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Biochemical Studies on Moringa Oleifera Leaves Extract

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Abstract
Phytochemical screening were determined in *Moringa oleifera* leaves. The total polyphenols and flavonoids contents of *Moringa oleifera* leaves have the highest of total polyphenols and flavonoids contents, which were 129.44mgGAE/g and 20.43mgGAE/g, respectively. Methanolic and aqueous extracts of plant leaves was antioxidant activity by used (FRAP, LPO, OH, DPPH and ABTS) The methanolic extract of *Moringa oleifera* leaves have the highest of reducing power which was ranged from 0.818 to 3.021 at the concentrations of 20 and 80 mg/ml, respectively. Also, by used (LPO, OH, DPPH and ABTS), were the highest antioxidants activity for methanolic extract respectively. Moreover, The methanolic extract of *Moringa oleifera* leaves produced the highest growth inhibition (20 and 17mm) for against *Escherichia coli* and *Bacillus subtillis* at 4mg/ml, respectively. While, the aqueous extracts highest growth inhibition (13mm) of against *St.coccus aureus* at 4mg/ml.
Keywords: Phytochemical , polyphenols, flavonoids, plant extracts, antioxidant activity and antibacterial.

INTRODUCTION

Moringa oleifera commonly known as (family: Moringaceae) horse radish tree or drumstick tree is both nutritional and medicinal with some useful minerals, vitamins, amino acids. Almost all the parts of this plant: root, bark, gum, leaf, fruit, Leaves, seed and seed oil have been used for various ailments in the indigenous medicine of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, hematological and hepatorenal disorders. Administration of *Moringa oleifera* leaf extract inhibited the growth of pathogenic gram positive and gram negative bacteria and antioxidant activity. Sreelatha and Padma (2009).

Moringa oleifera plant shows the presence of phytochemical constituents like alkaloids, flavonoids, carbohydrates, glycosides, proteins, saponins, tannins and terpenoids in different solvent extracts. Patel *et al.*, (2014).

Free radical damage is one of the most prominent causes of devastating diseases that are responsible for killing millions of people in the world and this can manifest as heart attacks and cancers. Free radicals naturally occur in the body as a result of chemical reactions during normal cellular processes such as conversion of food into energy in the body. Antioxidants are powerful free radical scavengers in the human body. Several researches on antioxidants in biological systems have confirmed their neutralizing effects on oxidative stress that predispose the human body to lethal diseases and thus, generating keen interest in assessment of antioxidant potentials of consumable food compounds antioxidants comprise a number of chemical compounds. Ahiakpa; et al., (2013).

Medicinal plants having various phytochemicals and bioactive components such as trace metal ions, vitamins, alkaloids, carotenoids, polyphenols, fats, carbohydrates, and proteins are involved in enhancing long-term health benefits. Antioxidant activity of plants have been monitored, using FRAP, ABTS and DPPH. They quench, scavenge and suppress the formation of reactive oxygen species (ROS) and oppose their actions. Sravanthi and Rao (2014).

MATERIALS AND METHODS

Plant materials

Leaves sample from each species was air dried in the shade and ground into a fine powder. The powdered air dried leaves which was divided into two extracts: First extract: Powdered air dried leaves (1 Kg) from Leaves sample was extracted by soaking at room temperature for six times with methanol (10 L), then the methanolic extracts were concentrated to nearly dryness under reduced pressure using the rotary evaporator at 45°C to achieve the crude methanol extract which kept for further investigation **(El-Khateeb *et al.*, 2014)**. The yields of extract was 22.08%, for *Moringa oleifera*. Second extract: Powdered air dried Leaves (1 Kg) of dried samples was extracted with distilled water by boiling at temperature from 80 to 100°C in reflux for 3 h to achieve an initial extract. The extract was filtered after cooling to room temperature. Finally, the extract was lyophilized and preserved at −20°C until further use **(Kim *et al.*, 2011)**. The yield of aqueous extract was 12.32%, for *Moringa oleifera*.

Preliminary phytochemical tests of crude methanolic and aqueous extracts of investigated leaves:
Preliminary phytochemical tests they were carried out on the crude methanolic and aqueous extracts by boiling for 3 hours, extract to detect the presence of: terpenes, tannins, flavonoids, saponins, alkaloids, carbohydrate and/or glycosides, phenolic glycosides and resins.
Detection of terpenes were detected according to method adopted by **Harborne (1988).** A small amount of crude aqueous plant extract was dissolved in chloroform, then a few drops of concentrated sulfuric acid were added carefully on the wall of test tube to form two separated layers, the resulted yellow ring changed to orange then red indicating the presence of terpenes.
Detection of tannins were detected by the method described by **Harborne (1988).** Few milliliters of distilled water were added to few milliliters of aqueous extract and filtrate, then ferric chloride solution (5%) was added to the filtrate. The presence of tannins yellowish green color was obtained.
Detection of flavonoids were detected according to **Harborne (1988).** A small amount of crude plant extract was macerated in hydrochloric acid (1%) over night, then sodium hydroxide solution (10%) was added to the filtrate, the appearance of yellow color indicates the presence of flavonoids.
Detection of saponins were detected according to **Harborne (1988).** The aqueous crude plant extract was vigorously shaken developing a voluminous froth which persisted for almost one hour indicate the presence of saponins.
Detection of carbohydrate and/or glycosides in crude plant extract were detected using Molish's reagent according to **Harborne (1988).** Some drops of α-naphthol in ethyl alcohol were added to 1ml of crude Boiling Water extract, then 1ml of concentrated sulfuric acid was added carefully without shaking, a purple ring was appeared indicating the presence of carbohydrate and/or glycosides in crude plant extract.
Detection of alkaloids were detected according to **Harborne (1988)** by adding 2ml of diluted hydrochloric acid to 1ml of plant extract. Then five drops of ***Wagner's reagent** were added to 1ml of the previous solution and shaking after addition of each drop. After leaving for sometimes, the formed precipitate indicating the presence of alkaloids.
Detection of phenolic glycosides were detected according to **Harborne (1988)** by the following technique: some drops of concentrated sulfuric acid were added to 1ml of plant extract, a red color was produced which disappear when water was added.
Detection of resins were detected according to the methods described by **Harborne (1988).** The crude boiling water extract was boiled on water bath for 20 minutes and distilled water was added to extract, a white precipitate was formed in presence of resins.
Total polyphenols content of investigated Leaves:
Total phenolic contents of air dried leaves were determined by using Folin–Ciocalteu reagent method according to **Lin and Tang (2007)**, the following experiment has been achieved at Chemistry department Faculty of Agriculture, Mansoura University. About 0.1g of air dried Leaves was dissolved, separately in 1 ml distilled water. Aliquots of 0.1 ml from previous solution was taken and mixed with exactly 2.8 ml of distilled water, 2.0 ml of (2% w/v) sodium carbonate and finally 0.1 ml of 50% (v/v) of Folin–Ciocalteu reagent was added. Mixture was incubated for 30 minutes at room temperature and the absorbance of the resulting color was measured at 750 nm against distilled water as blank, using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer. For quantitatively determination a standard curve of gallic acid (0-200mg/l) was prepared in the same manner. Total phenol contents were expressed as milligram gallic acid equivalent (GAE)/g based on dry weight.
Total flavonoids content of investigated Leaves:
Total flavonoids content of air dried leaves were determined calorimetrically using aluminum chloride as described by **Chang *et al.*, (2002)**, the following experiment has been achieved at Chemistry department Faculty of Agriculture, Mansoura University. About of 0.1g of air dried Leaves were dissolved in 1ml of distilled water. Resulting solution (0.5 ml) was mixed with 1.5 ml of 95% ethyl alcohol, 0.1 ml of 10% aluminum chloride (AlCl3), 0.1ml of 1M potassium acetate (CH3COOK) and 2.8 ml of distilled water. After incubation at room temperature for 40 min, the reaction mixture absorbance was measured at 415nm against distilled water as blank, using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer. Quercetin was chosen as a standard of flavonoids for making the standard curve (0–50mg/l). The concentration of total flavonoids contents was expressed as milligram quercetin equivalent (QE)/g based on dry weight.
Determination of Reducing power, (FRAP) radical scavenging activity:
Reducing power of methanolic leavess extracts was determined according to the method of **Oyaizu (1986)**, The following experiment has been achieved at Chemistry department Faculty of Agriculture, Mansoura University.: Extract (0–100mg) from each sample in 0.20mol phosphate buffer, pH 6.6 (2.5ml) was added to 2.5ml potassium ferricyanide (10mg/ml), mixture was incubated at 50°C for 20min. Trichloroacetic acid (TCA) (2.5ml, 100mg/ml), was added to the mixture then centrifuged at 650g for 10 minutes. The supernatant (2.5ml) was mixed with distilled water (2.5ml) and 0.5ml ferric chloride solution (1mg/ml) was added and the absorbance of the resultant color was measured using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer at 700nm. Higher absorbance of the reaction

mixture indicated greater reducing power.

Determination of Lipid peroxidation (LPO) measurement of MDA-TBARS:

MDA analysis of plant extracts. was made according to the method **Wu *et al.*, (2000).** For this purpose: 0.05 M TRIS-HCl pH 7.4/0.15 M KCl and 0.2% Tween 20 with a buffer solution containing 1 mM hydrogen peroxide FR and 3 mL were prepared daily. LPO for the measurement, 1 mL samples on after receipt of 0,6% TBA solution and 2 mL distilled water was added and vortexes. Then 90°C for 30 minutes and the reaction was allowed resulting pink color was extracted with 3 mL of n-butanol. Samples were centrifuged and the supernatant fraction obtained after centrifugation of the color density was measured in a spectrophotometer at 532 nm.

Determination of Hydroxyl radical scavenging activity:

Hydroxyl radical scavenging activity was measured by the salicylic acid method **(Smirnoff and cumbes 1989)** with some modifications7. Briefly the plant extracts were dissolved in distilled water at (10, 25, 50,100)µg/ml. A 1ml extract was mixed with 1ml of 9mmol/l salicylic acid, 1ml of 9mmol /l ferrous sulphate and 1ml of 9mmol /l hydrogen peroxide. The reaction mixture was incubated for 60 min at 370c in a water bath after incubation the absorbance of the mixtures was measured at 510nm using a UV/Vis spectrophotometer. The %hydroxyl radical scavenging activity of test sample was determined accordingly in comparison with negative control. Negative control was without antioxidant or extract. Gallic acid was taken as the positive control.

Determination of (DPPH) radical scavenging activity:

The DPPH free radical scavenging activity of Asteraceae (Family compositae) Leaves extracts at different concentrations were measured from bleaching of the purple colour of (2.2 Diphenyl -1-picryl hydrazyl) was based on the method of **Pratap *et al.*, (2013)**. Exactly 0.1 ml solution of different concentration of extract was added to 1.4 ml of DPPH and kept in dark for 30 min. The absorbance was measured at 517 nm, using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer. And the percentage inhibition was calculated by using the following Equation.

$$\text{Percentage inhibition (\%)} = (\text{A Blank} - \text{A Test}) / \text{A Blank}) \times 100$$

Determination of ABTS radical scavenging activity:

ABTS (2,2'-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) assay was based on the method of **Re *et al.*, (1999)** with slight modifications. 2ml of ABTS solution (1mg/1ml 0.1M phosphate buffer, pH 7.0) were added to 3ml of MnO2 (25mg/ml in previous phosphate buffer). Mixture was shaken and centrifuged for 10 minutes, clear supernatant was separated. Exactly 1mg of crude methanolic Leaves was dissolved in a mixture solvent (1ml) of methanol and previous phosphate buffer in the ratio of 1:1. Resultant extract solution (20µl) was added to the ABTS solution mixture, as previously described. Positive control sample was prepared exactly in the same manner but differ only in the addition of 20µl of 2mM ascorbic acid, instead of extract solution. Blank sample was prepared exactly in the same manner but differ only in the addition of 20µl of distilled water, instead of extract solution. Absorbance of the resulting greenish-blue solution was recorded at wave length 734nm, using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer. The decrease in absorbance is expressed as a percentage of inhibition which was calculated from the following equation:

$$\text{\% Inhibition} = \frac{\text{A Blank} - \text{A Test}}{\text{A Blank}} \times 100$$

Crude methanolic and aqueous extracts of investigated leaves as Antibacterial activity:

To study the effect of investigated leaves extracts, three cultures of bacteria namely*: Escherichia coli, Staphylococcus aureus* and *Bacillus subtillis* were used according to **Shan *et al*., (2007)**. Briefly, nutrient agar media and 30 ml were poured in the each Petri plate. Three wells of diameter 0.7 centimeter cut in each plate with the help of cork borer and then sealed with nutrient agar. In each prepared plate, extracts of the same concentration were poured in all wells using micro-pipette and three concentrations were made of each plant extract e.g. concentration of methanolic and aqueous extracts of *Moringa oleifera* were (1, 2 and 4µg/ml), in separate nutrient agar plates. Then, plates were incubated at 37˚C for 24 hrs. The diameter of inhibition zones were calculated as percentages.

Statistical analysis

Statistical analyses of all experimental data were done using the statistical software package **(CoStat, 2005).** All comparisons were first subjected to one way analysis of variance (ANOVA) and significant differences between treatment means were determined using Duncan's multiple range test at $P<0.05$ as the level of the significance (**Duncan, 1955)**.

RESULTS

Preliminary phytochemical tests of crude aqueous and methanolic extracts of investigated leaves:

Table (2) represented the phytochemical constituents of crude methanolic and aqueous extracts of *Moringa oleifera leaves*. The crude methanolic and aqueous extracts of investigated leaves were rich in terpenes, tannins, flavonoids, alkaloids, carbohydrate or glycosides and phenolic glycosides within the acceptable limits. But, aqueous extract

was poor in resins.

The methanolic extract was higher in focus than aqueous extract, where it is more polarity according to **Haghi *et al.,* (2014).**

Table (2) Phytochemical tests (Qualitative)

Tests / Leaves	Terpens	Tannins	Flavonoids	Saponins	Alkaloids	Carbohydrate or Glycosides	Phenolic glycosides	Resins
Methanolic	++	++	+++	++	++	+++	+++	+
Aqueous	++	++	++	++	++	++	++	-

Our data about *Moringa olifera* leaves were in agreement with those reported by **Vinoth *et al.,* (2012),** who found that the phytochemical analysis catecholic tannins were found in *Moringa olifera* in the solvents such as chloroform, ethanol and aqueous. The ethanol extract of *Moringa olifera* showed the presence of flavanoids, tannins, glycosides and terpinoids were found in presence of ethanol and aqueous extract. Alkaloids were observed only in chloroform extract of *Moringa olifera*. In all plant extracts found flavanoids except in chloroform extract of *Moringa olifera*. Saponin were observed in the chloroform and aqueous extract of *Moringa olifera*. Terpenoids were observed in the ethanol and water extract of *Moringa olifera*.

The data about *Moringa olifera leaves* were in agreement with those reported by **Patel *et al.,* (2014),** who established that Moringa oleifera is a good source of various phytochemicals like alkaloids, flavonoids, carbohydrates, glycosides, saponins, tannins and Terpenoids in the both extracts.

The results of aqueous and methanolic extracts of *Moringa olifera leaves* were agreed with those suggested by **Bamishaiye *et al.,* (2011),** who found that The Phytochemical screening showed that all leaves contain phenolics, Tannins, Alkaloids, Saponins, Flavonoids, Steroid and does not contain phylobatanin and tripertenes.

Total polyphenols and Total flavonoids content of investigated leaves:

Total polyphenols include several classes of phenolic compounds that are secondary plant metabolites and integral part of human and animal diets. Flavonoids are large group of the phenolic compounds consisting mainly of flavonols, flavanols and anthocyanins. Phenolic compounds can play an important role in preventing body cells and organs from injuries by hydrogen peroxide, damaging by lipid peroxides and scavenging or neutralizing free radicals **(Sroka and Cisowski, 2003).**

It has been reported that free radical scavenging and antioxidant activity of many medicinal plants are responsible for their therapeutic effect against cancer, diabetes, tissue inflammatory and cardiovascular diseases *(Cai et al., 2004).* Also, it was found that high total phenols content increase the antioxidant activity and there is a linear correlation between phenolic content and antioxidant activity in fig leaves extract **(Changwei *et al.,* 2008).**

Table (3) showed the total polyphenols (mgGAE/g) and total flavonoids (mgQE/g) contents of *Moringa oleifera* leaves. Data in Table (3) illustrated that, *Moringa oleifera* leaves contained average values of 129.44mgGAE/g and 20.43mgQE/g, dry weight for total polyphenols and total flavonoids, respectively.

Finally, *Moringa oleifera* leaves have the highest concentration of total polyphenols, that was 129.44mgGAE/g. While, The lowest concentration of total flavonoids, was 20.43mgQE/g.

Table (3) Total polyphenols and Total flavonoids content

Plant Leaves	Total polyphenols (mgGAE/g)	Total flavonoids (mgQE/g)
Moringa oleifera	**129.44**	**20.43**

Previous data for *Moringa oleifera* leaves were in agreement with those reported by **Pakade *et al.,* (2012),** who quantified that total flavonoids content was 21.15 mgQE/g of *for Moringa oleifera* leaves in india. While, the total phenolic contents in Mardaan 127.9 mgGAE/g.

The findings were higher than the results of *Moringa oleifera* leaves for total polyphenols contained was 129.44mgGAE/g, while were lowest for total flavonoids contained, was 20.43mgQE/g, recorded by by **Mukunzi *et al.,* (2011),** who found that the total polyphenols and total flavonoids contents in *M. oleifera* which were 30.02mgGAE/g and 52.18 mgQE/g, respectively.

The results were in the same line with those reported by **El-Awady *et al.,* (2016),** who stated that total phenolic contents ranged between 137.53 to 37.6 mg, While the total flavonoids contents ranged between 33.40 to 8.22 (mg QE/g) in *Moringa peregrine* and *Moringa oleifera* extracts, respectively.

Ayodele *et al.,* (2015), found in the dry leaf extracts of *Moringa oleifera* while the least values of total

flavonoid content (0.275±0.0015mg quercetin EQ/g) and total phenolic content (2.35±0.01 mg gallic acid/g), respectively.

Reducing power and Lipid peroxidation of leave extracts:

Efficiency of methanolic and aqueous leave extracts to reduce Fe^{+++} to Fe^{++} was determined according to the method of **Oyaizu (1986)**. Optical density of reaction mixture was measured at wave length 700nm using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer. The obtained data are presented in table (4) the absorbance showed the reducing power for different concentrations of crude methanolic and aqueous extracts of *Moringa oleifera* leaves. Data expressed as absorbance at 700nm for producing color as a result for using three concentrations (20, 40 and 80 mg/ml) for each sample. From table (4), some points could be deduced:

- The reducing power capacity increased with increasing the methanolic and aqueous extracts concentrations for all samples.
- *Moringa oleifera* leaves have the highest reducing power which was ranged from 0.818 to 3.021 for methanolic extract at the concentrations of 20 and 80 mg/ml, respectively. While, they have the reducing power of aqueous extract which was ranged from 0.519 to 2.227 at the concentrations of 20 and 80 mg/ml, respectively.
- High levels of reducing power indicated the presence of some compounds which could be considered electron donors and could react with free radicals to convert them into more stable products **(Arabshahi and Urooj, 2007)**.

The results of *Moringa oleifera* leaves in methanolic extract for reducing power was 0.519 at Concentration 20mg/ml, These findings was in the same line with those reported by **Sravanthi and Rao (2014)**, who found that antioxidant activity concentration, for reducing power, (510mg/g),dry wt, of *Moringa oleifera*.

Previous data of *Moringa oleifera* leaves for reducing power activity which ranged from (0.818 and 0.519) at Concentration 20mg/ml, in methanolic and aqueous extracts consecutively, which agreed with those obtained by **Ogbunugafor *et al.*, (2011),** who found reducing power activity ranged from (0.531 to 0.798mg/ml), at concentration 25 and 100(µg/mL) of *Moringa oleifera* seeds extract**.**

Ekaluo e*t al.*, (2015), who fount that reducing power of *M. oleifera*, ranged from 0.685 to 0.757at concentration 20 and 100 (µg ml^{1}), respectively. While, **Fakurazi *et al.*, (2012),** found antioxidant properties of *Moringa oleifera* leaves using FRAB, were 201.2 and 237.6µM TEAC/100g dw, in aqueous and ethanolic extracts respectively.

Present data agreed with those obtained by **Sultana *et al.*, (2009),** who mentioned that the reducing power of ethanol and aqueous leaves extract, were 2.50 and 2.88(expressed as absorbance values at 700nm), respectively.

Table (4) Reducing power and Lipid peroxidation

Leaves Extracts	Conc. mg/ml	FRAP at 700nm	LPO at 532 nm
***Methanolic* Extract**	**20**	**0.818**	**1.019**
	40	**2.028**	**0.717**
	80	**3.021**	**0.306**
Aqueous Extract	**20**	**0.519**	**1.530**
	40	**1.078**	**0.801**
	80	**2.227**	**0.403**

Lipid peroxidation (LPO) measurements using as standard Fenton Quercetin, Resvesterol reagents MDA-TBARS by the method of **Wu *et al.*, (2000)**. Optical density of reaction mixture was measured at wave length 532nm using a Spekol 11 (Carl Zeiss-Jena) spectrophotometer. The obtained data are presented in table (4) the absorbance showed the Lipid peroxidationfor different concentrations of crude methanolic and aqueous extracts of *Moringa oleifera* leaves. Data expressed as absorbance at 532nm for producing color as a result for using three concentrations (20, 40 and 80 mg/ml) for each sample. From table (4), some points could be deduced:

- The lipid peroxidation (LPO) capacity increased with increasing the methanolic and aqueous extracts concentrations for all samples.
- *Moringa oleifera* leaves have the highest lipid peroxidation which was ranged from 1.019 to 0.306 for methanolic extract at the concentrations of 20 and 80 mg/ml, respectively. While, have the lipid peroxidation of aqueous extract which was ranged from 1.530 to 0.403 at the concentrations of 20 and 80 mg/ml, respectively.
- ROS-mediated oxidation of membrane lipids result in the formation of lipid peroxidation of membrane (LPO) product as MDA (malondialdehyde) is generally considered to be degradation of polyunsaturated lipids. This compound is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells **(Yıldırım and Gökçe, 2014)**.

The results of *Moringa oleifera* leaves in methanolic extract for lipid peroxidation was 1.019 at

Concentration 20mg/ml, These findings was in the same line with those reported by **Florence *et al.,* (2014)**, who revealed that lipid peroxidation inhibition value (3.458±2.37), of *Moringa oleifera* leaves extract.

Previous data of *Moringa oleifera* leaves for lipid peroxidation which ranged from (0.818 and 0.519) at Concentration 10mg/ml, in methanolic and aqueous extracts consecutively. which agreed with those obtained by **Nanjappaiah and Shivakumar Hugar (2013),** who found the effects of *Moringa oleifera* seeds extract on LPO Levels, was (0.388mg/kg).

Sreelatha and Padma (2009), who fount that antioxidant profiles of *Moringa oleifera* (Concentration in micrograms (µg /ml) needed for 50% inhibition), IC_{50}ranged from 25.32 and 30.15 (µg/ml), in mature and tender leaf extract, respectively. Also, **Qwele *et al.,* (2013),** who found that lipid peroxidation of *Moringa oleifera* leaves, was 47.90%.

Determination of Antioxidant Activity Using the 2,2 Diphenyl-1-picrylhydrazyl (DPPH) and Hydroxyl radical (OH) Radical Scavenging Activity:

The capacity of *Moringa oleifera* leaves methanolic and aqueous extracts, radical scavenging ability using the stable radical DPPH and OH.

The antioxidant activity of methanolic and aqueous extracts prepared from the studied plant species are reported in Table (5).

The concentration of an antioxidant needed to decrease the initial DPPH concentration by 50% (IC_{50}) is a parameter widely adapted to measure the antioxidant activity **(Sanchez *et. al.* 1998).** The lower EC_{50} pointed to the higher antioxidant activity.

The antioxidant activity of the tested extracts were measured using DPPH radical scavenging activity. The antioxidants scavenging activities of DPPH are attributed to their hydrogen-donating abilities **(Biswas *et al.,* 2010).** VitaminC was used as the reference compound.

From table (5), it is clear that the scavenging effect (IC_{50}) of methanolic extracts for *Moringa oleifera* leaves have the most effective of inhibition percentage (0.015), followed by aqueous extract which was (0.018), respectively.

Different studies indicated that the electron donation capacity which reflecting the reducing power of bioactive compounds was associated with antioxidant activity (**Siddhuraju *et al.,* 2002**).

The data were parallel with that recorded by **Sravanthi and Rao (2014),** who determined the antioxidant capacity of *Moringa oleifera* leaves. The antioxidant activity for DPPH scavenging were (0.63mg/g dry wt).

The present data of *Moringa oleifera* leaves for antioxidant capacity are agreed with those found by **Sreelatha and Padma (2009),** who calculated the IC_{50} of antioxidant activity for methanolic extract of *Moringa oleifera* leaves using DPPH scavenging which was 18.15 and 19.12(µg/ml) of mature and tender leaf extract, respectively.

Mrudula *et al.,* (2014), The IC_{50} of antioxidant activities using DPPH assay of *M. oleifera* leaves was 69.52(µg/ml). **Das, *et al.,* (2012),** who found that The IC_{50} value of MOL extract for 2, 2-diphenyl-1-picrylhydrazyl radical scavenging was 18.54 µg $mL)^{-1}$. Also, **Qwele et al., (2013),** who found the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was 58.95±0.3% of *Moringa oleifera* leaves.

Table (5) Antioxidant capacity determined by ($DPPH^+$) and (OH^+) radical scavenging:-

Plant leaves	$DPPH^+$ IC_{50}	OH^+ radical (mgGAE/g)
Methanolic	**0.015**	**47.22**
Aqueous	**0.018**	**43.89**

From table (5), it is clear that the scavenging effect OH radical of methanolic extracts for *Moringa oleifera* leaves have the most effective as antioxidant activity percentage (47.22 mgGAE/g), followed by aqueous extract which was (43.89mgGAE/g), respectively.

The OH scavenging ability of the *M. oleifera* leaf extract could be attributed to the presence of polyphenols which are capable of donating hydrogen atoms to OH radicals, thus inhibiting the oxidation process **Zang *et al.,* (2003)**.

The present data of *Moringa oleifera* leaves for antioxidant capacity are agreed with those found by **Oboh *et al.,* (2015),** who found that The *Moringa oleifera* leaves extract scavenged NO^*, OH^*, chelated Fe^{2+}, and inhibited MDA production in a dose-dependent pattern with IC_{50} values of 1.36, 0.52, and 0.38mg/mL and 194.23 µg/mL, respectively.

The data were parallel with that recorded by **Mrudula *et al.,* (2014),** who found that IC_{50} values for radical scavenging activity assays of methanol extracts of *Moringa oleifera* was 331.62 (µg/ml).

Hanaa and gamal (2013), who established The *Moringa oleifera* seeds showed remarkable antioxidant activity, compared with that of commonly used antioxidants (α-tocopherol, BHT and BHA) as determined by OH radical scavenging, IC_{50} was 45.62 µg ml^{-1}.

Antioxidant capacity of leaves extracts determined by ($ABTS^{+}$) cation radical:

The capacity of *Moringa oleifera* leaves methanolic and aqueous extracts to scavenge the ABTS radical was determined separately and compared with the reduction of ascorbic acid as a control sample which is known as a strong reducing agent.

From table (6), it could be seen that all extracts showed different degrees of inhibition capacity, but their capacities were inferior than ascorbic acid which have the maximum inhibition (91.41%).

From the same table, it is clear that methanolic extract of *M. oleifera* leaves have the lowest absorbance value (0.062) with the highest value of inhibition percentage (93.48%) followed by aqueous extract which have (70.08%) as inhibition capacity.

Different studies indicated that the electron donation capacity which reflecting the reducing power of bioactive compounds was associated with high antioxidant activity **(Siddhuraju *et al.*, 2002).**

Table (6) Antioxidant capacity determined by ($ABTS^{+}$) cation radical

Plant leaves	Methanolic extract		Aqueous extract	
	Absorbance	% Inhibition	Absorbance	% Inhibition
Moringa oleifera	0.062	**92.48**	0.079	**70.08**
+Ve Control (Ascorbic acid)	**91.41**			
-Ve Control	**0**			

The present data of *M. oleifera* leaves for antioxidant capacity was (92.48%) for methanolic extract are agreed with those found by **Qwele *et al.*, (2013),** who mentioned that antioxidant potential of *Moringa oleifera* leaves, was 93.51%. Also, he present data of *M. oleifera* leaves for antioxidant capacity was (70.08%) for aqueous extract are agreed with those found by **Masika *et al.*, (2013),** who mentioned that antioxidant potential of Moringa oleifera leaves, was 68.6%.

Sravanthi and Rao (2014), who determined the antioxidant capacity of *Moringa oleifera* leaves. The antioxidant activity for DPPH scavenging were (5.0±0.3mg/g dry wt). Also, **Florence *et al.*, (2014),** who found that the antioxidant activity using ABTS *Moringa oleifera* was average 99.860%.

Effect of investigated leaves extracts as antibacterial agents:

The effect of various concentrations (1, 2 and 4µg/ml) of *Moringa oleifera* leaves crude methanolic and aqueous extracts on the growth inhibition of *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtillis*, are shown in table (7). It is clear that growth inhibition percentage increased gradually with increasing the concentration of the extracts for all microbial strains under investigation.

From table (7), it could be observed that *Moringa oleifera* leaves for methanolic extract produced the highest growth inhibition (20 and 17mm) against *Escherichia coli* and *Bacillus subtillis* at concentration 4µg/ml, respectively. While, the percentages of growth inhibition for *St.coccus aureus*extracts ranged from (9 to 13.5mm) in the same extract at concentration 1 and 4µg/ml, respectively.

Moreover, the *Moringa oleifera* leaves for aqueous extract produced the highest growth inhibition ranged from (8.5 and 13mm) against *St.coccus aureus* at concentration 1 and 4µg/ml, respectively. While, the percentages of growth inhibition of *Escherichia coli* and *Bacillus subtillis*, which were (10.25 and 10.5mm) in the same extract at concentration 4µg/ml, respectively.

Table (7) Growth Inhibition of Bacerial

Leaves extract	Extract	Concentration (µg/ml)	mm of growth inhibition for :		
			E. coli	***St. aureus***	***B. subtillis***
Moringa oleifera	**Methanolic**	**1**	11	9	10.5
		2	17	11.5	14
		4	20	14.5	17
	Aqueous	**1**	6.75	8.5	7.5
		2	8.5	10.25	9
		4	10.25	13	10.5

The results of several authors were agreed with that obtained, for instance, **Ashok *et al.*, (2014),** found that antimicrobial activities of *Moringa oleifera* Aqueous extracts on *E. Coli* and *St. aureus* were 12 and 10mm, respectively.

The results were highest compared with that obtained by **PAL *et al.*, (1995),** who found antibacterial

activity of ethanolic extract for *Moringa oleifera*, on *E. coli, B. subtillis* and *St.coccus* were 19, 15 and 13mm, consecutively. Also, **Bijal *et al.,* (2015),** who showed the maximum zone of inhibition against *E. coli* and *St. aureus* (28 and 26mm) of methanolic *Moringa* leaves extract. Likewise, the ethanol extract showed maximum zone of inhibition against *E. coli* and *S. aureus* (23 and 17mm) in *Moringa* flower, consecutively.

Marrufo *et al.,* (2013), who established the antimicrobial activity of the leaf essential oil of *Moringa oleifera* against *B. cereus and E. coli* were 10.7 and 6.7, at concentration 10µg, respectively. Moreover, **Rahman *et al.,* (2009),** who found the antibacterial activity of *Moringa oleifera* leaf aqueous extracts, were 17.25 and 12mm, for *B. subtillis* and *St. aureus*, respectively.

Conclusion

Phytochemical tests of investigated plants showed the highest content of Terpenes, Tannins, Flavonoids, Saponin, Glycosides, Alkaloids and phenolic glycosides. Medicinal plant extract containing high percentage of active compounds such as Polyphenols and Flavonoids for *Moringa oleifera* leaves. The impact of natural extracts as antioxidant tested using (DPPH-ABTS-FRAB-LPO-OH radical) showed high ability of these plants to scavenging the free radicals in laboratory. Moreover, methanolic extracts were the most effective bacterial inhibitor followed by aqueous extract on *E. coli, St. aureus and B. subtillis*.

REFERENCES

Ahiakpa, J. K.; Quartey, E. K, Amoatey, H. M, Klu, G. Y. P, Achel, D. G, Achoribo, E. and Agbenyegah, S. (2010). Total flavonoid, phenolic contents and antioxidant scavenging activity in 25 accessions of okra (*Abelmoschus spp* L.). Afr. J. Food Sci. Technol, Vol. 4(5) pp. 129-135.

Ashok, V. .; Pranita, A. G; Megha, P. J. and Neeta, A. D. (2014). Antimicrobial Activity of Indian Medicinal Plants: *Moringa oleifera* and *Saraca indica*. Int. J. Curr. Microbiol. App. Sci 3(6): 161-169.

Ayodele, O. D. and Dolapo, E. O. (2015). Total Antioxidant Activity, Total Phenolic and Total Flavonoid Content of Some Plant leaves in South-West Nigeria. International Journal of Scientific & Engineering Research, Volume 6, 418:427.

Bamishaiye, E. I.; Olayemi, F. F; Awagu, E. F. and Bamshaiye, O. M. (2011). Proximate and Phytochemical Composition of *Moringa oleifera* Leaves at Three Stages of Maturation. Advance Journal of Food Science and Technology 3(4): 233-237.

Bhumika, D. and Bijal, A. (2015). Antibacterial Activity and Phytochemical Screening of Different Parts of *Moringa oleifera* Against Selected Gram Positive and Gram Negative Bacteria. J Pharm Chem Biol Sci , Vol-3(3):421-425.

Biswas, M.; Haldar, P. K. and Ghosh A. K. (2010). Antioxidant and free-radical-scavenging effects of fruits of *Dregea volubilis.* J Nat Sci Biol Med.; 1(1): 29–34.

Chang, C. C.; Yang, M. H.; Wen, H. M. and Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis, 10, 178–182.

Changwei, A. O.; Anping, L.; Abdelnaser, A.; Tran, D. X. and Shinkichi, T. (2008). Evaluation of antioxidant and antibacterial activities of Ficus microcarpa L. leaf extract. Food Control, 19, 940-948.

CoStat program, Version 6.311 (2005). Cohort Software, 798 Lighthouse Ave. PMB 320, Monterey, CA, 3940, USA. http://www.cohort.com.

Duncan, D. (1955). Multiply range and multiple F test. Biometrics, 11, 1-42.

Ekaluo, U. B.; Ikpeme, E. V; Udensi, O. U; Ekerette, E. E; Usenm, S. O. and Usoroh, S. F. (2015). Comparative in vitro Assessment of Drumstick (Moringa oleifera) and Neem (*Azadiracta indica*) Leaf Extracts for Antioxidant and Free Radical Scavenging Activities. *Res. J. Med. Plant, 9 (1): 24-33*.

El-Awady, M. A.; Mohamed, M. H; El-Sayed, S. A. and Ahmed G. (2016). Comparison of the antioxidant activities, phenolic and flavonoids contents of the leaves-crud extracts of *Moringa peregrine* and *Moringa oleifera*. International Journal of Biosciences, Vol. 8, No. 1, p. 55-62.

El‐Khateeb, A. Y.; Azzaz, N. A. and Mahmoud, H. I. (2014). Phytochemical constituents, hypoglycemic and haematological effects of methanolic *Acalypha wilkesiana* leaves extract on streptozotocin‐induced diabetic rats. European Journal of Chemistry, 5 (3) 430‐438.

Fakurazi, S.; Syazana, A. S. and Palanisamy, A. (2012). *Moringa oleifera* Hydroethanolic Extracts Effectively Alleviate Acetaminophen-Induced Hepatotoxicity in Experimental Rats through Their Antioxidant Nature. *Molecules, 17*, 8334-8350.

Florence, I. F.; Akinloye, O. A. and Ilesanmi, O. S. (2014). Comparative Evaluation of *in vitro* Antioxidant Properties of *Cajanus cajan* Seed and *Moringa oleifera* Leaf Extracts. International J. of Biochemistry Research & Review, 4(2): 163-172.

Ganiyu Oboh,1 Adedayo O. Ademiluyi,1 Ayokunle O. Ademosun,1 Tosin A. Olasehinde,2 Sunday I. Oyeleye,1 Aline A. Boligon,3 andMargareth L. Athayde (2015). Phenolic Extract from *Moringa oleifera* Leaves Inhibits Key Enzymes Linked to Erectile Dysfunction and Oxidative Stress in Rats'

Penile Tissues. Biochemistry Research International,1:8.

Haghi, G.; Hatami, A.; Safaei, A. and Mehran. M. (2014). Analysis of phenolic compounds in *Matricaria chamomilla* and its extracts by UPLC-UV. Res. in Pharma. Scin, 31-37.

Hanaa, H. A. and Gamal, S. E. (2013). Characterization of Egyptian *Moringa peregrine* seed oil and its bioactivities. International Journal of Management Sciences and Business Research, Vol-2, 98:108.

Harborne, J. B. (1988). Phytochemical methods, 2nd ed. Published in USA by Chapman and Hall 29, west 35th street, New York.

Kim, I. S.; Yang, M. R; Lee, O. H. and Kang, S. N. (2011). Antioxidant Activities of Hot Water Extracts from Various Spices. *Int. J. Mol. Sci.* 2011, *12*, 4120-4131.

Lin, J. Y. and Tang, C-Y. (2007). Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chemistry, 101, 140–147.

Marrufo, T.; Filomena, N; Emilia, M; Florinda, F; Raffaele, C; Laura, D. M; Adelaide, B. A. and Vincenzo, D. F. (2013). Chemical Composition and Biological Activity of the Essential Oil from Leaves of *Moringa oleifera* Lam. Cultivated in Mozambique. *Molecules 18*, 10989-11000; doi:10.3390/molecules180910989.

Mrudula, C. M.; Ashish, A. P. and Ritu, R. (2014). Phytochemical quantification and antioxidant capabilities of *Moringaoleifera, Basellaalba* and *Centellaasiatica*leaf sources. International Journal of Innovative Research in Science, Engineering and Technology, V-3, 9243:9251.

Nanjappaiah, H. M. and Shivakumar, H. (2013). Evaluation of *Moringa oleifera* Seeds for Prophylactic and Curative Effects in CCl_4 Induced Rat Liver Injury. RGUHS J Pharm Sci | Vol 3, 41:46.

Ogbunugafor, H. A.; Eneh, F. U; Ozumba, A. N; Ezikpe, M. N. I; Okpuzor, J; Igwilo, I. O; Adenekan, S. O. and Onyekwelu, O. A. (2011). Physico-chemical and Antioxidant Properties of *Moringa oleifera* Seed Oil. Pakistan Journal of Nutrition 10 (5): 409-414.

Oyaizu, M. (1986). Studies on products of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine, Jpn. J. Nutr., 44, 307–315.

Pakade, V.; Ewa, C. and Luke, C. (2012). Comparison of antioxidant activity of *Moringa oleifera* and selected vegetables in South Africa. Research Article Antioxidant activity of Moringa oleifera and Vegetables, Page 1 of 5.

Patel, P.; Nivedita, P; Dhara, P; Sharav, D. and Dhananjay, M. (2014). Phytochemical Analysis and Antifungal Activity of *Moringa Oleifera*. Int J Pharm Pharm Sci, Vol 6, Issue 5, 144-147.

Pratap, C. R.; Vysakhi, M. V.; Manju, S.; Kannan, M.; Abdul, K. S. and Sreekumaran, N. A. (2013). In vitro free radical scavenging activity of Aqueous and Methanolic leaf extracts of *Aegle tamilnadensis (rutaceae)*. Int J Pharm Sci, 819-823.

Qwele, K.; Hugo, A; Oyedemi, S. O; Moyo, B; Masika, P. J. and Muchenje V. (2013). Chemical composition, fatty acid content and antioxidant potential of meat from goats supplemented with Moringa (*Moringa oleifera*) leaves, sunflower cake and grass hay. Meat Science 93, 455–462.

Rahman, M. M.; Mominul, I. S. M; Shamima, A. S; Soriful, I. M; M. Atikur; Mizanur, R. R. M. and Alam, M. F. (2009). Antibacterial Activity of Leaf Juice and Extracts of *Moringa oleifera* Lam. against Some Human Pathogenic Bacteria. CMU. J. Nat. Sci. Vol. 8(2), 219:227.

Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M. and Rice-Evans C. (1999). Antioxidant activity applying an improved ABTS radical. cation decolorization assay. Free Radical Biology & Medicine, Vol. 26, 1231–1237.

Sanchez, M. C.; Larrauri, A. and Saura, C. F. (1998). A procedure to measure the antiradical efficiency of polyphenols. Journal of the Science of Food and Agriculture, 76, 270–276.

Saroj K. Pal, Pulok K. Mukherjee, Kakali Saha, M. Pal And B.P. Saha (1995). Antimicrobial action of The Leaf Extract of *Moringa Oleifera* Lam. Ancient Science of Life, Vol No. XIV No.3, Pages 197-199.

Shan, B.; Caia, Y. Z.; Brooksb, J. D. and Corke, H. (2007). The in vitro antibacterial activity of dietary spices and medicinal herb extracts. International Journal of Food Microbiology, (112-119).

Siddhuraju, P.; Mohan, P. S. and Becker, K. (2002). Studies on the antioxidant activity of Indian laburnum (Casia fistula L.): a preliminary assessment of crude extracts from stem bark, leaves, flowers and fruits pulp. Food chemistry, 79, 61-67.

Smirnoff, N. and Cumbes, Q. J. (1989). hydroxyl radical scavenging activity of compatible solutes" photochemistry, 28:1057-1060.

Sravanthi, J. and Rao, S. G. (2014). Antioxidative studies in *Moringa oleifera* Lam. Annals of Phytomedicine 3(2): 101-105.

Sreelatha, S. and Padma, P. R. (2009). Antioxidant Activity and Total Phenolic Content of *Moringa oleifera* Leaves in Two Stages of Maturity. Plant Foods Hum Nutr, 64:303–311

Sroka, Z. and Cisowski, W. (2003). Hydrogen peroxide scavenging, antioxidant and antiradical activity of some

phenolic acids. Food Chem. Toxicol., 41, 753-758.

Sultana, B.; Farooq, A. and Muhammad, A. (2009). Effect of Extraction Solvent/Technique on the Antioxidant Activity of Selected Medicinal Plant Extracts. *Molecules,14,*2167-2180.

Vinoth, B.; Manivasagaperumal, R. and Balamurugan, S. (2012). Phytochemical Analysis and Antibacterial Activity of Moringa Oleifera Lam. International Journal of Research in Biological Sciences, 2(3): 98-102.

Wu, L.; Chang, L; Chen, S. N. and Fan, J. A. H. (2000). Antioxidant activity and melanogenesis inhibitory effect of the acetonic extract of Osmanthus fragrans: A potential natural and functional food flavor additive, LWT-Food Science and Technology, 42, 91513.

Yıldırım, I. and Gökçe, Z. (2014). The Determination of Antioxidant Vitamin Levels, Radical Scavenging Effect of Elaeagnus Angustifolia Methanol Extracts Against to MDA. Hacettepe J. Biol. & Chem., 42 (4), 551-556.

Zang, L. Y.; Cosma, G; Gardner, H; Castranova, V. and Vallyathan, V. (2003). Effect of chlorogenic acid on hydroxyl radical, Molecular and Cellular Biochemistry, Vol. 247, pp. 205–210.