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Phytochemical Screening, Antioxidant and Antibacterial Activity of Extracts Prepared from Fruit and Bark of *Moringa oleifera.*

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ABSTRACT

Moringa oleifera (family moringaceae) is used by various tribal communities and forest inhabitants for the treatment of a variety of diseases. The plant literature survey shows the plant possesses astringent, stimulant, diuretic, aphrodisiac, demulcent, and tonic effects and also helps in dysentery. It also possesses important pharmacological activity such as aphrodisiac, analgesic, anti-inflammatory and hepatoprotective activity in addition to anti-HIV activity and anticancer, antiangiogenic activity and hypotensive, hypoglycemic and antimicrobial activity. In present study methanolic extract of fruit and bark of *M. oleifera* was subjected to phytochemical analysis. Antioxidant potential of both extracts was evaluated by total antioxidant assay (TAC), reducing activity assay. Oxygen free radicals scavenging activity was evaluated by studying the effect on superoxide radical, hydrogen peroxide and hydroxyl radical. Extracts were also screened for their antibacterial property using disk diffusion method against *E. coli* and *B. subtilis*. It was observed that fruit extract contains more phenols and flavonoids than bark extract. This result was also reflected in antioxidant potential. Both the extracts showed significant antioxidant activity, but the potential was higher in fruit extract due to presence of higher content of polyphenolic compounds. Antimicrobial activity was also found in both the extracts. Results were compared with standard antibiotic. Thus, *M. oleifera* can be used to prevent free radical borne diseases.

Keywords: *Moringa oleifera*, phytochemical analysis, phenolic content, antioxidants, antimicrobial pathogens.

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INTRODUCTION

Plants have been used for medicinal purposes long earlier than prehistoric time.The term "herb" has been derived from the Latin word, *"herba"* and an old French word *"herbe".* In recent time, herb refers to any part of the plant like fruit, flower, stem, leaf, seed, bark, stigma or a root, as well as a non-woody plant. Earlier, the word "herb" turned into most effective applied to non-woody plants, together with come from trees and shrubs. These medicinal plants are also used as flavonoid, medicine, food or perfume and also in certain spiritual activities.Moringa has a few conventional and therapeutic employments. It is being consumed for over a quarter century Ghana and different parts of the world as a nutritional supplement. However, it is gotten to be famous in the whole society. Regardless of the nutraceutical significance, various parts of the plant have distinctive pharmacological action. Moringa tree has a tremendous procedure in treating malnutrition, particularly among newborn children and mothers. All the parts of this plant: leaf, blooms, root, seed bark, gum and seed oil have been used for different positions as a part of the indigenous treatment of South Asia, containing the treatment of inflammation and infectious diseases along with gastrointestinal, hematological, cardiovascular and hepatorenal scatters [1-3]. Different categories of compounds, for example, ascorbic, flavonoids, phenolics and carotenoids found in leaves act as a good source of natural antioxidant[4]. The leaf is very nutritious and contains huge quantities of rough protein (20-29%), vitamins and minerals [5, 6] and juice of leaves are being applied in eye contaminations. Moringa seeds are reported to indicate antimicrobial activity. The roots and seed separate have shown antimicrobial action [7]. The ethanolic essence of the leaves of Moringa was accounted for its antimicrobial exercises [8]. The plant is additionally surely understood for its different restorative properties, for example, reducing blood pressure, tumor healing properties, antifertility action, antibacterial activity [9, 10]. The aqueous extract and alcoholic extract of Moringa root-wood answered to decrease and prevent the enlargement of urinary stones [11]. A significant number of essential and auxiliary metabolites and pharmacological exercises have been accounted for the Moringa plant. Thus this survey contains vital and valuable data on botany, pharmacognosy, conventional use, phytochemistry, nutritional value and pharmacology of this useful plant.In present study, the methanolic bark and fruit extract of herbal plant (*Moringa oleifera*) were evaluated for phytochemical properties, antioxidant assay and antimicrobial activity.

MATERIALS AND METHODS

Fresh fruits and bark of *M. oleifera*were collected from U.P pollution control board, Vasundra, Ghaziabad, Uttar Pradesh, India. The fresh fruits and bark of *M. oleifera*were allowed to air dry at room temperature for five weeks. The dried plant materials were blended into powder and kept in clean air-tight containers for further use.

Extract preparation of plant materials

Powdered material was extracted successively with ethanol using Soxhlet apparatus at 90-95 °C for 15-24 hours in order to extract the polar and non-polar compound. The methanolic plant extracts were then redissolved in "tween 80" to get the solution of desired concentration which was subjected for further experiments.

Preparation of inoculums

Gram positive (*Bacillus. subtilis*) and gram negative (*E. coli*) bacteria were precultured in nutrient broth for overnight in a rotary shaker at 37°C. The cultures were used when 0.6 OD was obtained at A_{600}.

Qualitative phytochemical analysis

Study was carried out to identify the presence of phytochemicals present in methanolic extracts of fruit and bark of*Moringa oleifera.*

Detection of alkaloids

Wagner's test: Different conc. of the fruit and bark extract was treated with Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 100ml of distilled water) and observed for the formation of reddish brown precipitate.

Detection of phenolic compounds

Ferric chloride test: Different concentration of the extracts was treated with 5% $FeCl_3$ solution and observed for the formation of deep blue colour.

Detection of flavonoids

Aqueous NaOH test: Different concentrations of fruit and bark extracts was treated with 1N aqueous NaOH and observed for the formation of yellow-orange color.

Detection of Saponins

Foam test: Different concentrations of both the extract was vigorously shaken with water and observed for persistent foam.

Detection of tannins

Ferric chloride test: Different concentrations of plant extracts was stirred with 1ml of distilled water, filtered and few drops of ferric chloride was added to the filtrate. A blue-black, green, blue-green precipitate shows the presence of tannins.

Quantitative phytochemical analysis

Determination of total phenolic content

Total phenolic content was determined by the Folin-Ciocalteau method [12]. In brief, different conc. of fruit and bark extract was mixed with 4.9 ml distilled water, 0.5 ml of Folin Ciocalteu reagent was added to the mixture. After 5min of incubation, 5 ml of 7% of aqueous Na_2CO_3 solution was added. The mixture was allowed to stand for 30 minutes and the absorbance was measured at 760 nm using a UV-Vis Double Beam spectrophotometer. The standard curve was prepared by Gallic acid (mg/ml) in methanol: water (50:50, v/v). Total phenolic content was expressed as of Gallic acid equivalent mg/g GAE of extract.

Determination of flavonoid content

Aluminum chloride method was used for flavonoid determination [13]. Different concentrations of fruit and bark extract was mixed with 1.9ml distilled water, then 0.1 ml 10% aluminium chloride-hexa hydrate, 0.1 ml 1M potassium acetate and 2.8 ml of distilled water were added. The reaction mixture was incubated at room temperature for 40 min. The absorbance of the reaction mixture was measured at 415nm. Quercetin (mg/ml) was used as a standard. Total flavonoid content was expressed as mg/g QE of extract.

Antioxidant Activity of Plant Material

Total antioxidant activity

The total antioxidant activity was evaluated by using the method [14]. Plant extracts of fruit and bark were dissolved in tween-80 to form the concentration of 1mg/ml. Different concentrations of fruit and bark extracts was placed in a test tube, 0.3 ml of reagent solution (0.6 M Sulphuric Acid, 28 mM Sodium Phosphate, 4 mM Ammonium molybdate) was then added and the resulting mixture was incubated at 95^0C for 90 minutes. After the mixture was cooled to room temperature, the absorbance of both solution was measured by using UV-Visible spectrophotometer at 695 nm. The experiment was performed in triplicates. Antioxidant potential of fruit and bark extracts were compared with standard antioxidant ascorbic acid.

Reducing Power Activity

The reducing power was determined according to the method of [15].Different concentrations of fruit and bark extracts was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%). This was incubated at 50°C for 20 min. After the incubation, 2.5 ml of 10% trichloroacetic acid was added. The mixture was centrifuged at 650 rpm for 10 min. Then supernatant was mixed with 5 ml deionized water and 1 ml of 0.1% of ferric chloride, and the absorbance was measured at 700 nm. Higher absorbance indicates higher reducing power. The assays were carried out in triplicate and the results are expressed as mean values ± standard.

Superoxide radical scavenging assay

The method was used to determine O_2^- radical scavenging activity of samples [16]. Briefly, 1 ml of extract was added to 9 ml of 5 mM Tris-HCl buffer (pH 8.2). 40 µl of 4.5 mM pyrogallol was added to the mixture. The mixture was shaken and after 3 min just a drop of ascorbic acid (0.035%) was added to it. The absorbance of the reaction mixture was measured at 420 nm after 5 min (Similar concentration extract was used as the blank to eliminate interference). O_2^- radical scavenging activity was expressed by the oxidation degree of a test group in comparison to that of the control.

The percentage of scavenging effect was calculated using the following equation:

$$O_2^- \text{ radical scavenging } \% = [A_0\text{-}A_1/A_0] \times 100$$

Where A_0 is the absorbance of the Tris-HCl buffer with pyrogallol, A_1 is the absorbance of the extract addition.

Hydrogen peroxide decomposition

Hydrogen peroxide (H_2O_2) decomposition was determined according to the method of [17]. Hydrogen peroxide solution (a solution of hydrogen peroxide (34mM) was prepared in phosphate buffer, pH 7.0) was added to both extracts of different concentration against a blank solution containing phosphate buffer without hydrogen peroxide. The hydrogen peroxide decomposition was determined by absorption at 320 nm using a spectrophotometer. Ascorbic acid was used as standard.

The extent of H2O2 decomposition of the plant extracts was calculated as:

$$\% \text{ scavenging of hydrogen peroxide} = (A0 - A1)/ A0 \times 100$$

Where, A0 - Absorbance of control, A1 - Absorbance in the presence of plant extract

Measurement of hydroxyl scavenging activity

The method used to measure the hydroxyl radicals scavenging activity [18]. Reaction mixture contained 60 µl of 1.0mM $FeCl_2$, 90 µl of 1mM 1,10-phenanthroline, 2.4mL of 0.2M phosphate buffer (pH 7.8), 150µL of 0.17 M H_2O_2, and different concentrations of extracts. Adding H_2O_2 started the reaction. After incubation at room temperature for 5 min, the absorbance of the mixture was measured at 560 nm with double beam spectrophotometer. Results were compared with standard antioxidant ascorbic acid.

The percentage inhibition of hydroxyl scavenging activity was calculated using the following formula,

Rate % of inhibition = Absorbance (blank) – Absorbance (with extract) / Absorbance (without extract) x 100

Where, Absorbance (Test): Absorbance of the test (With extract) and Absorbance (Blank): Absorbance of the control (Without extract).

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Anti -microbial Activity

Disc diffusion method

Antibacterial activity of methanolic extracts of fruit and bark were investigated by the disc diffusion method [19]. Bacterial suspensions were used for preparing seeded Nutrient agar plates. Sterilized Whatmann filter no.1 discs were soaked with different concentrations of bark and fruit extracts of *Moringa oleifera* as well as antibiotic chloramphenicol followed by air drying. The air dry discs were then placed on different agar plates seed with *E coli* and *Bacillus subtilis*. The plates were then incubated at 37^0C for 24 hrs. The zone of inhibition around the discs were measuredafter 18-24 hours of incubation. The sensitivity of the microorganism species to the plant extracts was determined by measuring the size of inhibitory zones on the medium surface around the discs. Experiment was repeated twice in triplicates.

RESULTS AND DISCUSSION

Phytochemical Analysis (Qualitative)

Qualitative phytochemical analysis of fruit and bark extract of *Moringa oleifera* revealed the presence of secondary metabolites like alkaloids, flavonoids, phenolic, tannins and saponin compounds. Results are summarized in table no.1.

Table 1: Qualitative phytochemical analysis of fruit and bark extract

No.	Phytochemicals	Test	*Moringa oleifera* (fruit)	*Moringa oleifera* (bark)
1	Alkaloids	Wagner test	+	+
2	Flavonoids	Aqueous NaOH test	+	+
3	Phenolic comp.	Ferric chloride test	+	+
4	Tannins	Ferric chloride test	+	+
5	Saponins	Foam test	+	+

Key: "+" - positive "-"- negative

Quantitative study

Phytochemical constituents of fruit and bark extract of *Moringa oleifera* were investigated quantitatively and results are summarized below:

Total phenolic content

The fruit and bark extracts were tested for total phenolic content and results were shown in table 2. Results indicates that fruit extract of *Moringa oleifera* possess higher total phenolic content than bark extract. The phenolic was found to be 358 mg/gGAE in fruit extract and 320 mg/gGAE in bark extract. Results are compared with ascorbic acid standard.

Table 2: Total Phenolic Content of methanolic extracts of fruit and bark:

Total phenolic content	
Fruit (mg GAE/g of extract)	**Bark (mg GAE/g of extract)**
358 ± 0.278*	320 ± 0.278*

*Each value is expressed as mean ± standard deviation (n= 3); $P < 0.05$)

Total flavonoid content

The fruit and bark extracts were tested for total flavonoid content and results were shown in table 3. The fruit extract of *Moringa oleifera*showed higher total flavonoids content. The flavonoid content was found to be 300 mg/gQEin fruit extract and 258 mg/gQE in bark extract.

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Table 3: Total Flavonoid Content of methanolic plant extracts:

Total flavonoid content	
Fruit (mg QE/g of extract)	**Bark (mg QE/g of extract)**
300 ± 0.278*	258 ± 0.24*

*Each value is expressed as mean ± standard deviation (n= 3); $P < 0.05$)

In vitro antioxidant activity

Antioxidants are known to exhibit their biochemical effects through numerous mechanisms, including the prevention of chain initiation, reductive capacity and radical scavenging mechanisms. Several methods have been used to measure the antioxidant activity of biological materials. It is essential to use more than one method to evaluate antioxidant capacity of plant materials simply because of the complex nature of phytochemicals present in them. Therefore, in the present study, Total antioxidant activity, ferric reducing antioxidant power assay, superoxide anion radical scavenging activity, hydrogen decomposition assayand hydroxyl radical scavenging were done.

Total antioxidant activity

The Phosphomolybdenum method was used to assay total antioxidant activity of fruit and bark extracts under study. This method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm.

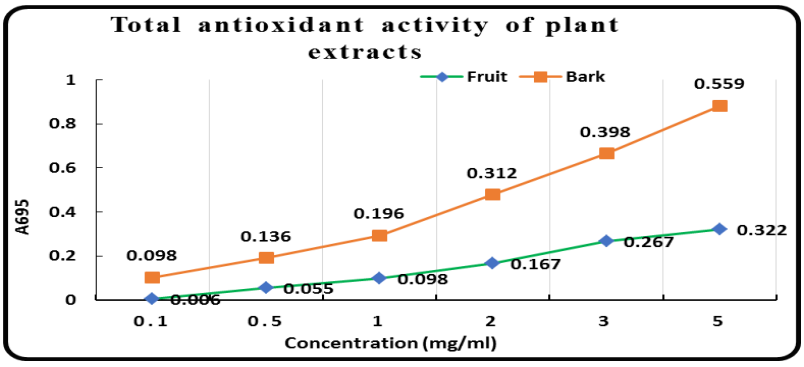

Figure 1: Total Antioxidant activity of fruit and bark extract of *Moringa oleifera*

The results in fig 1 shows the antioxidant activity of fruit and bark extracts. The results were compare with ascorbic acid standard. Itwas found that methanolic extracts of fruit and bark exhibit an increase in absorbance with increasing the concentration of extracts. Both extracts have good antioxidant potential and exhibited concentration dependent activity. Fruit extract show higher antioxidant activity as compare to bark extract.

Reducing Power Activity

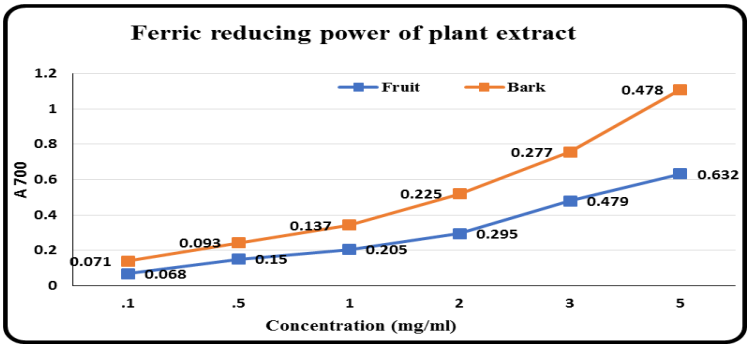

Figure 2: Reducing Activity of fruit and bark extract of *Moringa oleifera*

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For the measurements of the reducing ability, it has been investigated from the Fe^{3+} to Fe^{2+} transformation in the presence of extract samples. The results in fig 2 shows the reducing activity of fruit and bark extracts. The results were compare with ascorbic acid standard.

It was found that the tendency to reduce Fe^{3+} to Fe^{2+} steadily increase with increasing sample concentration of fruit and bark extracts. Both extracts have good reducing property and exhibited concentration dependent activity. Fruit extract show higher antioxidant activity as compare to bark extract of *Moringa oleifera*.

Superoxide anion scavenging activity

Superoxide anions are generated by the oxidation of pyrogallol, and the scavenging effects are expressed as the inhibition of pyrogallol autoxidation, so any substance existing in the reaction system that might affect the oxidation of pyrogallol might also affect the test results.

The resultsin table 4 shows the superoxide anion scavenging of fruit and bark extracts. The results were compare with standard ascorbic acid.Both the extracts have good superoxide scavenging activity. Gradual increase in the percentage activity with increase in concentration was found for both the extracts. The higher superoxide scavenging activity was observed for theFruit extract of *Moringa oleifera*was 57.04%, 67.90%, 72.94, 78.29%, 82.23 and 85.10%and scavenging activity with bark extract of *Moringa oleifera*was 30.60%, 47.29%, 51.25%, 61.71%, 68.24% and 74.87% at the concentration of 0.1, 0.5, 1.0, 2.0, 3.0 and 5.0 mg/ml respectively.

Table 4: Superoxide anion scavenging activity of methanolic fruit and bark extracts

Conc. (mg/ml)	Fruit extract (% inhibition)	Bark extract (% inhibition)
0.1	57.04 ± 0.12*	30.60 ± 0.12*
0.5	67.90 ± 0.20*	47.29 ± 0.20*
1.0	72.94 ± 0.18*	51.25 ± 0.19*
2.0	78.29 ± 0.02*	61.71 ± 0.03*
3.0	82.23 ± 0.15*	68.24 ± 0.15*
5.0	85.10 ± 0.22*	74.87 ± 0.21*

All values are expressed in % inhibition.
*Each value is expressed as mean ± standard deviation (n= 3); P < 0.05)

Hydrogen peroxide decomposition assay:

Hydrogen peroxide is a weak oxidizing agent and can activate few enzymes directly, usually by oxidation of thiol (-SH) groups. Hydrogen peroxide can cross cell membrane rapidly, once it enters the cell, H_2O_2 can probably react with Fe^{2+} and probably Cu^{2+} to form hydroxyl radical and this may be the origin of many of its toxic effects.

Table 5: Hydrogen peroxide decomposition assay of fruit and bark extract of *Moringa oleifera* -

Conc. (mg/ml)	Fruit extract % decomposition	Bark extract % decomposition
0.5	15.38 ± 0.002*	10.12 ± 0.006*
1.0	17.96 ± 0.005*	11.95 ± 0.004*
2.0	18.65 ± 0.001*	14.79 ± 0.005*
5.0	22.39 ± 0.006*	20.05 ± 0.001*

*Each value is expressed as mean ± standard deviation (n= 4); *P* < 0.07)
*P<0.06 when compared with ascorbic acid.

The results in table 5 shows the H_2O_2 decomposition assay of fruit and bark extracts. The results were compare with standard ascorbic acid. Both the extracts of Moringa have slight hydrogen peroxide decomposition property. The higher decomposition activity was observed for theFruit extract of *Moringa*

*oleifera*was 15.38%, 17.96%, 18.65% and 22.39% and scavenging activity of bark extract was 10.12%, 11.95%, 14.79% and 20.05% at the concentration of 0.5, 1.0, 2.0 and 5.0 mg/ml respectively.

Hydroxyl radical scavenging activity of plant extract

Hydroxyl radical is one of the ROS that easily reacts with biomolecules, such as amino acids, proteins and DNA. Therefore, removal of hydroxyl radicals can protect humans against some diseases. In this study, hydroxyl radical was generated by 1,10- phenanthroline/ H_2O_2 system to determine the hydroxyl radical scavenging capacity of fruit and bark extracts.

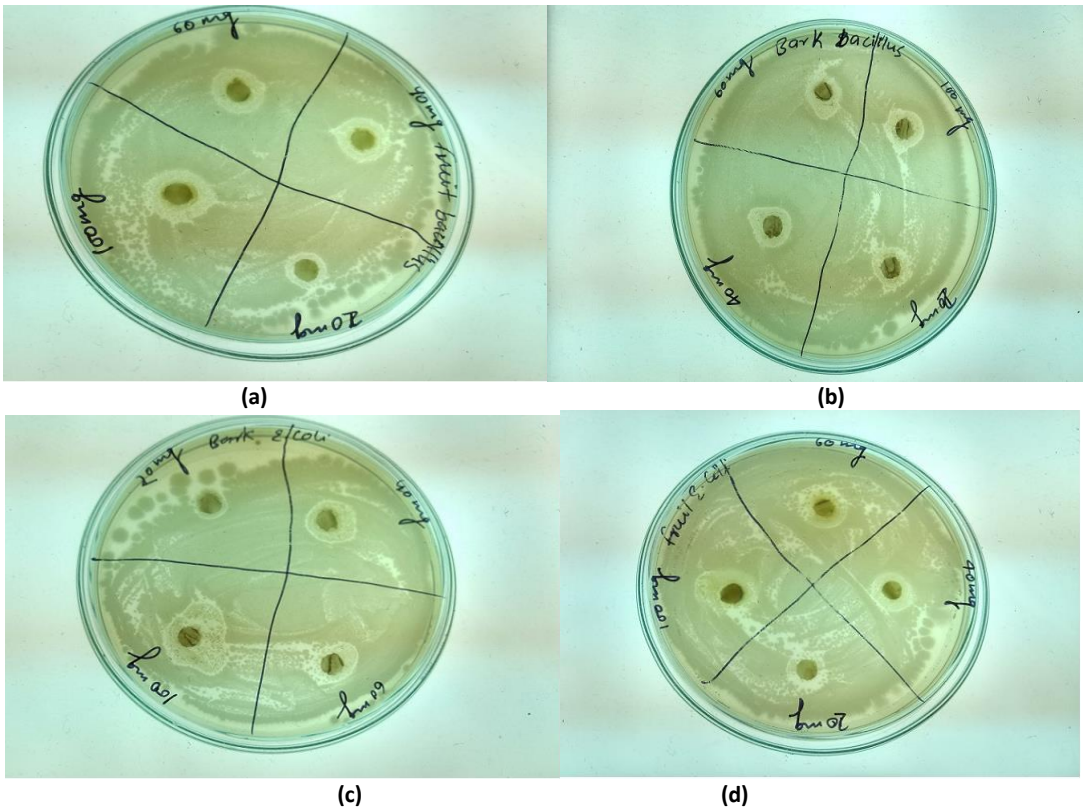
Table 6: Hydroxyl radical scavenging of fruit and bark extract

Concentration (mg/ml)	Fruit extract % inhibition	Bark extract % inhibition
0.5	12.15±0.012*	10.33 ± 0.005*
1.0	32.21±0.021*	27.96±0.011*
2.5	45.59±0.023*	42.55±0.014*
5.0	53.79±0.015*	48.93±0.011*

*Each value is expressed as mean ± standard deviation (n= 3); $P < 0.05$)
*P<0.08 when compared with ascorbic acid.

The results in table 6 shows the hydroxyl radical scavenging of fruit and bark extractsagainst hydroxyl radicals generated in a Fenton reaction system. Both the extracts have hydroxyl scavenging activity. Results were compared with ascorbic acid standard. The higher scavenging activity was observed for theFruit extract of *Moringa oleifera*was 12.15%, 32.21%, 45.59% and 53.79% and hydroxyl radical scavenging with bark extract of *Moringa oleifera* 10.33%, 27.96, 42.55% and 48.93% at the concentration of 0.5, 1.0, 2.5 and 5.0 mg/mlrespectively.

Anti- microbial Assay



(a) **(b)**

(c) **(d)**

Figure 3: In-vitro antibacterial activity of (a) Fruit extract against *Bacillus subtilis*. (b) Bark extract against *Bacillus subtilis*. (c) Bark extract against *E coli*. (d) Fruit extract against *E coli.*

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Natural antimicrobial compounds from plants act as therapeutics that can inhibit the growth of pathogens and have been used to overcome the side effects associated with the synthetic antimicrobial agents.

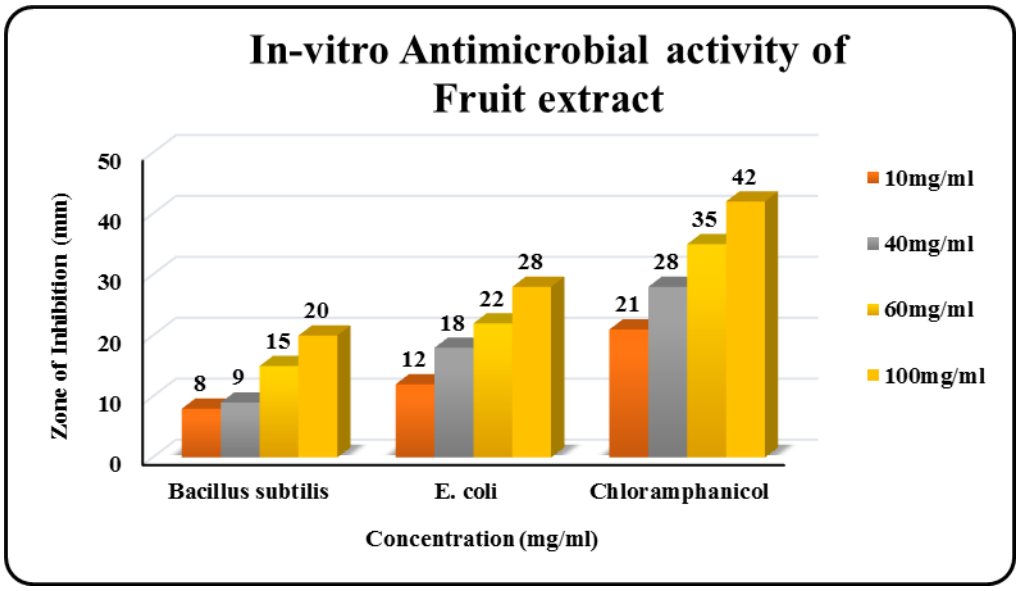

Figure 4: Antimicrobial activity of Fruit extract of *Moringa oleifera*

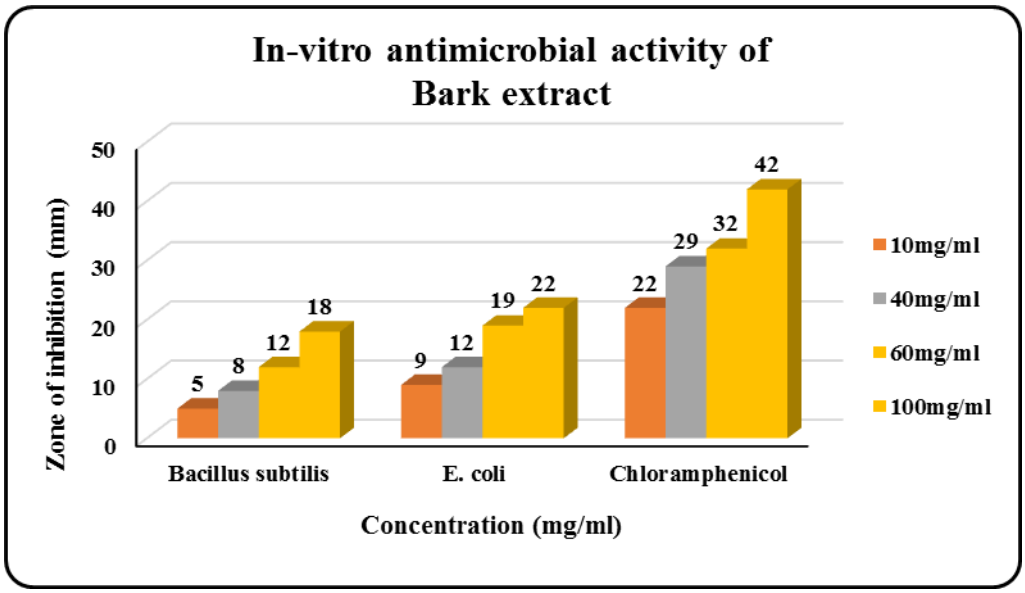

Figure 5: Antimicrobial activity of Bark extract of *Moringa oleifera*

Antimicrobial activity of methanolic extracts was tested using Agar disc diffusion method against Gram positive- *Bacillus subtilis* and Gram negative- *E. coli* bacteria. Present study revealed that fruit and bark extract of *Moringa oleifera* exhibit antibacterial effect on both Gram positive and gram negative bacteria (fig 3).

The results in fig 4 &5 shows the antimicrobial activity of fruit and bark extractsrespectively and results were compare with antibiotic chloramphenicol. Both the extracts have good antimicrobial activity. Antimicrobial activity was higher in fruit extract than bark extract on both the tested microorganisms. Test results shows 12, 18, 22 and 28mm zone of inhibition was observed with fruit extract and 9, 12, 19 and 22mm zone of inhibition was observed with bark extract at the concentration of 10, 40, 60 and 100 mg/ml against *E. coli*. In addition, 8, 9, 15 and 20mm zone of inhibition was observed with fruit extract and 5, 8, 12 and 18mm

zone of inhibition observed with bark extract at the concentration of 10, 40, 60 and 100mg/ml respectively against *Bacillus subtilis*.

CONCLUSION

Herbs have been used for medicinal purposes throughout history. Herbal bioactive, an important category of nutraceuticals, are commonly used by people who seek conventional health care. Therefore, Methanolic extract of both bark and fruit showed significant amount of phenol and flavonoid content. Phenol and flavonoid content was higher in fruit extract than bark extract.Because of this higher antioxidant activity, higher free radical scavenging activity was observed with fruit extract than bark extract. In addition, both extracts showed good antimicrobial activity against *Bacillus subtilis* and *E coli*.

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