In Vitro antioxidant and antibacterial activity of leaf extracts of Measa lanceolata

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ABSTRACT

This study investigated total phenolic contents (TPC), total flavonoid contents (TFC), antioxidant activity, and antibacterial activities of different leaf extracts of Measa lanceolata. The TPC and TFC of the extracts were determined by Folin-Ciocalteu and aluminum chloride methods, respectively. The 2, 2-diphenyl-1-picyrrhodanyl (DPPH) free radical scavenging, reducing power, and phosphomolybdenium assays were used to evaluate antioxidant activity. Antibacterial properties were assessed using the disc-diffusion assay based on minimum inhibitory concentration (MIC). Bacteria tested were three strains of gram-negative (Escherichia coli (ATCC-25922), Proteus vulgaris (ATCC-13315), and Pseudomonas aeruginosa (ATCC-43495)), and two strains of gram-positive (Staphylococcus aureus (ATCC-25923), and Enterococcus faecalis (ATCC-29212)). It was found that methanol extract contained the highest TPC (60.6 ± 4.4 mg of gallic acid equivalent/g of dried extract) and TFC (6.4 ± 0.6 mg of catechin equivalent/g of dried extract). Extract showed the strongest DPPH radical scavenging activity (EC50 = 76.7 ± 7.3 µg/mL), iron reducing power (EC50 = 74.0 ± 1.6 µg/mL), and total antioxidant activity (128 ± 4 mg of ascorbic acid equivalent per dried extract). Inhibition zones were observed in water and methanol extracts against bacterial strains, whereas Staphylococcus aureus (ATCC-25923), Escherichia coli (ATCC-25922), Pseudomonas aeruginosa (ATCC-43495), and Proteus vulgaris (ATCC-13315) were resistant against chloroform and ethyl-acetate extracts.

Introduction

Medicinal plants have been identified and used traditionally throughout the world from the beginning of human civilization. Medicinal plants are rich source of novel drugs that form the ingredients in traditional systems of medicine. More than 50,000 plant species are reported as being used across the globe for medicinal purposes. About 80% of the population in Africa primarily depends on traditional medicinal plants for their health care similarly, plants have been used as a source of traditional medicine since ancient times in Ethiopia to combat different ailments and human sufferings. The current account of medicinal plants of Ethiopia, as documented for national bio-diversity strategy and action plan shows that about 887 plant species were reported to be utilized in the traditional medicine.

Most of the developing countries depend on traditional medicinal plants for their health care. It is therefore, not surprising that some of these plants have chemical compounds of therapeutic value that may be used in the treatment of major diseases such as malaria, cancer and pathogenic microorganisms. As literature showed in Ethiopia more than 80% of the population uses plant-
based traditional medicine as their primary healthcare system and this wide use could be mainly attributed to the fact that it makes use of locally available wild plant resources. This is partly because modern medicinal services are unavailable to the vast majority of the rural people due to their high cost, lack of transport, and few healthcare centers. Interest has also increased in finding natural occurring antioxidants for use medicinal materials to replace synthetic antioxidants. In recent years, antibacterial resistance has become a major public health concern globally. One of the effective approaches could be the discovery and development of new antibacterial agents that have clinical significant importance from natural occurring phytochemicals and discover new antibacterial agents in the pharmaceutical in order to replace currently available antibacterial.

Measa lanceolata locally known as Abayyi, belongs to the family Myrsinaceae, is a well-known plant and widely distributed in many parts of Africa especially in Ethiopia. The leaf is traditionally used for the treatment of helminthes and bacterial infections in most rural areas of the country, since it is rich in biologically active constituents. In addition, the leaf of M. lanceolata is also used traditionally to eradicate the aquatic leeches. Few researchers conducted their research work on antioxidant and phytochemical content of leaf extracts of Measa lanceolata. So this research was conducted to investigate the total phenolic and flavonoid contents, antioxidant and antibacterial activities of various leaf extracts of Measa lanceolata.

Material and methods

Chemicals

Gallic acid, butylated hydroxyl toluene (BHT), Folin-Ciocalteu reagent, 2, 2-diphenyl-1–picryl hydrazyl (DPPH), catechin, ascorbic acid, were purchased from Sigma-Aldrich (St. Louis, USA). Analytical grade of Chloroform, ethyl acetate, methanol, water and ammonia solution, hydrochloric acid, sodium hydroxide, sulfuric acid, ferric chloride, sodium phosphate buffer, potassium ferric cyanide, trichloro acetic acid, ascorbic acid, ammonium molybdate, and muller–hinton agar were used.

Sample collection and preparation

Samples were collected in the month of December 2016 from west Guji zone, Oromia regional state, South Eastern Ethiopia. The sample authentication was carried out and the identification of the plant material based on the morphological criteria was confirmed at the National Herbarium, Addis Ababa University. The leaf was washed under distilled water to remove dust. The plant samples were dried in air for 10 days. Then, it was ground using electrical grander and then sieved (0.25 mm) to obtain uniform particle size. Sample was stored at – 20°C until used for further analysis.

Sample extraction

The chloroform, water, ethyl acetate, and methanol extracts of all were prepared by dissolving 10 g of the sample separately in 100 mL of each solvent. The contents were kept in orbital shaker for 8 hr at 120 rpm at room temperature. Then after, each extract was filtered using whatman no.1 filter paper and evaporated to dryness under vacuum at 40°C by using a rotary evaporator (Buchi, 3000 series, Switzerland). The extraction was done in triplicate for each solvent and the extracts were stored in a sealed plastic container at – 20°C until further investigation.

Total phenolic content (TPC)

The TPC was estimated by Folin-Ciocalteu method of Shan et al. with slight modification using gallic acid as standard. 0.1 mL of the extract (1 mg/mL), 1 mL Folin-Ciocalteu reagent (diluted ten times) was added and the mixture was left for 5 min and then 1 mL of sodium carbonate was added. The
absorbance of the resulting blue color was measured at 765 nm with a UV-visible spectrophotometer (JENWAY, 96500, UK) after incubation for 90 min at room temperature. The total phenolic content was estimated from gallic acid (1–100 µg/mL) calibration curve (y = 0.015 x + 0.081, R² = 0.991, p < .001) and results were expressed as mg gallic acid equivalent/g of dry extract (mgGAE/g).

**Total flavonoid content (TFC)**

The TFC was estimated using the method of Samidha. [16] The extract (1 mL, 1 mg/mL) was diluted with 1.25 mL distilled water and 75 µL 5% NaNO₂ was added to the mixture. After 6 min, 150 µL 10% AlCl₃ was added and then after 5 min, 1 mL 1 M NaOH was added to the reaction mixture. Then, the absorbance (pink in color), was determined at 510 nm versus prepared water blank. A standard curve was prepared using 5–1000 µg/mL of catechin. Results were expressed as milligram of catechin equivalents per milligram of dry extract of the plant extract. All the calculations were done using standard equation (y = 0.002x + 0.35, R² = 0.99, p < .001) obtained from standard calibration curve of catechin.

**Antioxidant activities**

**DPPH scavenging activity:** DPPH radical scavenging activity of the crude extracts was determined as described by Engeda et al. [17] Different concentrations (50 to 1000 µg/mL) of the extracts were taken in different test tubes. Freshly prepared DPPH solution (2 mL) was added in to each of the test tubes containing 1 mL of the extract. The reaction mixture and the reference (BHT) were vortexed and left to stand at room temperature in the dark for 30 min. Absorbance of the resulting solution was taken at 520 nm. Methanol was used as blank. The ability of the scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging = [(Ac-As)/Ac] x100

where Ac is the absorbance of the control and As is the absorbance in presence of the sample of the extracts. The antioxidant activity of each extract was expressed as EC₅₀. The EC₅₀ value is defined as the effective concentration (µg/mL) of extracts that scavenges the DPPH radical by 50%.

**Ferric ion reducing power:** The presence of antioxidants in the extract causes the reduction of the yellow ferric cyanide complex to the ferrous form which can be monitored by measuring the formation of Perl’s Prussian blue at 700 nm. [18] One milliliter of extract solution (final concentration 50–1000 µg/mL) was mixed with 2.5-mL sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferric cyanide. Then, the mixture was incubated at 50°C for 20 min. Trichloro acetic acid (2.5 mL, 10%) was added to the mixture, which was then centrifuged at 3000 rpm (Centurion, 1000 series, UK) for 5 min. Finally, 2.5 mL of the supernatant solution was mixed with 2.5 mL of distilled water and 0.5 mL FeCl₃ (0.1%) and absorbance was measured at 700 nm. The EC₅₀ value (µg/mL) is the effective concentration at which the absorbance is 0.5 for reducing capacity and was calculated from the graph of absorbance at 700 nm against the sample concentration.

**Total antioxidant activity using phosphomolybdenum method:** The method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds or crude extract and subsequent formation of green Mo (V) complexes with a maximal absorption at 695 nm at acidic medium. [19] Plant extract (0.5 and 1 mg/mL) was mixed with 3 mL of reagent solution mixture containing equal amount of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The samples were incubated at 95°C for 90 min, cooled to room temperature and absorbance was measured at 695 nm and methanol (3 mL) was used as blank. The total antioxidant activity was expressed as milligram ascorbic acid equivalent/gram of dried extract (mgAAE/g) based on the calibration curve; (y = 0.0094x + 0.112, R² = 0.99).
**Antibacterial activity**

**Culture and maintenance of bacterial strains:** The antimicrobials activity of each extract was evaluated using five bacterial strains. Two strains were gram positive (Staphylococcus aureus (ATCC-25923), and Enterococcus faecalis (ATCC-29212)) and three of them gram negative (Escherichia coli (ATCC-25922), Proteus vulgaris (ATCC-13315), and Pseudomonas aeruginosa (ATCC- 43495)).

Pure cultures of the test organisms were obtained from Ethiopian Biodiversity institute, Addis Ababa, Ethiopia, October, 2016. Bacteria were maintained on nutrient agar medium. Each bacteria culture was further maintained by subculturing regularly on the same medium and stored 4°C before use.

**Disc diffusion assay:** Antibacterial properties of the extracts were measured using the disc–diffusion method.\(^{[20]}\) Inoculums (100 μL) were spread evenly onto 20 mL Mueller–Hinton agar set in 90 mm Petri dishes using a sterile cotton swab. Sterilized paper disc (6 mm diameter) was impregnated with extract (20 μL) using a micropipette and firmly placed onto the inoculated agar ensuring even distribution to avoid overlapping of zones. Streptomycin susceptibility discs were used as positive controls. In the assay, each inoculums suspension (20 mL) was spread evenly over the entire nutrient agar (Muller–Hinton Agar) surface by sterile collection swab. Then, discs of diameter 6 mm were sterilized at 121°C for 15 min and loaded with prepared positive control (Gentamicin 10 μg/mL) and extract solutions of *Measa lanceolata* at various concentrations. The impregnated discs were dried for 3–5 min and dispensed onto the surface of the inoculated plates with flamed forceps. The plates were then labeled and incubated at 37°C for 24 hr. Diameter (millimeter) of zone of inhibition was measured using digital caliper.

**Minimum inhibitory concentration (MIC):** The minimum inhibitory concentration (MIC) of the extracts was measured by agar dilution method of Nasso et al\(^{[21]}\) with minor modification. Each extract was diluted by twofold. The agar and the plant extracts were mixed thoroughly in sterile container and dispensed to Petri dish labeled with a given concentration of diluted plant extract. The plates were incubated at 37°C for 24 h, after which all plates were observed for growth. The minimum diluted of fraction inhibiting the growth of each organism was taken as the MIC.

**Statistical analysis**

The data were subjected to analysis of variance (ANOVA), Origen 8 software and Duncan’s multiple range tests were used for mean separation at \(p < .05\). Linear regression analysis was used to calculate IC\(_{50}\) value.

**Result and discussions**

**Total phenolic and flavonoid contents**

The total phenolic contents in various solvent extracts from the leaf of *Measa lanceolata* varied widely, ranging from 1.1 to 60.6 mg GAE/g of dried extract (Table 1). It was observed that methanol extract (60.6 ± 4.4 mg GAE/g) showed the highest and chloroform extract (1.1 ± 0.2 mg GAE/g) showed the lowest TPC. The TPC followed the order: methanol > water > ethyl acetate > chloroform extracts.

**Table 1.** Total phenols (TPC) and Total flavonoid (TFC) contents of *Measa lanceolata* extracted with different solvents.

| Extraction solvent | TPC (mg GAE/g) | TFC (mg CE/g) |
|--------------------|----------------|---------------|
| Methanol           | 60.6 ± 4.4\(^{c}\) | 6.4 ± 3.5\(^{b}\) |
| Water              | 52.3 ± 7.1\(^{c}\) | 2.4 ± 4.2\(^{a}\) |
| Ethyl acetate      | 10.0 ± 2.4\(^{b}\) | 4.5 ± 3.4\(^{a}\) |
| Chloroform         | 1.1 ± 0.2\(^{a}\) | 3.1 ± 0.7\(^{b}\) |
There was no significant difference ($p > .05$) in TPC between methanol and aqueous extracts but these values were significantly higher ($p < .05$) than that of ethyl acetate and chloroform extracts ($p < .05$). TPC of methanol extract obtained from this study was lower than that of reported from South Africa.$^{[22]}$ Similarly, the methanol extract was the richest source of TFC ($p < .05$) and decreased in the order of methanol > water > ethyl acetate > chloroform extracts (Table 1). There was no significant difference ($p > .05$) in TPC among water, ethyl acetate, and chloroform extracts but these values were significantly lower ($p < .05$) than that of methanol extract. Similarly, the TFC of the present study was lower than that of sample collected from South Africa.$^{[22]}$ The lower content of phenolic compounds in chloroform extract compared to methanol may be explained by the low solubility of polyphenols in this solvent.$^{[23]}$ These differences in the amount of TPC may be due to varied efficiency of the extracting solvents to dissolve different compounds. Several studies showed that TPC determined differed with polarity of solvent used in extraction.$^{[24–27]}$ Results of the present study showed that among all the solvent extracts of *Measa lanceolata*, methanol extract had the highest TPC and TFC.

Results are expressed as milligram of gallic acid equivalents per gram of dried extract (mg GAE/g) and milligram of catechin equivalents per gram of dried extract (mg CE/g). Values are expressed as mean ± SD ($n = 3$). Different letters in column after the mean indicate significant differences at $p < .05$.

**Antioxidant activity**

**DPPH scavenging activity**: DPPH radical scavenging assay is the most common method used in the study of antioxidant activity of plant extracts. It results in the formation of stable free radical which can be detected by common spectrophotometric technique. Decrease in absorbance shows the more efficient antioxidant activity of the extract in terms of hydrogen atom donating capacity. This assay is more indirect type as it measures the inhibition of reactive species (free radicals) generated in the reaction mixture and its results depend on the type of reactive species used.$^{[28]}$ The concentration of the plant extracts required to scavenge DPPH showed a dose dependent response. Antioxidant activity of all extracts as measured by ability to scavenge (DPPH) free radicals was compared with the standards butylated hydroxyl toluene (BHT). As the result shows that (Figure 1) the concentration of sample increased, the percentage inhibition of DPPH radical also increased. It was observed that methanol of leaf extract had higher activity than water, ethyl acetate, and chloroform extracts of the

![Figure 1. DPPH radical scavenging activity (%) of methanol, ethyl acetate, chloroform and water, extracts from air-dried leaf *Measa lanceolata* and control (BHT). Each value is expressed as mean ± standard deviation ($n = 3$).](image-url)
The EC$_{50}$ values of *Measa lanceolata* extracts were calculated from graph of percentage scavenging activity against concentration of the extracts (Table 2). The EC$_{50}$ was actually used to examine the antioxidant effectiveness of the samples. The lower the EC$_{50}$ value, the higher is the scavenging potential. The IC$_{50}$ values ranged from 76.7 ± 7.3 μg/mL for methanol extract to 282 ± 50 μg/mL for chloroform extract. The strongest scavenging activity (lower IC$_{50}$ value) was recorded for methanol extract which appeared more than three folds stronger than that of chloroform extracts and two times stronger than that of the ethyl acetate extracts and two times stronger than that of the water extracts. The EC$_{50}$ values of petroleum ether, aqueous, chloroform and ethyl acetate extracts were significantly different ($p < .05$), but these values were significantly higher (weaker DPPH scavenging) than ($p < .05$) the DPPH scavenging activity of methanol extract. Whereas the DPPH scavenging activity of methanol extracts was not significantly different from that of BHT. But this value is weaker than the DPPH scavenging activity of *M. Lanceolata* reported from South Africa.$^{[22]}$

**Ferric ion reducing power:** The ferric ion reducing power was used to evaluate the antioxidant properties of the extracts based on their ability to reduce ferricyanide complex to Perl’s Prussian blue colored ferrocyanide complex.$^{[29]}$ In this assay Fe$^{3+}$ – Fe$^{2+}$ transformation in the presence of the extracts was investigated. The reducing power of the extracts may serve as a significant indicator of its potential antioxidant activity.$^{[30]}$ In this assay, the yellow color of the test solution changes to various shades green and blue colors of ferrous ion, depending on the reducing power of test specimen. The presence of reductones, which have been shown to be an impart antioxidant action by breaking the free radical chain by donating a hydrogen atom. The presence of antioxidants in the sample extracts might cause the reduction of Fe$^{3+}$/Ferric cyanide complex to ferrous form which can be monitored by spectrophotometric ally at 700 nm.$^{[31]}$ The trends of reducing potential of different solvent extracts of *Measa lanceolata* was presented in (Figure 2), the greater the intensity of the blue color, the greater was the absorption; consequently, it has strong antioxidant properties. Similar to DPPH scavenging activity the methanol extract of *Measa lanceolata* in this assay also, showed the strongest iron reducing power activity. At the concentration of 1 mg/mL, the reducing power of *Measa lanceolata* leaf extracts decreased in the order of methanol > water > ethyl acetate and chloroform with values of 1.5 ± 0.7 nm, 1.4 ± 0.5 nm, 0.7 ± 0.3 nm, and 0.6 ± 0.3 nm, respectively. The EC$_{50}$ values of ferric reducing powers of *Measa lanceolata* were shown in Table 2. The EC$_{50}$ values of water, ethyl acetate, and chloroform extracts were found to be significantly higher (weaker DPPH scavenging) ($p < .05$) than the IC$_{50}$ value of ascorbic acid, while that of the methanol extract was found to be similar ($p > .05$) to the IC$_{50}$ value of ascorbic acid.

**Total antioxidant activity using phosphomolybdenum assay:** The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of green phosphate/Mo (V) complex with absorption at 695 nm.$^{[32]}$ The total antioxidant capacity of different solvent extracts of *Measa lanceolata* was also evaluated by the phosphomolybdenum method and expressed as ascorbic acid equivalents (AAE) per gram of dried extract. The total antioxidant activity

| Extract           | DPPH scavenging | Ferric reducing power |
|-------------------|-----------------|-----------------------|
| Methanol          | 76.7 ± 7.3$^{a}$| 74.0 ± 1.6$^{a}$      |
| Water             | 148.6 ± 2.3$^{b}$| 158 ± 22$^{b}$       |
| Ethyl acetate     | 204 ± 12$^{c}$  | 429 ± 8$^{c}$        |
| Chloroform        | 282 ± 50$^{d}$  | 928 ± 89$^{d}$       |
| BHT               | 33.2 ± 6.4$^{a}$| -                     |
| Ascorbic acid     | -               | 64.3 ± 4.2$^{a}$     |
For methanol, ethyl acetate, water, chloroform; at 1 mg/mL were found to be (128 ± 4 mg AAE/g), (99 ± 7 mg AAE/g), (57 ± 7 mg AAE/g), and (40.0 ± 3.2 mg AAE/g) respectively. At 1 mg/mL, the total antioxidant activity of methanol extract was significantly stronger (p < .05) than the total antioxidant activity of water, ethyl acetate and chloroform extracts. Similarly, at 0.5 mg/mL, the total antioxidant activity of methanol extract was the strongest (p < .05) whereas chloroform, and water extracts showed significantly the weakest activity (p < .05). This result is consistent with the strongest total antioxidant effect of methanol extract as determined by the DPPH assay and the weakest total antioxidant activity was found in the water extract. No significant difference (p > .05) was found in the total antioxidant activity of water and chloroform extracts (p > .05). However, these values were significantly lower (p < .05) than the total antioxidant activity of ethyl acetate and methanol extracts.
**Antibacterial activity**

Evaluation of antibacterial activity of these plant extracts was recorded in Tables 3 and 4. All of the examined extracts showed varied inhibitory activity against all strains, of the tested bacteria. (*Staphylococcus aureus* (ATCC-25923) was the most sensitive against water and methanol extracts, but *Escherichia coli* (ATCC-25922), *Pseudomonas aeruginosa* (ATCC-43495) *Staphylococcus aureus* (ATCC-25923), and *Proteus vulgaris* (ATCC-13315) were the most resistance against chloroform and ethyl acetate. At 100 mg/mL, the highest average inhibition zone (12.8 ± 0.4 mm) was recorded in methanol extract against *Proteus vulgaris* (ATCC-13315). Whereas the lowest inhibition zone was recorded (6.0 ± 0.5 mm) in the same extract in *Escherichia coli* (ATCC-25922) strain. At the same concentration, the highest average inhibition zone (10.8 ± 0.1 mm) was recorded for water extract against *Staphylococcus aureus* (ATCC-25923). In general, water and methanol extracts of *Measa lanceolata* showed higher antibacterial activity, whereas ethyl acetate and chloroform extracts showed weaker antibacterial activity. The Methanol and aqueous extracts of the present study

| Extract | Conc. (mg/mL) | S. aure | E. coli | P. aeru. | E. fac. | p. vulg. |
|---------|--------------|---------|---------|----------|---------|---------|
| Water   | 100          | 11.8 ± 1.0 | 6.8 ± 0.3 | 6.7 ± 0.5 | –       | 8.5 ± 0.7 |
|         | 50           | 9.5 ± 0.7  | 6.2 ± 0.3 | 6.1 ± 0.1 | –       | 6.8 ± 1.1 |
|         | 25           | 8.3 ± 0.3  | 6.1 ± 0.1 | 6.1 ± 0.1 | –       | 6.2 ± 0.1 |
|         | 12.5         | 6.5 ± 0.7  | –        | –        | –       | –       |
|         | 6.3          | 6.3 ± 0.2  | –        | –        | –       | –       |
| Methanol| 100          | 10.0 ± 1.4 | 8.5 ± 0.7 | –        | 10.5 ± 0.7 | 12.8 ± 0.4 |
|         | 50           | 8.1 ± 0.2  | 7.8 ± 0.4 | –        | 7.8 ± 0.4 | 10.2 ± 1.2 |
|         | 25           | 7.3 ± 0.4  | 7.1 ± 0.4 | –        | 6.8 ± 0.4 | 7.8 ± 0.2 |
|         | 12.5         | 6.3 ± 0.4  | 6.2 ± 0.3 | –        | 6.1 ± 0.1 | 6.3 ± 0.1 |
|         | 6.3          | 6.1 ± 0.1  | 6.0 ± 0.5 | –        | –       | –       |
| Chloroform | 100        | –      | –        | –        | 11.5 ± 1.4 | –       |
|         | 50           | –      | –        | –        | 11.3 ± 1.8 | –       |
|         | 25           | –      | –        | –        | 10.3 ± 1.0 | –       |
|         | 12.5         | –      | –        | –        | 8.5 ± 0.7 | –       |
|         | 6.3          | –      | –        | –        | 6.4 ± 0.2 | –       |
| Ethyl acetate | 100        | –      | –        | –        | 9.0 ± 1.4 | –       |
|         | 50           | –      | –        | –        | 7.5 ± 0.7 | –       |
|         | 25           | –      | –        | –        | 6.3 ± 0.4 | –       |
|         | 12.5         | –      | –        | –        | 6.3 ± 0.4 | –       |
|         | 6.3          | –      | –        | –        | –       | –       |
| Streptomycin | 100        | –      | –        | –        | 27.0 ± 1.4 | 14.0 ± 1.4 |
|           | –            | –      | –        | –        | 17.0 ± 0.7 | 21 ± 3 |

**Table 3.** Antibacterial activity of *Measa lanceolata* leave extracts at various concentrations.

Negative (-ve), shows that the solvents used could not inhibit the growth of the selected organisms

**Note:** S. aure, E. coli, P. aeru, E. fac., and p. vulg. are *Staphylococcus aureus* (ATCC-25923), *Escherichia coli* (ATCC-25922), *Pseudomonas aeruginosa* (ATCC-43495), *Enterococcus faecalis* (ATCC-29212) and *Proteus vulgaris* (ATCC-13315) respectively.

| Extract  | S. aure | E. coli | P. aeru | E. fac. | p. vulg. |
|----------|---------|---------|---------|---------|---------|
| Water    | 3.1     | 12.5    | 12.5    | –       | 12.5    |
| Methanol | 3.1     | 3.1     | –       | 6.3     | 6.3     |
| Chloroform | –      | –       | –       | 3.1     | –       |
| Ethyl acetate | –      | –       | –       | 12.5    | –       |

**Table 4.** Minimum inhibitory concentrations (MIC) of *Measa lanceolata* leaf extracts.

**Note:** S. aure, E. coli, P. aeru, E. fac., and p. vulg. are *Staphylococcus aureus* (ATCC-25923), *Escherichia coli* (ATCC-25922), *Pseudomonas aeruginosa* (ATCC-43495), *Enterococcus faecalis* (ATCC-29212) and *Proteus vulgaris* (ATCC-13315) respectively.
showed higher inhibition zone than that of *Measa lanceolata* studied by Berhan et al[^33] and Chemweno et al.,[^34] but showed lower inhibition zones than *Measa lanceolata* 80% methanol and acetone leaf extracts.[^35,36]

The minimum inhibitory concentration values of the *Measa lanceolata* leaf extract were summarized in Table 4. The MIC values for tested bacterial strains were in the range of 3.1 mg/mL and 12.5 mg/mL. The methanol and water extracts showed the strongest activity against *Staphylococcus aureus* (ATCC-25923) and *Escherichia coli* (ATCC-25922) with values of MIC 3.1 mg/mL. Furthermore, *Enterococcus faecalis* (ATCC-29212) appeared the most sensitive microorganisms tested for chloroform extract with MIC value of 3.1 mg/mL. But, (*Staphylococcus aureus* (ATCC-25923), *Escherichia coli* (ATCC-25922), *Pseudomonas aeruginosa* (ATCC-43495), and *Proteus vulgaris* (ATCC-13315) were resistant against chloroform and ethyl acetate extracts. Hence, these sensitivity differences between Gram-positive and Gram-negative bacteria to the extract of different extracts might be due to the structural and compositional differences in membranes between the two groups.[^37] Indeed, Gram-negative bacteria are more resistant to antibiotics because they possess impermeable outer membrane; consequently, the levels of antibiotics in the cell are reduced.[^38,39]

**Conclusion**

The results obtained showed the in-vitro studies of methanol extract of *Measa lanceolata* showed a stronger antioxidant activity, which is directly related to the total phenolic and flavonoid contents. Methanol and water extracts showed strongest antimicrobial activity as compared to the chloroform and ethyl acetate extracts. While *Staphylococcus aureus* (ATCC-25923), *Escherichia coli* (ATCC-25922), *Pseudomonas aeruginosa* (ATCC-43495), and *Proteus vulgaris* (ATCC-13315) showed resistant against chloroform and ethyl acetate extracts. Generally, methanol, water, ethyl acetate, and chloroform extracts exhibited the highest antibacterial activity against the gram-positive bacterial strains. The study revealed that *Measa lanceolata* contain considerable amount of phenolic compounds and has significant antioxidant and antimicrobial activities, which can be used as easily accessible source of natural antioxidants and in pharmaceutical applications. Further studies are necessary on the isolation and characterization of individual compounds to elucidate their different antioxidant and antimicrobial mechanisms. The isolated compounds need to be evaluated in scientific manner using scientific animal models and clinical mechanisms of action in search of bioactive molecules.

**Acknowledgments**

The authors would like to acknowledge the financial assistance provided by Office of Vice President for Research and Technology Transfer of Dilla University, Ethiopia and also School of nutrition, Food Science and Technology, College of Agriculture, Hawassa University, Ethiopia for the use of laboratory facilities. The authors declare no conflicts of interest regarding the publication of this paper.

**Funding**

This work was supported by the Office of the Vice President for Research and Technology Transfer [010/16].

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