Phytochemical screening, \textit{in vitro} antioxidant and antibacterial activities of essential oil from \textit{Myrtus communis} L.

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Received on: 15-04-2017; Revised on: 29-05-2017; Accepted on: 20-06-2017

abstract

Introduction: \textit{Myrtus communis} L. (family - Myrtaceae) is an evergreen aromatic medicinal plant which has been used since ancient times for medicinal, food and spice purposes. The main purpose of this study was to determine the phytochemical analysis, antioxidant activity and antibacterial activity of the essential oil of \textit{Myrtus communis} leaves. Methods: The present study was to assess the qualitative phytochemical analysis (Alkaloids, phenols, flavonoids, saponins, tannins, steroids, terpenoids, amino acids, reducing sugars, anthraquinones, and volatile oils), antioxidant potential of \textit{Myrtus communis} by 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) and antibacterial activities by disc diffusion method. Results: The antioxidant activity of the essential oil was studied by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method and its activity was compared with ascorbic acid as a standard antioxidant. The essential oil and standard showed their maximum activity (88.33 \% inhibition) and 98.33 \% inhibition at 200 \mu g/mL respectively. Moreover, the antibacterial activity of the essential oil was also evaluated using disc diffusion method against \textit{S. aureus}, \textit{E. coli} and \textit{S. typhi}. showed high activity against \textit{E. coli}. Conclusion: The essential oil of \textit{Myrtus communis} leaves is a potential source of natural antioxidants and antibacterial compounds which are used for the treatment of various diseases caused by free radicals and microbes. Thus, it is a promising candidate for antioxidant and antibacterial agents.

Key Words: \textit{Myrtus communis} L.; Phytochemical analysis; antioxidant activity; DPPH; Antibacterial; \textit{S. aureus}; \textit{E. coli}; \textit{S. typhi}.

1. Introduction:

Medicinal plants are the backbone of traditional medicines and variety of bioactive substances present in medicinal plants are widely used against various diseases. About 80\% of the population in various developing countries depends on traditional medicine for human alleviation due to its fewer side effects. It is the property of most of the plant-based drugs to be simple, effective and offering a broad spectrum of activity with greater emphasis on preventive action. In addition to that large numbers of secondary metabolites are also produced by some of the higher plants. The demand for natural food constituents has resulted in broad research on naturally occurring antioxidants which are able to deactivate highly reactive free radicals. As a base for further pharmacological studies, there is a need to screen medicinal plants for their secondary metabolites and bioactive compounds. In the last century, roughly 121 pharmaceutical products were formulated based on the traditional knowledge obtained from various sources.[1].

Myrtle (\textit{Myrtus communis} L.) is an evergreen shrub aromatic plant belonging to the Myrtaceae family. It grows spontaneously throughout the Mediterranean area and has been used for medicinal, foodstuff and spice purposes since ancient times. The fruit and leaves are traditionally used as disinfectant, antiseptic and hypoglycemic agents.[2]. In folk medicine the fruit of the plant is used in the treatment of various transmittable diseases such as diarrhea and dysentery, while the leaves are used as antibacterial and anti-inflammatory agents, as a mouthwash, for treatments of candidiasis, for healing wounds, as well as in the therapy of urinary diseases.[3,4]. The leaves contain tannins, flavonoids such as quercitin, catechin and myricetin derivatives and volatile oils.[5,6]. Essential oils are gaining remarkable interest for their potential multipurpose use as antioxidant, antibacterial, and antiseptic agent; the essential oil obtained from the leaves was used in the past for the treatment of lung disorders.[7-10]. Therefore the present study is aimed to analyze the presence of phytochemical, \textit{in vitro} antioxidant and antibacterial activity of the essential oil of \textit{Myrtus communis} L. leaves.
2. MATERIALS AND METHODS

2.1. Collection of plant material
Fresh leaves were collected from healthy and uninfected Myrtus communis tree from Chelekot, which is about 16 km southwest Mekelle town in January 2013. The leaves were washed under running tap water followed by using distilled water to eliminate dust and other foreign particles and to cleanse the leaves thoroughly and it was dried under shade. The plant material was authenticated and specimen herbarium was deposited at Addis Ababa University, Biology department, National herbarium of Ethiopia and it is given the voucher specimen number of 81858.

2.2. Phytochemical analysis
Crude methanolic extract of Myrtus communis leaves was prepared by applying maceration extraction technique for preliminary phytochemical analysis. The crude extract and essential oil of Myrtus communis leaves were qualitatively analyzed for the presence of alkaloids, phenols, flavonoids, glycosides, saponins, tannins, steroids, terpenoids, amino acids, anthraquinones, volatile oils and reducing sugars according to the methods of Trease and Evans[11], Harborne[12].

2.2.1. Test for alkaloids
0.05g of the crude extract was dissolved in 5 % hydrochloric acid and it was filtered using filter paper. Then iodine and Wagner’s tests were used to test the presence of alkaloids.

**Iodine Test:** 1 mL test solution was taken and 5 drops of 5 % (w/v) iodine solution were added. There was no formation of blue coloured solution, which indicates the absence of alkaloids

**Wagner’s test:** 2 mL of the filtrate was treated with Wagner’s reagent (Iodine in Potassium Iodide). There was no formation of reddish brown precipitate which indicates the absence of alkaloids.

2.2.2. Test for phenols
**Ferric Chloride Test:** 0.05g of the extract was treated with 4 drops of 1 % (w/v) ferric chloride solution. Formation of bluish black colour was observed that indicates the presence of phenols.

2.2.3. Test for flavonoids
**Alkaline reagent test:** 0.025g the crude extract was treated with few drops of 10 % (w/v) sodium hydroxide solution. Intense yellow colour was formed and it became colourless on addition of dilute hydrochloric acid, which indicates the presence of flavonoids.

**Shinoda test:** To 3 mL extract, 4 fragments of magnesium metal were added in a test tube, followed by dropwise addition of concentrated HCl. Formation of magenta colour indicated the presence of flavonoids.

2.2.4. Test for glycosides
**Alkaline reagent test:** To small amount of the extract, 1 mL water was added and it was shaken well. Then aqueous solution of NaOH was added to it. Formation of yellow colour was observed which shows the presence of glycosides.

2.2.5. Test for saponins
**Froth Test:** 0.05 g of the extract was taken, and then it was diluted with distilled water to 20 mL and it was shaken this in a graduated cylinder for 15 minutes. There was no formation of 1 cm layer of foam that indicates the absence of saponins.

2.2.6. Test for tannins
**Ferric chloride test:** Small amount of the extract was mixed with distilled water and it was heated on water bath and it was filtered. Few drops of 0.1% ferric chloride solution were added to it and a blue-black coloration was occurred which shows the presence of tannins.

2.2.7. Test for steroids
**Liebermann Burchard’s test:** 2 mL of acetic anhydride was added to a mixture 0.5 g of the sample with 2mL of 5 % (v/v) H₂SO₄ solution. Colour change from violet to green was observed which shows the presence of steroids.

2.2.8. Test for terpenoids
0.2g of the crude extract was mixed with 2mL of chloroform and 3 mL of concentrated sulphuric acid added slowly. A reddish brown coloration at the interface was formed which indicates the presence of terpenoids.

2.2.9. Test for amino acids
**Xanthoproteic Test:** 0.05g of the extract was taken and it was treated with 5 drops of concentrated Nitric acid. Yellow colour was formed. Thus, amino acids are present in the extracts.

2.2.10. Test for reducing sugars
**Fehling’s test:** To 0.05g of the crude extract, 1 mL of water and 8 drops of Fehling’s solution were added and it was heated over water bath. Brick red precipitate was formed that indicates the presence of reducing sugars.

2.2.11. Test for anthraquinones
0.025 g of the extract was taken and 7 drops 10% (v/v) HCl solution was added. The mixture was boiled on water bath for few minutes and it was filtered and allowed to cool. Equal volume of CHCl₃ and 7...
drops of 10% (v/v) ammonia solution were added to the filtrate and the mixture was heated over water bath. No rose pink color was formed which shows the absence of anthraquinones.

2.2.12. Test for volatile oils
2 mL of extract was shaken with 0.1 mL of 10 % (w/v) NaOH solution and a small quantity of dilute HCl. A white precipitate was formed. Formation of the white precipitate indicates the presence of volatile oils.

2.3. Determination of the antioxidant activity of the essential oil of Myrtus communis leaves using DPPH assay
The antioxidant activity of the essential oil was determined using 1, 1-diphenyl-2-picrylhydrazyl (DPPH)\[13\]. Different amounts (25, 50, 75, 100, 150 and 200 µg/mL) of the essential oil in methanol solution were prepared. 1 mL from each of the prepared essential oil solutions was taken in six test tubes and 1mL of 90µM of DPPH in methanol solution was added to each of the essential oil solutions. The mixtures were incubated at room temperature for 70 minutes. The UV-Visible spectrophotometer was made zero using methanol and the absorbance of DPPH in methanol was measured at 517 nm wave length until its absorbance remains constant. After 70 minutes the absorbance of each sample was measured at 517nm using UV-Visible spectrophotometer. Ascorbic acid was used as a reference standard at concentrations ranging from 25 - 200 µg/mL. All measurements were performed in triplicates. The percentage of inhibition is calculated using the following formula:

\[
\text{Inhibition (\%)} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

Where; A is absorbance, \(A_{\text{control}}\) is absorbance of DPPH solution in methanol and \(A_{\text{sample}}\) is absorbance of sample in the presence of DPPH.

2.4. Statistical analysis
The data for antioxidant and antibacterial activity were expressed as average of three measurements and all the remaining data were expressed as mean ± standard deviations of triplicates.

3. RESULTS AND DISCUSSION

3.1. Phytochemical analysis
The difference in the phytochemical constituents (Table 1) of the extracts could be due to the difference in the extraction techniques. The crude methanolic extract of Myrtus communis leaves contain phenolic compounds such as phenols, flavonoids and tannins. Thus, this extract could be potential sources of antioxidants and antibacterial compounds because phenolic compounds are known for their different pharmaceutical activities such as antioxidant and antibacterial activity. Moreover, the leaves of the plant are a good source of essential oils and these essential oils are used in different...
application such as perfumery, food additives, cosmetics and in chemotherapy to treat different diseases like bronchitis, cough, tuberculosis, astringent, acne and related skin infections[14].

Table: 1 Phytochemical analysis of the crude extracts and hydrodistilled essential oil of Myrtus communis leaves

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Phytochemicals</th>
<th>Method of testing</th>
<th>MeOH extract</th>
<th>Hydrodistilled essential oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Iodine test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Phenols</td>
<td>Ferrie chloride test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Flavonoids</td>
<td>NaOH test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Glycosides</td>
<td>Alkaline reagent test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>Forth test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Tannins</td>
<td>Ferrie chloride test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Steroids</td>
<td>Liebermann-Burchard’s test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Terpenoids</td>
<td>Liebermann-Burchard’s test</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Amino acids</td>
<td>Xanthoproteic test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Reducing sugars</td>
<td>Fehling’s test</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>Volatile oils</td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12</td>
<td>Anthraquinones</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: #: means present and -: means not present/absent/3.

3.2. Antioxidant activity of the essential oil of Myrtus communis leaves by DPPH free radical scavenging assay

The antioxidant (free radical scavenging) activity of the hydrodistilled essential oil of Myrtus communis leaves has been studied by its ability to reduce 1,1-diphenyl-2-picrylhydrazyl (DPPH); a stable free radical and the results are shown in table 2.

Table 2. Percent Inhibition of the hydrodistilled essential oil and ascorbic acid on DPPH at different concentrations

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (µg/mL)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential oil</td>
<td>25</td>
<td>61.90</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>66.67</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>67.38</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>73.81</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>78.09</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>88.33</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>25</td>
<td>91.90</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>94.40</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>94.64</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>95.48</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>96.78</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>98.33</td>
</tr>
</tbody>
</table>

DPPH radical scavenging ability is widely used to evaluate the antioxidant activity of medicinal plants in a relatively short time. Any molecule that can donate an electron or hydrogen to DPPH can react with it and thereby bleach the DPPH absorption. DPPH is a purple colour dye having absorption maxima at 517 nm and upon reaction with a hydrogen donor (antioxidant) the purple colour fades or disappears due to conversion of it to stable 1,1-diphenyl-2-picrylhydrazine (DPPH-H) resulting in decrease in absorbance[15]. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability. The results from table 5 indicate that the radical scavenging activity (% inhibition) of the essential oil from Myrtus communis L.leaves showed maximum activity of 88.33 % inhibition at the concentration of 200µg/mL. It was noticed that the scavenging activities of the essential oils were increased with the increased of the essential oils concentrations. And the standard, ascorbic acid showed its maximum activity of 98.33 % inhibition at the same concentration. In this study, the antioxidant activities of essential oils of Myrtus communis L. leaves was compared with ascorbic acid (vitamin C) as a reference standard antioxidant compound and the results are summarized in figure 1. It was found that the essential oils of Myrtus communis L. leaves analyzed showed good antioxidant capacities but lower than vitamin C.

![Figure 1. Comparative DPPH radical scavenging activity of the essential oil and ascorbic acid.](image)

The radical scavenging activity was found to increase with increasing concentrations in both the essential oil and the reference standard. Antioxidant activities of essential oils from aromatic plants are mainly attributed to the active compounds present in them. This can be due to the high percentage of main constituents, but also to the presence of other constituents in small quantities or to synergy among them. Thus, antioxidant activity of the essential oil could be due to the presence of 1,8-cineole, linalool, α-terpineol and methyl eugenol because these compounds can donate proton to DPPH.

Myrtus communis L. essential oils were able to reduce the stable, purplescolored radical DPPH into yellow colored (DPPH-H). This could be due to the chemical composition of the essential oil, as the essential oil contained mainly phenolic species (oxygenated monoterpenic hydrocarbons) such as 1,8-cineole, linalool, α-terpineol, and methyl eugenol.
Previous published reports on the antioxidant activity of the essential oil of the plant leaves showed the essential oil of the leaves of this plant have significant antioxidant potential. GhadirZayZafoonet et al.[19] reported the essential oil of Myrtus communis leaves are promising source of natural antioxidants properties as determined by using Photochemiluminescence (PCL) assay this was due to the presence of phenolic species in the essential oil extracts such as cineole, α-pinene, linalool, limonene, nerol, bornyl acetate, geranyl acetate, eugenol and farnesyl alcohol. Moreover, Neda Mimica Dukic et al.[17] also reported the essential oil of the leaves of the plant have good antioxidant activity. It was shown 1,8-cineole and methyl eugenol are the compounds most responsible for the radical scavenging activity of the entire oil. Thus, the present study is in agreement with these studies.

### 3.3. Antibacterial activity of the essential oil of Myrtus communis leaves

The results for the in vitro antibacterial activity of the essential oil of Myrtus communis leaves are shown in table 3. The antibacterial activity of the essential oil was tested against one Gram positive bacterial strain; Staphylococcus aureus, and two Gram negative bacterial strains; Escherichia coli and Salmonella typhi using disc diffusion method and its antibacterial activity was compared with the antibiotic drugs; amoxicillin and penicillin and the activity was measured in terms of diameter of zone of inhibition (in millimetres).

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Zone of inhibition (in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Essential oil</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>8.47</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>16</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>6.3</td>
</tr>
</tbody>
</table>

Key: - means no effect, size of disc 5mm, all the results are mean values (n = 3)

The results showed that the hydrodistilled essential oil (neat) applied with the same concentration have variable antibacterial effect against the tested microorganisms. As shown in table 3, the essential oil inhibited the growth of all the tested bacterial strains and the antibiotic drugs; amoxicillin inhibited the growth all the examined microorganisms and penicillin inhibited the growth of Staphylococcus aureus and Escherichia coli but it was not inhibited the growth of Salmonella typhi. Moreover, as can be seen from the table above, the essential oil inhibited more the growth of Escherichia coli. The results also revealed the antibacterial activity of the essential oil against Staphylococcus aureus was more than the standard antibiotic agents, amoxicillin and penicillin. Moreover, the antibacterial effect of the essential oil against Escherichia coli was more than penicillin and nearly equal to amoxicillin. Thus, according the results of this study the essential oil of the leaves of this plant have a potent antibacterial activity against all the tested microorganisms and the strongest antibacterial activity was observed for Escherichia coli. The antibacterial activity of the essential oil could be due to the large amount eucalyptol (33.795 %), linalool (29.217 %), linalyl anthranilate (9.048 %) and α-terpene (7.158 %) or due to synergetic effect.

The results of this study was compared with the previous published research papers and the published reports showed that the essential oil of Myrtus communis leaves have strong antibacterial activity. Rasibool et al.[18] reported the essential oil of the plant leaves extract have a potential antibacterial activity against E. coli, K. pneumoniae, B. subtilis, B. licheniforms, C. albicans and S. cerevisiae. Yadegarina et al.[19] have demonstrated the activity of Myrtus communis L. essential oil against E. coli, S. aureus and Candida albicans. Salvagniniet al.[20] also reported that the essential oil of Myrtus communis leaves extract showed significant antimicrobial activity against S. aureus, S. epidermidis, E. coli, B. subtilis and Serratia marcescens. Akin et al.[21] reported the essential oil of the leaves extract of the plant showed significant antibacterial activity against S. aureus, E. coli and pS. Aeruginosa. Furthermore, Marry et al.[22] reported the essential oil of leaves of the plant have potent antibacterial activity against the food pathogenic microorganisms E. coli, S. typhimurium and Aspergillus niger.

The results of the present study are in line with the previous literatures published on the antibacterial activity of the essential oil of Myrtus communis leaves extract. But it is difficult to compare the data of with the literatures because several variables influence the results such as chemical composition due to environmental factors (such as geographical location, temperature, day length, nutrients, age of the plant parts used, etc) of the plant. Since the biological activity of the plant extract depends on its chemical composition and amount of chemical constituents of the bioactive components.

### 4. CONCLUSION

The essential oil of Myrtus communis leaves can be a promising candidate as potential alternatives for synthetic antioxidants and bactericides for use in food industry, cosmetic industry and perfume industry along with their possible application in pharmaceutical industry for prevention and treatment of pathogenesis caused by microbes and free radicals.

ACKNOWLEDGEMENT

The authors sincerely thank to the Department of Chemistry, Mekelle University, Mekelle, Ethiopia for providing laboratory facilities,
Medicinal plant was authenticated by Department of Biology, National herbarium of Ethiopia, Addis Ababa University, Department of Chemistry, Aksum University, Axum, Ethiopia and Ministry of Education, Ethiopia for providing constant support.

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Source of support: Nil ; Conflict of interest: None Declared