phytochemistry, 2012: 1(4), pp. 94.

[18] Oliveira, J.T.A., Silveira, S.B., Vasconcelos, I.M., Cavada, B.S. and Moreira, R. A. Compositional and nutritional attributes of seeds from the multiple purpose tree Moringa oleifera Lamarck. Journal of the Science of Food and Agriculture. 1999: 79(6), pp.815–820.

[19] Aslam, M. F., Anwar R., Nadeem U., Rashid T.G., Kazi A. and Nadeem M. Mineral composition of Moringa oleifera leaves and pods from different regions of Punjab, Pakistan Asian J. Plant Sci. 2005: 4, pp 417–42.

[20] Saalu L. C., Osinubi A. A., Akinbami A. A, Yama, O. E., Oyewopo, A. O. and Enaibe, B. U. Moringa oleifera Lamarck (drumstick) Leaf Extract Modulates the Evidences of Hydroxyurea –Induced Testicular Derangement. International Journal of Applied Research in Natural Products, 2011: 4 (2), pp. 32-45.

[21] Hettiarachchy, S. N., Min Zhang, Ronny H., Arvind, K., Apputhury, Praisoody, M. D., Arumugam M. and Chandrasekhara R. M. Phytochemicals, antioxidant and antimicrobial activity of Hibiscus sabdariffa, Centella asiatica, Moringa oleifera and Murraya koenigii leaves. Journal of Medicinal Plants Research, 2011: 5(30), pp. 6672-6680.

[22] Luqman, S., Srivastava, S., Kumar, R., Maurya, A. K. and Chanda, D. Experimental Assessment of Moringa oleifera Leaf and Fruit for its Antistress, Antioxidant, and Scavenging Potential Using In Vitro and In Vivo Assays. Evid Based Complement Alternat Med. 2012: 519084.

[23] Farooq F., Meenu R., Avinash T., Abdul Arif K. and Farooq S. Medicinal properties of Moringa oleifera: An overview of promising healer. Journal of Medicinal Plants Research, 2012: 6(27), pp. 4368-4374.

[24] Jameel F., Vijayalakshmi, Satish Kumar M. C., Sanjeeva, Prabhath K. G., Benegal A., Udupa A. L. and Rathnakar U. P. Antiurolithiatic activity of aqueous extract of bark of Moringa oleifera (Lam.) in rats. Health, 2010: 2(4), pp. 352-355

[25] Kim MK, Lee H.S., Kim E.J., Won N.H, Chi Y.M, Kim B.C. and Lee K.W. Protective effect of aqueous extract of Perilla frutescens on tert-butyl hydroperoxide-induced oxidative hepatotoxicity in rats. Food and Chemical Toxicology, 2007: 45, pp. 1738–1744

[26] Singleton, V. L. & Rossi. J. A. Jr, Colorunetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. Amer. J. Enol. Viticult. 1965: 16, pp. 144-58.

[27] Oyas Ahmed Asimi, N. P. Sahu and A.K. Pal, Antioxidant activity and antimicrobial property of some Indian spices. International Journal of Scientific and Research Publications, 2013: 3(3), pp. 1-8.

[28] Perumal S., Pillai S., Lee W. C., Mahmud R and Ramanathan S. Determination of Minimum Inhibitory Concentration of Euphorbia hirta (L.) Extracts by Tetrazolium Microplate Assay. Journal of Natural Products, 2012: 5,pp. 68-76.

[29] Chenielle D., Lois R., Alison N., Sylvia M., John L. and Mohammed A. Antibacterial and antifungal analysis of crude extracts from the leaves of Callistemon viminalis. Journal of Medical and Biological Science, 2009: 3(1), pp. 1-7.

[30] Houghton P. and Raman A. Laboratory Handbook for the Fractionation of Natural Extracts. Springer US, Science, 1998. 199 pages

[31] Shabir G., Anwar F, Sultana B, Khalid ZM, Afzal M, Khan QM and Ashrafuzzaman M. Antioxidant and antimicrobial attributes and phenolics of different solvent extracts from leaves, flowers and bark of Gold Mohar [Delonix regia (Bojer ex Hook.) Raf]. Molecules, 2009:14, pp. 2167-2180.

[32] Qader S. Wasman., Abdulla M. A., Lee S. C., Nigar N., Zain M. M. and Hamdan S. Antioxidant, Total Phenolic Content and Cytotoxicity Evaluation of Selected Malaysian Plants. Molecules, 2011: 16, pp. 3433-3443

[33] Paine A. and Davan AD, Defining a tolerable concentration of methanol in alcoholic drinks. Hum Exp Toxicol, 2001:20(11), pp. 563-8.

[34] Patil SD and Jane R. Antimicrobial activity of Moringa oleifera and its synergism with Cleome viscosa. Int. J. of Life Sciences, 2013: 1(3), pp. 182-189.

[35] Siddiqui S, Verma A, Rather AA, Jabeen F and Meghvansi MK, Priliminary phytochemicals analysis of some important medicinal and aromatic plants. Advan. Biol. Res., 2009: 3(5-6), pp. 188-195.

[36] Cowan M. M. Plant Products as Antimicrobial Agents. Linical Microbiology Reviews, 1999: 12(4) pp. 564–582.

[37] Khanahmadi M, Rezazadeh SH and Taran M, In vitro antimicrobial and antioxidant properties of Smyrnium cordifolium Boiss. (Umbelliferae) extract. Asian J. Plant Sci., 2010: 9(2) pp. 99-103.

[38] Baldemir AM, Coskun and Yildiz S. Antimicrbial activity of Ferula halophla. FABAD J. Pharm. Sci., 2006: 31, pp. 57-61

[39] Karou D., Dicko M. H., Jacques S. and Traore A. S. Antioxidant and antibacterial activities of polyphenols from ethnomedicinal plants of Burkina Faso. African Journal of Biotechnology, 2005: 4 (8), pp. 823-828.

[40] Marrufo T., Filomena N., Emilia M., Florinda F., Raffaele C., Laura D. M., Adelaide B. A. and Vincenzo D. F. Chemical composition and biological activity of the essential oil from leaves of Moringa oleifera lam. Cultivated in mozambique. Molecules, 2013: 18, pp. 10989-11000.

[41] Silvestro L, Weiser JN, Axelsen PH. Antibacterial and antimembrane activities of cecropin A in Escherichia coli. Antimicrob Agents Chemother. 2000: 44(3), pp. 602-7.

[42] Pascal J. D., Kareen S., Benoit G. and Mazza G. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. International Journal of Food Microbiology, 2002: 74, pp. 101 – 109.

AUTHORS

First Author - Esayas Welday Tekle, Fish Nutrition, Biochemistry and Physiology Division, Central Institute of Fisheries Education, Mumbai, Maharashtra, India. Email- e_welday@yahoo.com

Second Author - Dr. N. P. Sahu, Principal Scientist and HOD, Fish Nutrition, Biochemistry and Physiology Division, Central Institute of Fisheries Education, Mumbai, Maharashtra, India. Email- npsahu@cife.edu.in

Third author - Dr. Makesh M., Senior Scientist, Aquatic Environment and Health Management Division, Central Institute of Fisheries Education, Mumbai, Maharashtra, India. Email- mmakesh@cife.edu.in

Correspondence author- Dr. N. P. Sahu, Principal Scientist and HOD, Fish Nutrition, Biochemistry and Physiology Division, Central Institute of Fisheries Education, Mumbai, Maharashtra, India. Email- npsahu@cife.edu.in