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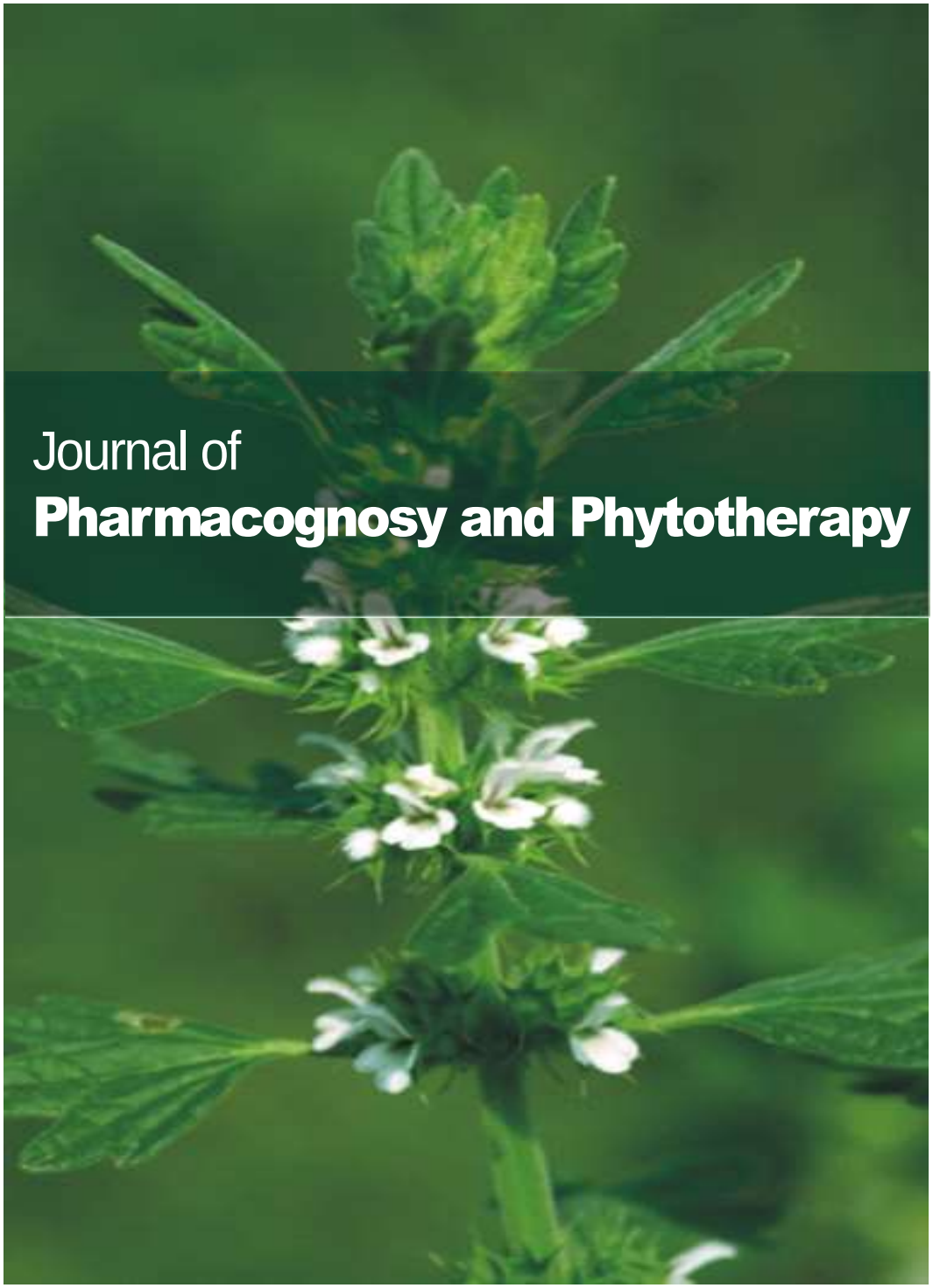

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*Full Length Research Paper*

# Antimicrobial activities from extracts of seven medicinal plant species against multidrug-resistant bacteria and fungi

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**This is the first to report the antimicrobial effects of extracts of seven endemic medicinal plants. Flower extracts of *Onopordum jordanicolum* exhibited antibacterial activity against *Staphylococcus aureus*, MRSA, *Klebsiella pneumonia*, and *Proteus mirabillis*. Ethanol extract displayed significant antibacterial activity against *S. aureus* with the best MIC and MMC values. *Onopordum blancheanum* flower extracts produced antibacterial activity against *S. aureus*, *Escherichia coli*, *K. pneumonia*, and *P. mirabillis*. Ethanol extract of *Aethionema carneum* leaves exhibited antibacterial activity against all test bacteria except MRSA and produced significant antibacterial activity against *E. coli* with the best MIC and MMC values. Methanol and acetone extracts of *Delphinium ithaburense* leaves showed significant antibacterial activity against *K. pneumonia*. Aqueous extract of *Lathyrus hirticarpus* leaves revealed a broad spectrum of antibacterial activity. Aqueous and acetone extracts of *Orchis sancta* flowers showed significant antibacterial activity against MRSA and *Pseudomonas aeruginosa*, respectively. Aqueous and methanol extracts from *Papaver umbonatum* flowers exhibited significant antibacterial activity against *Streptococcus pyogenes* with the best MIC and MMC values. For antifungal activity, it was found that *Aspergillus brasiliensis* and *Candida albicans* were inhibited by aqueous extracts of *A. carneum* and *P. umbonatum*, acetone extract of *D. ithaburense*, ethanol extract of *L. hirticarpus*, methanol extracts of *O. blancheanum* and *O. sancta*. Interestingly, acetone extract of *O. jordanicolum* displayed significant antifungal activities against *A. brasiliensis* and *C. albicans* with the best MIC and MMC values. Phytochemical screening of promising extracts revealed the presence of alkaloids, flavonoids, saponins, and/or tannins which might be responsible for their antimicrobial activity.**

**Key words:** Antimicrobial, phytochemical, *carneum*, *ithaburense*, *hirticarpus*, *Onopordum*, *sancta*, *umbonatum*.

## INTRODUCTION

Traditional medicines that are based on medicinal plants still play an essential role in health care. Medicinal plants are an important source of drugs and a huge number of bioactive medicines against different diseases are developed from plants origin (Sahoo et al., 2010). Secondary metabolites derived from medicinal plants

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**Table 1.** Selected plant species for this study and their uses in folk medicine.

| Scientific name | Common name | Plant family | Uses in folk medicine |
|---|---|---|---|
| *Aethionema carneum* | Ferrite curled | Brassicaceae | Treatment of meningitis |
| *Delphinium ithaburense* | Tabor | Renonculaceae | Treatment of intestinal worms, fluid retention, poor appetite, and insomnia |
| *Lathyrus hirticarpus* | Indian Vetch | Fabaceae | Not determined, but used in food, feed, fodder, and ornamental purposes |
| *Onopordum blancheanum* | Cotton Thistle | Asteraceae | Treatment of hypertension and colon problems, and as food source |
| *Onopordum jordanicolum* | Cotton Thistle | Asteraceae | Treatment of kidney problems |
| *Orchis sancta* | Holy Orchid | Orchidaceae | Treatment of sore throat, digestive problems, diarrhea, and gum disease |
| *Papaver umbonatum* | Corn Poppy | Papaveraceae | Treatment of earache, toothache, neuralgia, coughs, insomnia, and poor digestion |

hold great promise in the treatment and prevention of different types of diseases caused by several pathogenic microorganisms. Recently, more than one hundred secondary plant metabolites derived from plants are used worldwide as drugs to treat different diseases (Jagatheeswari et al., 2013).

Increasing antibiotic resistance by pathogenic bacteria is developing at an alarming rate, as the rate of discovery for new antibiotics has been on the decline (Hopwood, 2007). Therefore, medicinal plants shift the attention of scientists and researchers to herbal medicine to find effective antimicrobial agents for treatment of diseases caused by multidrug resistant pathogens like bacteria and fungi (Parungao et al., 2007).

Although natural products derived from medicinal plants are used for thousands of years to treat different diseases, most medicinal plants around the world are not yet explored for their medicinal activities (Hassan, 2012). Therefore, seven endemic medicinal plants in Jordan (*Aethionema carneum*, *Delphinium ithaburense*, *Lathyrus hirticarpus*, *Onopordum blancheanum*, *Onopordum jordanicolum*, *Orchis sancta*, and *Papaver umbonatum*) were selected in the present study and evaluated for their antimicrobial activities against human pathogenic bacteria and fungi (Table 1). Few phytochemical investigations about *A*. *carneum* have been described in the literature. This annual plant has a unique character, which produce glucosinolate (mustard oil) compounds (Adigizel, 2000).

Nevertheless, the significant antimicrobial activity of this plant is not known. The plant *D*. *ithaburense* has been utilized for the treatment of various problems (Table 1). Many species of *Delphinium* have numerous toxic alkaloids (Gardner and Pfister, 2007). However, few phytochemical investigations about *D*. *ithaburense* have been described in the literatures and the antimicrobial effects were not identified. The medicinal plant *L*. *hirticarpus* is grown in the northern part of Jordan. This plant is commonly called Indian vetch. Seeds of members of the genus *Lathyrus* are toxic if ingested in high quantity (Grela et al., 2000). Until today, there is no antimicrobial study related to this plant. Two species of *Onopordum* from Asteraceae family were investigated in this study, *O*. *blancheanum* and *O*. *jordanicolum*. The *O*. *blancheanum* distributed throughout the Mediterranean areas (Ronel et al., 2009) and it is commonly known as cotton thistle. The *O*. *jordanicolum* is a Jordanian local plant and it is commonly called camel thistle. This plant species is found in Jordan Eastern desert. There are no previous studies that assessed the antimicrobial activity of *O*. *blancheanum* and *O*. *jordanicolum*. The orchid *O*. *sancta* belong to the family Orchidaceae. This family is the largest family of the plant kingdom, comprising more than 30,000 species (Jin-Ming et al., 2003).

In Jordan, there is a unique species of orchids called *O*. *sancta* commonly known as holy orchid and considered as an elegant perennial plant. The existence of alkaloids in orchid and considered as an elegant perennial plant.
The existence of alkaloids in orchid constituents suggests that orchids possess some biological activity against disease and cancer (Bulpitt et al., 2007). Even so, the antimicrobial activity of *O*. *sancta* was not evaluated. The native plant to Jordan, *P*. *umbonatum*, is grown wildly in many parts of the world. Flowers of *P*. *umbonatum* are used for treating various mild pains (Table 1). Recently, it was revealed that active phytochemicals found in *P*. *umbonatum* are antioxidants and contain various phenolic compounds (Bernáth, 2006). Nonetheless, no previous antimicrobial investigations of *P*. *umbonatum* have been described in the literature. Therefore, no

previous studies were performed concerning the use of those medicinal plants selected in this study as sources of antibacterial and antifungal agents. Thus, this study is established to determine the antibacterial and the antifungal activities of those selected medicinal plants.

## MATERIALS AND METHODS

### Plant materials collection

Plant materials and specimens were collected in March, 2014 from different locations of Jordan. The collected plant specimens were identified by a taxonomist specialist, Mr. Refad Khawaldeh, at Jordan Royal Botanical Garden where the voucher specimens are deposited and conserved for future reference; *A*. *carneum* (Voucher specimen no. Azzam 079/2014) was collected from Dana Preservation in Tafila, *D*. *ithaburense* (Voucher specimen no. Obeidat 082/2014) was collected from Princess Tasneem bint Ghazi Technological Research Station in Al-Salt, *L*. *hirticarpus* (Voucher specimen no. Azzam 087/2014) and *O*. *blancheanum* (Voucher specimen no. Azzam 093/2014) were collected from Sharhabil Bin Hassneh EcoPark in Irbid, *O*. *jordanicolum* (Voucher specimen no. Obeidat 081/2014) was collected from Jordan East desert, and *O*. *sancta* (Voucher specimen no. Obeidat 085/2014) and *P*. *umbonatum* (Voucher specimen no. Obeidat 090/2014) were collected from the Royal Botanical Garden in Jarash. Collected plant materials (roots, leaves, fruits, and flowers) from each plant species were washed and dried in the shade in an aerated place at room temperature until complete drying. The dried plant material was ground into a fine powder for initiating the extraction process.

### Preparation of plant extracts

Each powdered plant material was soaked in each of acetone, Ethanol, methanol, and hot water solvents (plant material to solvent ratio was 1:10, w/v) and extracted for two weeks at room temperature with shaking at 150 rpm. All extracts were filtered through White canvas and filter paper. The collected filtrates of the extracts were dried by evaporation until dryness. The dried extracts were weighed and dissolved in 0.05% dimethyl sulphoxide (DMSO) to a final stock concentration of 200 mg/ml. All crude extracts were purified by filtration through 0.22 μm filter units and kept at -20°C until use.

### Test microorganisms

A total of 10 antibiotic-resistant test microorganisms including three Gram positive bacteria (*Streptococcus pyogenes* ATCC 8668, *Staphylococcus aureus* ATCC 25923, and Methicillin resistant *Staphylococcus aureus* ATCC 95047 (MRSA)), five Gram negative bacteria (*Salmonella typhimurium* ATCC 14028, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 27253, *Klebsiella pneumonia* ATCC 7700, and *Proteus mirabillis* ATCC 12453) and two fungi (*Aspergillus brasiliensis* ATCC 16404 and *Candida albicans* ATCC 10231) were used in this study. The antibiotic-resistance patterns for each test microorganism were determined previously by Obeidat (2017).

### Preparation of inoculums

Test bacteria and test fungi were grown in nutrient broth (NB) at 37°C for 24 h and in sabouraund dextrose broth (SDB) at 28°C for 48 h, respectively. Bacterial and fungal cultures were serially diluted and adjusted to achieve $2\times10^6$ colony forming units (CFU/ml) for bacteria and $2\times10^5$ spore/ml for fungi (Ceylan et al., 2008).

### Antimicrobial activity

The antibacterial activity of plant extracts was screened in triplicates against test bacteria by using the agar well diffusion method (Perez et al., 1990). An inoculum of 100 µl bacterial suspension was swapped uniformly to solidified nutrient agar (NA) plates and the plates were allowed to dry for 5 min. Holes of 5 millimeters (mm) in diameter were made in the seeded agar using sterile cork borer. Aliquot of 50 µl from each plant extract was added into each hole on the seeded medium and allowed to stand for one hour for proper diffusion and incubated at 37°C for 24 h. The antibacterial activity was evaluated by measuring the inhibition zone diameter in mm around the wells. The antifungal activity of plant extracts was performed in triplicates against test fungi in the same manner as described for bacteria but by using sabouraund dextrose agar (SDA) plates and incubation at 28°C for 48 h.

### Determination of the minimum inhibitory concentration and minimum microbial concentration

The minimum inhibitory concentration (MIC) and the minimum microbial concentration (MMC) were determined for plant extracts that showed the most significant antimicrobial activity according to the modified methods previously described by Obeidat (2011). All samples were examined in triplicates.

### Phytochemical screening

The phytochemical components of promising extracts (extracts that exhibited broad spectrum antibacterial activity and extracts that showed the most significant antifungal activity) from the selected medicinal plants in this study were performed according to the standard protocols; Mayer's test for alkaloids (Siddiqui and Ali, 1997), sodium hydroxide test for flavonoids (Roopashree et al., 2008), foam test for saponins (Roopashree et al., 2008), and ferric chloride test for tannins (Iyengar, 1995).

### Statistical analysis

All measured inhibition zones were expressed as the mean ± standard error (SE). For statistical evaluation of data for generated inhibition zones, one-way ANOVA (Tukey's studentized range) was applied using the program IBM SPSS statistics 19.0 for Windows. Significant differences were considered significant at $P < 0.05$.

## RESULTS

Antimicrobial activities were estimated by measuring the average diameters of the formed inhibition zones around wells. The inhibitory effects of plant extracts prepared by different solvent (water, Ethanol, methanol, and acetone) from *A*. *carneum*, *D*. *ithaburense*, *L*. *hirticarpus*, *O*. *blancheanum*, *O*. *jordanicolum*, *O*. *sancta*, and *P*. *umbonatum* were investigated against 10 antibiotic-resistant test microorganisms including eight bacteria and two fungi. In general, most plant extracts of selected plants in this study showed a broad spectrum of antimicrobial activity. Although all plant parts of selected

**Table 2.** Antibacterial activity of extracts from selected medicinal plants against pathogenic Gram-positive bacteria.

| Plant species | Plant part | Solvent | Inhibition Zone (mm)* | | |
|---|---|---|---|---|---|
| | | | ***S. pyogenes*** **ATCC 8668** | ***S. aureus*** **ATCC 25923** | **MRSA ATCC 95047** |
| *Aethionema carneum* | Leaf | Water | $0^{a}$ | $0^{a.}$ | $0^{a}$ |
| | | Ethanol | $20.33\pm1.20^{fg}$ | $21.33\pm1.67^{fgh}$ | $0^{a}$ |
| | | Methanol | $18.00\pm1.15^{def}$ | $22.67\pm0.88^{h}$ | $0^{a}$ |
| | | Acetone | $0^{a}$ | $0^{a}$ | $0^{a}$ |
| *Delphinium ithaburense* | Leaf | Water | $13.33\pm0.33^{b}$ | $20.67\pm2.58^{efgh}$ | $18.33\pm0.67^{c}$ |
| | | Ethanol | $0^{a}$ | $0^{a}$ | $0^{a}$ |
| | | Methanol | $0^{a}$ | $0^{a}$ | $0^{a}$ |
| | | Acetone | $0^{a}$ | $22.67\pm1.33^{gh}$ | $0^{a}$ |
| *Lathyrus hirticarpus* | Flower | Water | $17.00\pm0.58^{d}$ | $20.00\pm1.73^{efg}$ | $0^{a}$ |
| | | Ethanol | $15.67\pm0.88^{c}$ | $18.67\pm1.86^{def}$ | $0^{a}$ |
| | | Methanol | $17.33\pm0.33^{d}$ | $19.33\pm1.20^{ef}$ | $0^{a}$ |
| | | Acetone | $0^{a}$ | $21.67\pm1.45^{fgh}$ | $0^{a}$ |
| *Onopordum blancheanum* | Flower | Water | $0^{a}$ | $30.67\pm0.67^{i}$ | $0^{a}$ |
| | | Ethanol | $20.33\pm2.19^{efgh}$ | $29.67\pm1.45^{i}$ | $0^{a}$ |
| | | Methanol | $0^{a}$ | $14.67\pm1.67^{bc}$ | $22.00\pm2.52^{de}$ |
| | | Acetone | $0^{a}$ | $22.33\pm0.67^{gh}$ | $23.67\pm1.86^{e}$ |
| *Onopordum jordanicolum* | Flower | Water | $22.33\pm0.67^{h}$ | $16.00\pm2.52^{cde}$ | $26.33\pm0.33^{f}$ |
| | | Ethanol | $15.00\pm1.15^{c}$ | $13.67\pm2.33^{bc}$ | $20.67\pm2.85^{cde}$ |
| | | Methanol | $15.33\pm1.67^{cd}$ | $30.33\pm0.33^{i}$ | $14.33\pm2.19^{b}$ |
| | | Acetone | $0^{a}$ | $12.67\pm2.67^{bc}$ | $19.67\pm1.45^{cd}$ |
| *Orchis sancta* | Flower | Water | $20.33\pm0.45^{g}$ | $15.67\pm1.67^{cd}$ | $26.33\pm1.67^{f}$ |
| | | Ethanol | $19.33\pm0.33^{ef}$ | $20.00\pm1.00^{f}$ | $0^{a}$ |
| | | Methanol | $0^{a}$ | $0^{a}$ | $0^{a}$ |
| | | Acetone | $0^{a}$ | $0^{a}$ | $0^{a}$ |
| *Papaver umbonatum* | Flower | Water | $27.33\pm0.88^{i}$ | $20.33\pm1.20^{f}$ | $0^{a}$ |
| | | Ethanol | $17.33\pm1.67^{cde}$ | $21.00\pm1.15^{fgh}$ | $0^{a}$ |
| | | Methanol | $30.67\pm2.85^{i}$ | $20.67\pm1.67^{fgh}$ | $0^{a}$ |
| | | Acetone | $0^{a}$ | $13.33\pm0.33^{b}$ | $0^{a}$ |

*Inhibition zone diameters are expressed as Means±SE. The means±SE within column followed by the same letter are not significantly different (Tukey's studentized range test: α = 0.05).

plants were screened for the potential antimicrobial activity, it was found that antimicrobial activity was produced from leaves of *A*. *carneum* and *D*. *ithaburense* from flowers of *L*. *hirticarpus*, *O*. *blancheanum*, *O*. *jordanicolum*, *O*. *sancta*, and *P*. *umbonatum* (Table 2).

It was found that flower extracts of *O*. *jordanicolum* were the best source for antimicrobial agents. All test microorganisms were affected by at least one extract. It was found that all flower extracts of *O*. *jordanicolum* exhibited antibacterial activity against Gram-positive bacteria except acetone extract which did not show antibacterial activity against *S*. *pyogenes* (Table 2). Methanol and water extracts were significantly the most active extracts against *S*. *aureus* and MRSA, respectively (Table 2).

Ethanol extract of *O*. *jordanicolum* exhibited antibacterial activity against Gram-negative bacteria (Table 3). On the other hand, water and ethanol extract of *O*. *blancheanum*, which belongs to the same plant genus, showed a broad spectrum of antimicrobial activity with significant antibacterial activity against *S*. *aureus*.

As shown in Table 2, the aqueous and methanol extracts of *P*. *umbonatum* flowers exhibited significant antibacterial activity against *S*. *pyogenes*. Remarkably, it was found that leaf extracts of *D*. *ithaburense* (methanol and acetone extracts) and *A*. *carneum* (water and ethanol extract) produced significant antibacterial activity against *K*. *pneumonia* (Table 3).

It was observed that *S*. *typhimurium* (Gram-negative bacterium) followed by MRSA (Gram-positive bacterium) and *P*. *aeruginosa* (Gram-negative bacterium) are the least sensitive among all test microorganisms used in this study (Table 2 and 3). The bacterium *S*. *typhimurium* was affected by only three extracts (water and ethanol extract of *A*. *carneum* and ethanol extract of *O*. *jordanicolum*); ethanol extract of *A*. *carneum* gave the most significant antibacterial activity (Table 3), whereas eight extracts inhibited MRSA growth (Table 2).

**Table 3.** Antibacterial activity of extracts from selected medicinal plants against pathogenic Gram-negative bacteria

| Plant species | Plant part | Solvent | Inhibition Zone (mm)* | | | | |
|---|---|---|---|---|---|---|---|
| | | | ***S. typhimurium*** **ATCC 14028** | ***E. coli*** **ATCC 25922** | ***P. aeruginosa*** **ATCC 27253** | ***K. pneumonia*** **ATCC 7700** | ***P. mirabillis*** **ATCC 12453** |
| *Aethionema carneum* | Leaf | Water | 16.67±0.33$^{b}$ | 19.33±0.67$^{e}$ | 29.67±0.88$^{f}$ | 30.67±1.33$^{j}$ | 20.67±0.88$^{d}$ |
| | | Ethanol | 19.33±0.33$^{c}$ | 30.67±2.58$^{h}$ | 13.33±0.67$^{b}$ | 29.33±0.33$^{j}$ | 23.00±1.00$^{ef}$ |
| | | Methanol | 0$^{a}$ | 29.00±1.15$^{h}$ | 0$^{a}$ | 24.33±0.33$^{i}$ | 22.33±0.67$^{ef}$ |
| | | Acetone | 0$^{a}$ | 16.33±2.19$^{cd}$ | 21.00±1.15$^{d}$ | 0$^{a}$ | 25.33±0.67$^{g}$ |
| *Delphinium ithaburense* | Leaf | Water | 0$^{a}$ | 18.67±1.67$^{de}$ | 16.66±0.50$^{c}$ | 13.00±1.00$^{bc}$ | 23.67±2.33$^{efg}$ |
| | | Ethanol | 0$^{a}$ | 22.00±2.52$^{defg}$ | 0$^{a}$ | 0$^{a}$ | 22.33±0.33$^{e}$ |
| | | Methanol | 0$^{a}$ | 22.33±0.33$^{fg}$ | 14.33±2.19$^{bc}$ | 31.67±1.67$^{j}$ | 25.00±2.00$^{fg}$ |
| | | Acetone | 0$^{a}$ | 20.67±0.88$^{efg}$ | 0$^{a}$ | 30.33±0.67$^{j}$ | 0$^{a}$ |
| *Lathyrus hirticarpus* | Flower | Water | 0$^{a}$ | 11.67±1.86$^{b}$ | 20.67±2.33$^{de}$ | 13.33±2.19$^{bc}$ | 30.33±1.20$^{h}$ |
| | | Ethanol | 0$^{a}$ | 0$^{a}$ | 0$^{a}$ | 14.33±1.20$^{c}$ | 22.67±2.33$^{defg}$ |
| | | Methanol | 0$^{a}$ | 22.33±1.20$^{g}$ | 0$^{a}$ | 17.67±1.86$^{def}$ | 20.33±0.67$^{d}$ |
| | | Acetone | 0$^{a}$ | 0$^{a}$ | 0$^{a}$ | 18.00±1.15$^{def}$ | 19.33±2.19$^{cd}$ |
| *Onopordum blancheanum* | Flower | Water | 0$^{a}$ | 19.00±0.58$^{d}$ | 12.67±2.33$^{b}$ | 20.67±1.86$^{fgh}$ | 20.67±0.88$^{d}$ |
| | | Ethanol | 0$^{a}$ | 18.67±2.67$^{cdefg}$ | 12.33±1.45$^{b}$ | 22.33±1.67$^{hi}$ | 21.66±0.50$^{de}$ |
| | | Methanol | 0$^{a}$ | 18.33±0.67$^{d}$ | 0$^{a}$ | 21.33±0.67$^{h}$ | 25.67±1.33$^{g}$ |
| | | Acetone | 0$^{a}$ | 15.67±1.67$^{c}$ | 0$^{a}$ | 24.67±1.33$^{i}$ | 18.67±0.67$^{c}$ |
| *Onopordum jordanicolum* | Flower | Water | 0$^{a}$ | 0$^{a}$ | 0$^{a}$ | 22.33±2.19$^{hi}$ | 18.33±0.88$^{c}$ |
| | | Ethanol | 16.67±0.67$^{b}$ | 19.00±1.00$^{de}$ | 23.33±0.67$^{e}$ | 18.33±1.67$^{defg}$ | 12.67±0.67$^{b}$ |
| | | Methanol | 0$^{a}$ | 12.67±0.67$^{b}$ | 12.67±2.67$^{b}$ | 12.00±1.00$^{b}$ | 24.67±1.67$^{fg}$ |
| | | Acetone | 0$^{a}$ | 14.67±2.67$^{bc}$ | 0$^{a}$ | 23.33±3.67$^{ghi}$ | 22.33±0.67$^{ef}$ |
| *Orchis sancta* | Flower | Water | 0$^{a}$ | 20.33±1.67$^{e}$ | 13.00±1.00$^{b}$ | 0$^{a}$ | 30.67±1.67$^{h}$ |
| | | Ethanol | 0$^{a}$ | 20.67±2.67$^{defg}$ | 0$^{a}$ | 18.33±0.67$^{ef}$ | 25.33±0.33$^{g}$ |
| | | Methanol | 0$^{a}$ | 19.67±2.67$^{defg}$ | 0$^{a}$ | 20.67±1.67$^{fgh}$ | 25.66±0.50$^{g}$ |
| | | Acetone | 0$^{a}$ | 22.33±1.20$^{g}$ | 28.67±2.33$^{f}$ | 14.67±2.67$^{bcd}$ | 24.33±1.45$^{fg}$ |
| *Papaver umbonatum* | Flower | Water | 0$^{a}$ | 21.00±2.00$^{efg}$ | 0$^{a}$ | 0$^{a}$ | 21.00±1.15$^{de}$ |
| | | Ethanol | 0$^{a}$ | 20.33±1.20$^{efg}$ | 0$^{a}$ | 0$^{a}$ | 20.33±0.88$^{d}$ |
| | | Methanol | 0$^{a}$ | 21.33±2.19$^{efg}$ | 20.33±0.67$^{d}$ | 14.33±2.19$^{bc}$ | 20.67±2.33$^{cdef}$ |
| | | Acetone | 0$^{a}$ | 20.67±1.67$^{efg}$ | 0$^{a}$ | 0$^{a}$ | 21.33±1.20$^{de}$ |

*Inhibition zone diameters are expressed as Means±SE. The means±SE within column followed by the same letter are not significantly different (Tukey's studentized range test: α = 0.05).

Interestingly, all extracts of *O*. *jordanicolum* exhibited anti-MRSA activity. The highest significant anti-MRSA activity was produced from aqueous extracts of *O*. *jordanicolum* and *O*. *sancta* flowers. It was found that only 13 extracts (out of 32) exhibited antibacterial activity against *P*. *aeruginosa* (Table 3). The most significant antibacterial activity against *P*. *aeruginosa* was obtained from aqueous extract of *A*. *carneum* and acetone extract of *O*. *sancta*. However, the most sensitive bacteria were *P*. *mirabillis* and *E*. *coli*, which were affected by most plant extracts (Table 3). The growth of *P*. *mirabillis* was inhibited by all plant extracts except acetone extract of *D*. *ithaburense*. The bacterium *E*. *coli* was insensitive to only three plant extracts; ethanol and acetone extracts of *L*. *hirticarpus* and aqueous extract of *O*. *jordanicolum*.

For antifungal activity, it was observed that the test fungi was inhibited by few plant extracts (Table 4). For instance, it was observed that only water extract of *A*. *carneum* leaves, ethanol extract of *L*. *hirticarpus* flowers, and methanol extract of *O*. *sancta* flowers exhibited antifungal activity against *A*. *brasiliensis* and *C*. *albicans* (Table 4). However, it was found that all flower

**Table 4.** Antifungal activity of extracts from selected medicinal plants against pathogenic fungi.

| Plant species | Plant part | Solvent | Inhibition Zone (mm)* | |
|---|---|---|---|---|
| | | | ***A. brasiliensis* ATCC 16404** | ***C. albicans* ATCC 10231** |
| *Aethionema carneum* | Leaf | Water | 11.66±0.57$^{b}$ | 15.66±0.57$^{d}$ |
| | | Ethanol | 0$^{a}$ | 0$^{a}$ |
| | | Methanol | 0$^{a}$ | 0$^{a}$ |
| | | Acetone | 0$^{a}$ | 0$^{a}$ |
| *Delphinium ithaburense* | Leaf | Water | 15.33±0.88$^{e}$ | 21.33±1.33$^{e}$ |
| | | Ethanol | 0$^{a}$ | 0$^{a}$ |
| | | Methanol | 0$^{a}$ | 0$^{a}$ |
| | | Acetone | 22.00±0.58$^{f}$ | 23.67±0.88$^{f}$ |
| *Lathyrus hirticarpus* | Flower | Water | 0$^{a}$ | 0$^{a}$ |
| | | Ethanol | 11.33±0.88$^{b}$ | 12.33±0.33$^{c}$ |
| | | Methanol | 0$^{a}$ | 0$^{a}$ |
| | | Acetone | 0$^{a}$ | 0$^{a}$ |
| *Onopordum blancheanum* | Flower | Water | 12.00±1.15$^{b}$ | 0$^{a}$ |
| | | Ethanol | 13.33±0.67$^{c}$ | 0$^{a}$ |
| | | Methanol | 15.00±0.58$^{e}$ | 12.67±1.20$^{bc}$ |
| | | Acetone | 0$^{a}$ | 11.33±0.33$^{b}$ |
| *Onopordum jordanicolum* | Flower | Water | 11.33±0.33$^{b}$ | 11.33±0.88$^{bc}$ |
| | | Ethanol | 13.67±0.88$^{cd}$ | 17.67±1.45$^{d}$ |
| | | Methanol | 14.33±0.33$^{de}$ | 20.67±0.67$^{e}$ |
| | | Acetone | 23.67±1.20$^{f}$ | 24.67±1.73$^{f}$ |
| *Orchis sancta* | Flower | Water | 0$^{a}$ | 0$^{a}$ |
| | | Ethanol | 0$^{a}$ | 0$^{a}$ |
| | | Methanol | 11.33±0.88$^{b}$ | 12.67±1.20$^{bc}$ |
| | | Acetone | 0$^{a}$ | 0$^{a}$ |
| *Papaver umbonatum* | Flower | Water | 15.33±0.67$^{e}$ | 14.67±0.67$^{d}$ |
| | | Ethanol | 12.33±0.80$^{bc}$ | 0$^{a}$ |
| | | Methanol | 0$^{a}$ | 0$^{a}$ |
| | | Acetone | 0$^{a}$ | 12.33±0.33$^{c}$ |

*Inhibition zone diameters are expressed as Means±SE. The means±SE within column followed by the same letter are not significantly different (Tukey's studentized range test: α = 0.05).

extracts of *O*. *jordanicolum* exhibited antifungal activity.

Furthermore, acetone extract of *O*. *jordanicolum* flowers produced the highest significant antifungal activity against *A*. *brasiliensis* and *C*. *albicans*. It was interesting to notice that acetone extract of *D*. *ithaburense* leaves also exhibited significant antifungal activity against both test fungi (Table 4). It was found that methanol extract of *O*. *blancheanum* and aqueous extract of *P*. *umbonatum* exhibited high antifungal activities.

Significant antibacterial activities, expressed as MIC and MMC, of promising plant extracts against test bacteria are shown in Table 5. Extracts of selected plants were among the most active with MIC values ranging from 4 to 32 mg/ml and MMC values ranging from 8 to 64 mg/ml. The antibacterial activities with the best MIC and MMC values were produced significantly by ethanolic extracts of *A*. *carneum* against *E. coli* and *O*. *blancheanum* against *S*. *aureus* and by methanolic extracts of *O*. *jordanicolum* against *S*. *aureus* and *P*. *umbonatum* against *S*. *pyogenes* (Table 5). Whereas, MIC and MMC values of the most active antifungal plant extracts ranged from 32 to 64 mg/ml and from 64 to 128 mg/ml, respectively.

The antifungal activities with the best MIC and MMC values were produced significantly by acetone extracts of *O*. *jordanicolum* followed by *D*. *ithaburense* against *A*.

**Table 5.** Minimum inhibitory concentration and minimum microbial concentration of the most significant active extracts of selected medicinal plants

| Plant species | Plant part | Solvent | MIC (MMC)* in mg/ml | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1** | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| *Aethionema carneum* | Leaf | Water | - | - | - | - | - | 8 (64) | 16 (16) | - | - | - |
| | | Ethanol | - | - | - | - | 4 (8) | - | 32 (32) | - | - | - |
| | | Methanol | - | - | - | 32 (64) | 4 (16) | - | - | - | - | - |
| *Delphinium ithaburense* | Leaf | Methanol | - | - | - | - | - | - | 8 (8) | - | - | - |
| | | Acetone | - | - | - | - | - | - | 16 (64) | - | 64 (64) | 64 (128) |
| *Lathyrus hirticarpus* | Flower | Water | - | - | - | - | - | - | - | 8 (8) | - | - |
| *Onopordum blancheanum* | Flower | Water | - | 8 (16) | - | - | - | - | - | - | - | - |
| | | Ethanol | - | 4 (8) | - | - | - | - | - | - | - | - |
| *Onopordum jordanicolum* | Flower | Water | - | - | 16 (64) | - | - | - | - | - | - | - |
| | | Methanol | - | 4 (8) | - | - | - | - | - | - | - | - |
| | | Acetone | - | - | - | - | - | - | - | - | 32 (64) | 32 (32) |
| *Orchis sancta* | Flower | Water | - | - | 32 (32) | - | - | - | - | 16 (32) | - | - |
| | | Acetone | - | - | - | - | - | 16 (64) | - | - | - | - |
| *Papaver umbonatum* | Flower | Water | 8 (8) | - | - | - | - | - | - | - | - | - |
| | | Methanol | 4 (8) | - | - | - | - | - | - | - | - | - |

*MIC is the minimum inhibitory concentration. MMC is the minimum microbial concentration.
**1; *S. pyogenes* ATCC 8668, 2; *S. aureus* ATCC 25923, 3; MRSA ATCC 95047, 4; *S. typhimurium* ATCC 14028, 5; *E. coli* ATCC 25922, 6; *P. aeruginosa* ATCC 27253, 7; *K. pneumonia* ATCC 7700, 8; *P. mirabillis* ATCC 12453, 9; *A. brasiliensis* ATCC 16404, 10; *C. albicans* ATCC 10231.

*brasiliensis and C. albicans*.

The preliminary phytochemical screening of extracts with broad-spectrum antibacterial activity revealed the presence of alkaloids in ethanol extract of *A. carneum* leaves, *O. blancheanum* and *O. jordanicolum* flowers as well as in methanol extract of *P. umbonatum* flowers (Table 6), flavonoids in all selected medicinal plant extracts, saponins in aqueous extracts of *L. hirticarpus* and *O. sancta* flowers in addition to ethanol extract of *O. blancheanum* and *O. jordanicolum* flowers, tannins in ethanol extract of *O. blancheanum* and *O. jordanicolum* flowers and in methanol extract of *P. umbonatum* flowers (Table 6). The screening of phytochemical constituents of extracts with the most significant antifungal activity is illustrated in Table 7. The presence of alkaloids and tannins was detected in all extracts except aqueous extracts of *A. carneum* leaves and *P. umbonatum* flowers. Flavonoids were detected in all promising plant extracts. Saponins were found in methanol extracts of *O. blancheanum* and *O. sancta*, acetone extract of *O. jordanicolum*, and ethanol

**Table 6.** Screening of phytochemical components of selected medicinal plants' extracts which displayed broad-spectrum antibacterial activity.

| Scientific name | Plant part | Solvent | Alkaloids | Flavonoids | Saponins | Tannins |
|---|---|---|---|---|---|---|
| *Aethionema carneum* | Leaf | Ethanol | + | + | - | - |
| *Delphinium ithaburense* | Leaf | Water | - | + | - | - |
| *Lathyrus hirticarpus* | Flower | Water | - | + | + | - |
| *Onopordum blancheanum* | Flower | Ethanol | + | + | + | + |
| *Onopordum jordanicolum* | Flower | Ethanol | + | + | + | + |
| *Orchis sancta* | Flower | Water | - | + | + | - |
| *Papaver umbonatum* | Flower | Methanol | + | + | - | + |

**Table 7.** Screening of phytochemical components of selected medicinal plants' extracts which displayed the most significant antifungal activity.

| Scientific name | Plant part | Solvent | Alkaloids | Flavonoids | Saponins | Tannins |
|---|---|---|---|---|---|---|
| *Aethionema carneum* | Leaf | Water | - | + | - | - |
| *Delphinium ithaburense* | Leaf | Acetone | + | + | - | + |
| *Lathyrus hirticarpus* | Flower | Ethanol | + | + | + | + |
| *Onopordum blancheanum* | Flower | Methanol | + | + | + | + |
| *Onopordum jordanicolum* | Flower | Acetone | + | + | + | + |
| *Orchis sancta* | Flower | Methanol | + | + | + | + |
| *Papaver umbonatum* | Flower | Water | - | + | - | - |

extract of *L*. *hirticarpus* flowers.

## DISCUSSION

Antibiotic resistance of many human pathogens including bacteria and fungi has become a major clinical and public health problem. Therefore, the antimicrobial activity of seven endemic medicinal plants that have not been evaluated previously was investigated and determined in the current study. Four solvents were used in the extraction of bioactive compounds from different plant parts. Generally, it was revealed that most prepared extracts from leaves of *A*. *carneum* and *D*. *ithaburense* and from flowers of *L*. *hirticarpus*, *O*. *blancheanum*, *O*. *jordanicolum*, *O*. *sancta*, and *P*. *umbonatum* exhibited promising antibacterial and antifungal activities. Thus, this study provides valuable information on the abilities of the extracts of selected medicinal plants to yield bioactive compounds that could be potentially used to treat diseases caused by multidrug-resistant bacteria and fungi.

The efficacy of plant extracts evaluated as antimicrobial agents was dependent on the solvent of extraction. In general, ethanol was the best extracting solvent from *A*. *carneum* leaves and from flowers of *O*. *blancheanum* and *O*. *jordanicolum*, produced broad-spectrum and/or significant antibacterial activity (Tables 2 and 3). Whereas, extraction of *D*. *ithaburense* leaves, *L*. *hirticarpus* flowers, and *O*. *sancta* flowers by hot water was found to be the best method for extraction of antibacterial active compounds while methanol appeared to be the appropriate solvent for extraction of antibacterial agents from *P*. *umbonatum* flowers. For production of broad spectrum antifungal activity, it was observed that acetone is the best extracting solvent from most selected medicinal plants. Obeidat et al. (2012) reported that ethanol is the best extracting solvent for plant leaves. Alzoreky and Nakahara (2003) found that both methanol and acetone proved to be suitable solvents for extraction of bioactive inhibitory effects from medicinal plants. Eloff (1998) and Cowan (1999) revealed that methanol was more efficient than acetone in extracting phytochemicals from plant materials. In accordance with those contradictory findings and in agreement with Obeidat et al. (2012), the results of this study indicate that the antimicrobial efficacy depends on selected plant species, plant part, solvent type, test microorganism, and phytochemical components.

The obtained results show that most plant extracts displayed antibacterial activity against all test bacteria except *S*. *typhimurium* which was affected only by three extracts. In contrast to previous findings (Ripa et al., 2009; Obeidat et al., 2012), this study showed in general no significant differences in susceptibility of Gram-positives and Gram-negative bacteria to plant extracts but unfortunately most extracts produced no or even limited antibacterial activity against *S*. *typhimurium*.

The antimicrobial activity of *A*. *carneum* is evaluated for the first time in this study. It was found that ethanol and methanol extracts of *A*. *carneum* leaves exhibited antibacterial activity against Gram-positive bacteria *S*. *pyogenes* and *S*. *aureus*. However, none of the extracts displayed anti-MRSA effects. Aqueous and ethanol extract were found to produce antibacterial activity against all test Gram-negative bacteria. Significant antibacterial activity was given from water and ethanol extract against *K*. *pneumonia*. The best significant antibacterial activity against *P*. *aeruginosa* was obtained from water extract of *A*. *carneum*. Interestingly, the least sensitive bacterium *S*. *typhimurium* was inhibited by water and ethanol extract of *A*. *carneum*. All *A*. *carneum* extracts exhibited antibacterial activity against *P*. *mirabillis* and *E. coli*. In addition, ethanolic extract of *A*. *carneum* showed significant antibacterial activity against *E. coli* with the best MIC and MMC values. For antifungal activity, it was found that *A*. *brasiliensis* and *C*. *albicans* were inhibited by water extract of *A*. *carneum*. Preliminary phytochemical screening of active extracts obtained from *A*. *carneum* leaves demonstrated the presence of alkaloids and flavonoids in ethanolic extract that exhibited a broad spectrum antibacterial activity and flavonoids in aqueous extract that exhibited the most significant antifungal activity. Therefore, the antimicrobial activity of *A*. *carneum* could be attributed to the presence of flavonoids and alkaloids in leaf extracts.

Although no antimicrobial investigations about *D*. *ithaburense* have been described in the literatures, its antibacterial and antifungal activities are identified in the current study. Water extract of *D*. *ithaburense* leaves, which was found to contain flavonoids, produced a broad spectrum antibacterial activity against all test bacteria except *S*. *typhimurium*. Methanol and acetone extracts of *D*. *ithaburense* leaves showed the highest significant antibacterial activity against *K*. *pneumonia*. In some related species, it was revealed that green synthesis of silver nanoparticles using *D*. *denudatum* root extract exhibits antibacterial activities (Suresh et al., 2014). The essential oil from the flowers of *D*. *formosum* showed moderate antibacterial activity against *E. faecalis* and *S*. *aureus*, no antibacterial activity against *E*. *coli*, and no anticandidal activity (Güleç et al., 2007). However in the current study, leaf extracts of *D*. *ithaburense* exhibited both antibacterial activity against *E*. *coli* and anticandidal activity against *C*. *albicans*. The bacterium *E*. *coli* was found to be sensitive to all *D*. *ithaburense* extracts, while acetone extract of *D*. *ithaburense* leaves that contains different phytochemicals including alkaloids, flavonoids, and tannins exhibited the most significant antifungal activity against *A*. *brasiliensis* and *C*. *albicans*.

The medicinal plant *L*. *hirticarpus* was not previously screened for its potential as a source of antimicrobial agents. It was found that water extract prepared from *L*. *hirticarpus* flowers exhibited antibacterial activity against all test bacteria except MRSA and *S*. *typhimurium*. This broad spectrum antibacterial activity might be due to detected flavonoids and saponins in *L*. *hirticarpus* flowers. Moreover, it was found that only MRSA and *S*. *typhimurium* were insensitive to all extracts of *L*. *hirticarpus* flowers. Even though MRSA was resistant to *L*. *hirticarpus* extracts, *S*. *aureus* was sensitive to all plant extracts. Likewise, *K*. *pneumonia* and *P*. *mirabillis* were inhibited by all plant extracts. It was observed that ethanol extract of *L*. *hirticarpus* flowers exhibited antifungal activity against *A*. *brasiliensis* and *C*. *albicans*. Interestingly, alkaloids, flavonoids, saponins, and tannins were detected in ethanol extract. Thus, the significant antifungal activity of ethanol extract of *L*. *hirticarpus* flowers could be due to the presence of these phytochemical compounds.

Two *Onopordum* species were investigated in this study, *O*. *blancheanum* and *O*. *jordanicolum*. Those *Onopordum* species were not examined previously for their effectiveness as antimicrobial agents. It was revealed that flower extracts of *O*. *jordanicolum* produced broad spectrum antimicrobial activities and appeared to be the best source for antimicrobial agents among tested plant species in this study. Ethanol extract of *O*. *jordanicolum* flowers showed antibacterial activity against all test bacteria with the best MIC and MMC values produced by methanol extract for *S*. *aureus*. Furthermore, all *O*. *jordanicolum* extracts produced antibacterial activity against *S*. *aureus*, MRSA, *K*. *pneumonia*, and *P*. *mirabillis*. Both test bacteria *S*. *aureus* and MRSA, which belongs to the same species were inhibited significantly by methanol and aqueous extracts of *O*. *jordanicolum* flowers, respectively. Interestingly, it was detected that all flower extracts of *O*. *jordanicolum* displayed antifungal activity against *A*. *brasiliensis* and *C*. *albicans* with the best MIC and MMC values for acetone extract. For *O*. *blancheanum*, aqueous and ethanol extract of *O*. *blancheanum* flowers displayed significant antibacterial activity against *S*. *aureus* with the best MIC and MMC values for ethanol extract. All *O*. *blancheanum* extracts produced antibacterial activity against *S*. *aureus*, *E*. *coli*, *K*. *pneumonia*, and *P*. *mirabillis*. It was noticed that methanol extract exhibited antifungal activity against *A*. *brasiliensis* and *C*. *albicans*. Zare et al. (2014) illustrated that methanol extract of the related species *O*. *acanthium* showed significant antibacterial activity against *S. epidermidis* and *M. luteus*, while n-hexane extract demonstrated negligible to no inhibitory activity against Gram-negative and Gram-positive bacteria. Ugur et al. (2011) reported that chloroform and ethanol extract of *O*. *caricum* has a potential to inhibit the growth of *S. maltophilia* and *S*. *aureus*. Thus, the antimicrobial results in this study are competitive and more promising than that reported previously for other *Onopordum* species. It was clearly observed that extracts of both *Onopordum* species investigated in this study contain various phytochemical types including alkaloids, flavonoids, saponins, and tannins that are known to exhibit anti-

bacterial and antifungal activities.

The orchid *O*. *sancta* is a unique species in Jordan. However, its antimicrobial activity was not studied before this study. The four extracts of *O*. *sancta* flowers exhibited antibacterial activity against *E*. *coli* and *P*. *mirabillis*. Significant anti-MRSA activity was produced from aqueous extract of *O*. *sancta* flowers. Acetone extract of *O*. *sancta* gave the highest significant antibacterial activity against *P*. *aeruginosa*. It was found that only methanol extract of *O*. *sancta* flowers showed antifungal activity against *A*. *brasiliensis* and *C*. *albicans*. On the other hand, it was reported that methanolic extract of the related species *O*. *latifolia* showed antifungal activity against *C*. *albicans* and antibacterial activity against multidrug-resistant clinical isolates including *E*. *coli*, *S*. *aureus*, and *Enterococcus* sp. (Avasthi et al., 2013). Alkaloids, flavonoids, saponins, and tannins were detected in methanol extract and only flavonoids and saponins were detected in water extract of *O*. *sancta*. This result is in agreement with Avasthi et al. (2013) who demonstrated that phytochemical analysis of the active fractions obtained from *O*. *latifolia* contained flavonoids, steroids, and tannins. Therefore, the antimicrobial activity of *O*. *sancta* might be attributed to the presence of these phytochemicals.

The antimicrobial activity of *P*. *umbonatum* has not been performed. Therefore, this plant was selected in this study for determination of its antimicrobial activity. Water and methanol extract prepared from *P*. *umbonatum* flowers exhibited significant antibacterial activity against *S*. *pyogenes* with the best MIC and MMC values. Although there is no antibacterial activity against MRSA, the other related test bacteria *S*. *aureus* was found to be sensitive to all flower extracts of *P*. *umbonatum*. Similarly, *E*. *coli* and *P*. *mirabillis* were inhibited by all extracts of *P*. *umbonatum*. In addition, methanol extract exhibited antibacterial activity against *P*. *aeruginosa* and *K*. *pneumonia*. Aqueous extract produced the best antifungal activity against *A*. *brasiliensis* and *C*. *albicans*. This result is in accordance with Kostic et al. (2010) who indicated that ethanol extract of the related species *P*. *rhoeas* exhibited antifungal activity against *C*. *albican* and antibacterial activity against *E*. *coli*, *S*. *aureus*, and *P*. *aeruginosa*. Alkaloids, flavonoids, and tannins were detected in methanol extract of *P*. *umbonatum*, and only flavonoids were detected in the aqueous extract. This is in agreement with the previous analysis of the phytochemical constituents of *P*. *umbonatum* by Bernáth (2006) who revealed the presence of flavonoids and clinically useful alkaloids.

The result of this study shows the existence of different phytochemicals in selected medicinal plants such as alkaloids, flavonoids, saponins, and tannins that are known to exhibit various antimicrobial activities. It was previously reported (Cushnie and Lamb, 2005; Lim et al., 2006; Maatalah et al., 2012) that alkaloids, flavonoids, saponins, and tannins possessing various antimicrobial activities including antibacterial and antifungal activities. Therefore, the antimicrobial activity of promising medicinal plant extracts investigated in this work could be due to the presence of these phytochemicals.

In conclusion, the results of this study represent the first report on the antibacterial and antifungal activities of aqueous, ethanol, methanol, and acetone extracts prepared from seven endemic medicinal plants (*A*. *carneum*, *D*. *ithaburense*, *L*. *hirticarpus*, *O*. *blancheanum*, *O*. *jordanicolum*, *O*. *sancta*, and *P*. *umbonatum*) against human frequent pathogens. The promising extracts from selected plants in this study may be used in the development of future drugs and in the treatment of infectious diseases caused by human pathogenic bacteria and fungi that exhibited antibiotic resistance. Further phytochemical analysis is required to characterize all bioactive constituents and their amounts in active medicinal plant extracts.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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*Full Length Research Paper*

# The impact of seasonal variation on the volatile oil profile of leaves of *Severinia buxifolia* (Poir.) and its antimicrobial activity

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**The effect of the time of collection on the quality and quantity of the oil obtained from *Severinia buxifolia* leaves as well as its antimicrobial activity was studied. The chemical composition of the hydro-distilled oils of the leaves of *S. buxifolia* (Poir.)Tenore, collected at the four seasons was determined by GC/MS analysis. Moreover, antimicrobial activity was studied, for the oil sample regarding the yield and quality, against selected bacteria and yeast. The highest oil yield was obtained from the leaves collected during winter (0.5%) followed by autumn (0.308%), however, those collected in spring and summer scored almost the same yield; (0.26%) and (0.283%) respectively. Limonene was the most abundant hydrocarbon in winter (35.5%), and amounted to 29.3% in summer, whereas, spring and autumn samples constituted 21.15% and 19.17% of limonene respectively. α-Santalene, accounted to 20.87% in autumn sample followed by the winter sample (18.93%), then 13.56% in the spring sample and recorded its lowest concentration in the summer sample (8.1%). Furthermore, γ-elemene was detected in a lesser extent amounting to 7.75% in the spring sample, 7.33% in autumn sample, 6.28% in the winter sample and 5.54% in the summer sample. Based on the above results, as regards to limonene content, *S. buxifolia* leaf oil collected in winter was chosen for further antimicrobial study. The agar disc diffusion method was adopted for screening the antibacterial activity of the selected oil sample. Results show moderate effect against *Escherichia coli*, and *Listeria monocytogenes*. Nevertheless, it showed weak activity against *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilus,* MRSA, and *Candida albicans.* The MIC of the volatile oil against *L. monocytogenes was* 4 and 1 µl/ ml against *S. aureus, P. aeruginosa, B. subtilus,* MRSA and *C. albicans.***

**Key words:** *Severinia buxifolia* (Poir.) Tenore, Rutaceae, GC/MS analysis, limonene, *in vitro* antimicrobial activity.

## INTRODUCTION

Rutaceae is best known for the exotic genus citrus, because of its commercially consumed fruits. Other groups of the same subfamily of Citrus are commonly cultivated as ornamentals in America, including species of *Atalantia*, *Clausena*, *Murraya*, and *Swinglea*.

Genus *Atalantia* includes approximately 20 species

(Roskov et al., 2017). Amongst *Atalantia* species, *Atalantia buxifolia* or *Severinia buxifolia* (Poir.) Tenore is the plant of our interest. *S. buxifolia* (*Atalantia buxifolia*) is commonly known as the thorny, evergreen shrub; it is also known as Chinese box-orange or Box-leaved *Atalantia*. The Flora of China has moved this species to *A. buxifolia* (Poir.) Oliv. (Wu et al., 2008). In Hainan province of China, the roots of *A. buxifolia* are used in folk medicine for the treatment of influenza, cough, malaria, and stomachache by the people of Li nationality (Yang, 2012).

Essential oils are complex mixtures including important compounds where each contributes to the beneficial or adverse effects of these oils. Essential oils have massive consumptions as raw materials in several areas, including perfumes, cosmetics, aromatherapy, phototherapy, spices and nutrition Buchbauer, 2000). Accordingly, it was of deep interest to have strong background regarding the essential oil composition since this permits for a better and specially directed application.

Infectious diseases are major causes of death worldwide. Infections with bacteria are associated with high morbidity and mortality especially with immuno-compromised patients (Driscoll et al., 2007; Del Toro et al., 2006). The proposed strategies to avoid and govern infectious diseases include public health improvements in sanitation and hygiene, as well as the use of antimicrobial agents (WHO, 2001). The resistance of microorganisms to antibiotic has become an important alarm to the patients as well as a scientist (Westh et al., 2004) in addition to the side effects of these antibiotics. This directed researchers to explore new chemotherapeutic agents to combat the infections caused by drug-resistant microbes and to reduce the harm caused by antibiotics (Bocanegra-Garcı´a et al., 2009; Giamarellou, 2006). Volatile oils obtained from plants have been recognized for many years as antimicrobial agents.

Direct addition of essential oil from aromatic plants to food products evidenced antimicrobial effect (Costa et al., 2015). Nowadays, due to consumer complaint from artificial preservatives, attention to volatile oils and their application in food preservation has been considered. Many reports dealt with the wide range of application for essential oils as antiseptic, antibacterial, antiviral, antioxidant, anti-parasitic, antifungal, and insecticidal agents (Burt, 2004; Dorman and Deans, 2008). Hence, essential oils can serve as a powerful device to minimize the bacterial resistance (Stefanakis et al., 2013).

This work was carried out to evaluate the oil produced from the plant and the effect of seasonal fluctuation on the yield and composition of the essential oil of the leaves, furthermore comparing the identified components and the major constituents detected in the studied samples, with the aim to rationalize the effect of climate conditions on the yield and quantity. The antimicrobial activity of the oil sample giving the highest yield and quality was studied.

## MATERIALS AND METHODS

### Plant material

Sample leaves of *S. buxifolia* (Poir.) Tenore used in this study were collected in May to June during the years 2013 to 2014 (collected in four different seasons throughout the year) from the Orman Garden, Giza, Egypt. The plant was authenticated by Dr. Mohamed El-Gebaly, senior botany specialist at the Orman Garden, Giza, Egypt. Voucher specimens (26-05-2015) were kept at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Egypt.

### Microbial strains

The tested bacteria and fungi were supplied by the Antimicrobial Unit, National Research Center, Egypt. Bacterial strains were *Bacillus subtilus* (ATCC6051), *Staphylococcus aureus* (ATCC 6538), *Methicillin-resistant Staphylococcus aureus* (MRSA) (laboratory collection), *Listeria monocytogenes* (ATCC2180-1A) representing pathogenic gram positive bacteria, and *Escherichia coli* (ATCC 8739) and *Pseudomonas aeruginosa*(ATCC 9027) representing pathogenic gram negative bacteria and *Candida albicans* (RCMB 05035) representing fungi. Ciprofloxacin (Pharco Pharm. Cairo Egypt, and Amphotericin B obtained from Sigma-Aldrich (Merk) was used as reference drugs.

### Preparation of the volatile oil

For preparation of essential oil, fresh leaf samples were collected at three months intervals along the four seasons (winter, spring, summer and autumn) between May 2013 to June 2014 and separately subjected to hydro distillation in a Clevenger's apparatus for 3 h, according to the procedure described in the Egyptian Pharmacopeia. The obtained oils were separately dried over anhydrous sodium sulphate and carefully stored in a refrigerator for further chemical and biological studies. The percentage yields were calculated on a dry weight basis, and the oils were kept in a refrigerator for further analysis. The specific gravities and refractive indices were determined according to the Egyptian Pharmacopoeia (CAPA, 2005) procedures. All stated values were the average of three determinations.

### Determination of percentage yield of the volatile oil

The yield of the volatile oils was calculated as weight/weight (g/kg), on fresh weight basis.

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**Table 1.** Yield and physical characters of essential oil of the leaves of *S. buxifolia* (Poir.) Ten. at four different seasons.

| Physical properties | Seasons (%) | | | |
|---|---|---|---|---|
| | Winter | Spring | Summer | Autumn |
| Odour | Lemon-like odour | | | |
| Colour | Pale yellow | | | |
| Yield % (w/w, g/kg) | 0.5 | 0.283 | 0.26 | 0.308 |
| Specific gravity (g/cm$^3$ at 25°C) | *0.86 | *0.84 | *0.81 | *0.83 |
| Refractive index (recorded at 20°C) | *1.4694 | *1.4653 | *1.4624 | *1.4642 |

*Results are average of three determinations.

**GC/MS analysis of the volatile oil content**

Volatile oil prepared from *S. buxifolia* leaves were subjected to GC/MS analysis. Volatile oil prepared was subjected to GC/MS analysis. The injection volume was 1 µl/ml and the instrument was controlled by the Shimadzu Class-5000 Version 2.2 software containing a NIST62 (National Institute of Standards and Technology) MS library. Volatiles were separated on a DB5-MS column (30 m length, 0.25 mm inner diameter, and 0.25 ml film (J&W Scientific, Santa Clara, California). Injections were made in the split mode for 30 s, and the gas chromatograph was operated under the following conditions: injector 220°C and column oven 40°C for 3 min, then programmed at a rate of 12°C/min to 180°C, kept at 180°C for 5 min, and finally ramped at a rate of 40°C/min to 220°C and kept for 2 min; He carrier gas was at 1 mL/min. The transfer line and ion–source temperatures were adjusted at 230 and 180°C, respectively. The HP quadrupole mass spectrometer was operated in the electron ionization mode at 70 eV. The scan range was set at 40 to 500 m/z. The percentages of different components in each oil sample were determined by computerized peak area measurements relative to each other. Volatile components were identified using the procedure described (Farag and Wessjohann, 2012). The peaks were first deconvoluted using AMDIS software (www.amdis.net) and identified by its retention indices (RI) relative to *n*-alkanes (C6–C20), mass spectrum matching to NIST, WILEY library database.

**Evaluation of the antimicrobial activity**

***In-vitro qualitative screening susceptibility test***

The selected volatile oil sample of the leaves of *S. buxifolia* under investigation was screened for their antimicrobial activity against representatives of G+ bacteria: *B. subtillus*, *S. aureus*, Methicillin-resistant *S. aureus* (MRSA), L. *monocytogenes*, and G$^-$*E. coli*, *P. aeruginosa*, and yeast (*C. albicans*) applying the agar disc diffusion according to CLSI guidelines (2009).

The selected volatile oil was tested by impregnating sterile discs of Whatmann filter paper 1 (5 mm diameter) in 20 µl of the oils. 20 µl of dimethyl sulfoxide was used as a negative control. The reference standards ciprofloxacin and amphotericin-B were dissolved separately in dimethyl sulfoxide at a concentration of 20 µg/µl. The discs were then placed onto the surface of the plates containing the solid bacterial medium (Mueller–Hinton agar) or the fungal medium (Dox's medium) which has been heavily seeded with the spore suspension of the tested microorganisms. The plates were incubated at 37°C for 25 h in case of bacteria and at 25°C for 48 h in case of fungi. After incubation, the inhibition zones were measured in mm.

***Determination of the minimum inhibitory concentration (MIC)***

Minimum inhibitory concentration (quantitative assay) was evaluated for the volatile oil of *S. buxifolia*, based on the results obtained for the antimicrobial screening. Accordingly, *B. subtilus, S. aureus,* MRSA*, L. monocytogenes, E. coli, P. aeruginosa* and yeast (*C. albicans*) for *S. buxifolia* leaf oil. In brief, stationary phase cultures of bacterial strains were prepared at 37°C and used to inoculate fresh 5.0 ml culture to an OD600 of 0.05. The 5.0 ml culture was then incubated at 37°C until an OD600 was achieved from which standardized bacterial suspensions were prepared to a final cell density of 6 105 CFU/ml. Serial dilutions from the volatile oils were prepared and mixed with 5.0 ml of the standardized bacterial suspension then added to the plates and incubated for 24 h at 37°C. The colony forming units (CFU) were counted for each dilution (NCCLS, 2000).

***Agar dilution method***

The tested samples were serially diluted in molten medium equilibrated at 50°C with 2% glucose. One ml was added to each well in a 24-well plate with a flat bottom and allowed to solidify. The centre of each well was inoculated with 10 ml of the bacterial suspension. Drug free growth control was included. MIC was determined after 48 h at 35°C. MICs were defined as the lowest concentration that had granular appearing micro-colonies of growth instead of filamentous radiating colonies on solid agar.

## RESULTS AND DISCUSSION

This is the first report dealing with the effect of seasonal variation of the essential oils obtained from the fresh leaves of *S. buxifolia* (Poir.) Tenore.

Investigation of the oil obtained from the leaves of *S. buxifolia* at different seasons; winter, spring, summer and autumn revealed qualitative and quantitative differences. The highest oil yield was obtained from the leaves collected during winter (0.5%) followed by autumn (0.308%), while, those collected in spring and summer counted almost the same yield; 0.26 and 0.283% respectively (Table 1).

GC/MS analysis of the essential oil obtained from *S. buxifolia* leaves at four different seasons (Table 2) indicated that the identified constituents varied as regards to their abundance and concentration. The total identified

**Table 2.** Chemical composition of the essential oils of *S. buxifolia* leaves at four different seasons and their mass data.

| S/N | Rt | KI | $M^+$ | BP | Main fragments m / z | Compound | Area% | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Autumn | Winter | Spring | Summer |
| 1 | 7.680 | 913.3 | 136 | 93 | 41, 53, 67, 79, 105, 121 | *β* - trans-Ocimene | 0.44 | 0.45 | 0.65 | 0.82 |
| 2 | 7.7712 | 936.2 | 136 | 93 | 41, 53, 79, 107, 121 | *α*-Thujene | – | 0.3 | 0.41 | 0.55 |
| 3 | 7.9672 | 936.5 | 136 | 93 | 41, 53, 79, 107, 121 | *β*-Thujene | 0.23 | 0.66 | 0.43 | 0.36 |
| 4 | 8.251 | 937.7 | 136 | 93 | 41, 53, 67, 77, 105, 121 | *α*-Pinene | 0.1 | – | – | – |
| 5 | 8.530 | 975.8 | 136 | 93 | 41, 53, 69, 77, 121 | Sabinene | – | 0.08 | 0.1 | 0.07 |
| 6 | 8.6432 | 989 | 136 | 41 | 53, 67, 69, 79, 91 | *β*-Myrcene | 0.31 | 0.72 | 0.35 | 0.07 |
| 7 | 8.7910 | 1011 | 136 | 93 | 41, 53, 65, 77, 105, 121 | *δ*-3-Carene | – | – | 0.03 | 0.05 |
| 8 | 9.052 | 1027.7 | 134 | 119 | 41, 51, 65, 77, 91, 103 | *p*-Cymene | – | 0.03 | 0.04 | 0.1 |
| 9 | 9.1976 | 1033 | 154 | 59 | 43, 67, 81, 96 | Santolina alcohol | – | – | – | 0.05 |
| 10 | 9.3675 | 1034 | 136 | 68 | 41,53,68,79, 93,107, 121 | Limonene | 19.17 | 35.5 | 21.15 | 29.3 |
| 11 | 9.5390 | 1047.4 | 134 | 119 | 41, 51, 65, 77, 91, 103, 119 | Octen-1-ol | – | – | 0.19 | – |
| 12 | 9.8175 | 1061.9 | 136 | 93 | 41, 51, 65, 77 | *α*-Phellandrene | 0.13 | – | – | – |
| 13 | 10.1151 | 1062.1 | 136 | 93 | 43, 58, 65, 77, 105, 121 | *δ*-Terpinene | – | – | – | 0.16 |
| 14 | 10.5283 | 1063 | 136 | 93 | 41, 53, 65, 77, 121 | *β*-Phellanderene | 0.11 | 0.23 | – | – |
| 15 | 10.7522 | 1101 | 154 | 71 | 43, 55, 80, 93, 107, 121 | *β*-Linaloo | 0.04 | – | 0.16 | 0.15 |
| 17 | 10.9910 | 1169 | 156 | 71 | 41, 55, 81, 95, 109, 123 | Menthol | 0.3 | 0.25 | 0.19 | 0.1 |
| 18 | 11.2071 | 1172 | 154 | 71 | 43, 55, 77, 86, 93, 111, 136 | Terpinen-4-o | 0.13 | – | 0.17 | 0.2 |
| 19 | 11.5259 | 1173.0 | 170 | 71 | 43, 55, 82, 128, 141 | Hexenyl butanoate | – | 0.18 | – | – |
| 20 | 11.7814 | 1206 | 94 | 81 | 41, 54, 67, 95 | Norbornene | – | – | 0.8 | – |
| 21 | 11.940 | 1207 | 156 | 41 | 57, 70, 82, 95, 112, 128 | Decana | 0.96 | 0.93 | 0.84 | 1.17 |
| 22 | 12.5582 | 1274.6 | 152 | 41 | 53, 59, 69, 84, 94, 109, 119, 137 | Neral | 0.05 | 0.08 | – | – |
| 23 | 13.463 | 1345 | 204 | 121 | 41, 53, 67, 77, 93, 105, 136, 161, 175, 189 | *δ*-Elemene | 0.04 | – | 0.1 | 0.2 |
| 24 | 13.796 | 1359 | 202 | 145 | 41, 55, 65, 77, 91, 105, 119, 131, 145, 159, 174, 187 | Silphiperfol-5,7(14)-diene | 0.05 | 0.09 | 0.09 | – |
| 25 | 14.0061 | 1401.5 | 96 | 67 | 43, 57, 85 | Norbornane | 0.1 | – | 0.16 | 0.18 |
| 26 | 14.537 | 1411 | 184 | 67 | 43, 55, 79, 110, 123, 138, 166 | Z-2-Dodecenol | 0.13 | 0.15 | 0.17 | 0.13 |
| 27 | 14.7733 | 1431 | 204 | 94 | 41, 55, 69, 79, 107, 121, 133, 147, 161, 176, 189 | *α*-Santalene | 20.87 | 18.93 | 13.56 | 8.1 |
| 28 | 14.8725 | 1439 | 204 | 41 | 55, 69, 79, 93, 105, 120, 133, 147, 161, 175, 189 | *β*-Caryophyllene | 5.08 | 5.07 | 3.78 | 2.98 |
| 29 | 14.9125 | 1442 | 204 | 119 | 41, 55, 69, 77, 93, 105, 133, 147, 161 | Zingiberene | 0.43 | 0.37 | 0.3 | 0.22 |
| 30 | 15.0371 | 1446 | 164 | 164 | 41, 55, 65, 77, 91, 103, 121, 131, 149 | Isoeugenol | 0.4 | – | 0.13 | 0.19 |
| 31 | 15.1192 | 1459 | 204 | 94 | 41, 55, 67, 79, 107, 122, 133, 147, 161, 189 | Epi-*β*-Santalene | 2.07 | 1.6 | 1.96 | 0.83 |
| 32 | 15.2725 | 1472 | 204 | 94 | 41, 55, 67, 79, 107, 122, 133, 147, 161, 189 | *β*-Santalene | 2.52 | 1.98 | – | 1.23 |
| 33 | 15.323 | 1475 | 204 | 93 | 41, 53, 67, 80, 107, 121, 136, 147, 161, 189 | *α*-Humulene | 0.87 | 0.9 | 0.75 | 0.65 |
| 35 | 15.5634 | 1511.3 | 204 | 161 | 41,55, 67, 79, 91, 105, 119, 133, 147, 175, 189 | [+]-Valencene | 0.55 | – | – | – |

**Table 2.** Contd.

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 36 | 15.834 | 1515 | 204 | 121 | 41, 55, 67, 79, 93, 107, 136, 147, 161, 175, 189 | *γ* -Elemene | 7.33 | 6.28 | 7.75 | 5.54 |
| 37 | 15.895 | 1519 | 204 | 161 | 41, 53, 81, 93, 107, 119, 133, 147, 161, 189 | Germacrene A | – | – | 0.18 | 0.2 |
| 38 | 16.032 | 1530 | 204 | 105 | 41, 55, 69, 81, 91, 119, 133, 161, 175, 189 | *α* -Cadinene | 0.75 | – | 0.94 | 0.8 |
| 39 | 16.219 | 1531.6 | 204 | 119 | 41, 55, 69, 79, 93, 105, 133, 148, 161, 189 | γ-Cuprenene | – | 0.62 | – | 0.45 |
| 40 | 16.330 | 1534.5 | 222 | 69 | 41, 55, 69, 81, 93, 107, 136, 161, 189 | Nerolidol | – | – | 0.13 | – |
| 41 | 16.475 | 1550 | 222 | 59 | 43, 67, 81, 93, 107, 121, 135, 149, 161, 189 | Elemol | 0.21 | – | 0.28 | 0.1 |
| 42 | 16.55 | 1562 | 204 | 41 | 55, 69, 79, 93, 107, 119, 134, 147, 161, 189 | (Z)-*α*-Farnesene | 0.48 | – | 0.7 | 0.74 |
| 43 | 16.782 | 1575 | 220 | 43 | 55, 67, 79, 91, 105, 119, 131, 147, 159, 177, 187, 205 | Spathulenol | 0.28 | – | – | – |
| 44 | 16.903 | 1588 | 220 | 41 | 55, 69, 77, 93, 109, 121, 135, 149, 161, 177, 187,205 | Caryophylline oxide | – | – | – | 0.52 |
| 45 | 17.198 | 1600 | 220 | 159 | 41, 55, 67, 79, 91, 105, 118, 131, 177, 187, 202 | cis-*α*-Copaene-8-ol | 1.41 | 0.69 | 1.88 | 1.65 |
| 46 | 17.210 | 1608.2 | 222 | 41 | 55, 69, 81, 93, 107, 121, 135, 161, 179 | Z-Sesquilavandulol | – | – | 0.91 | 0.76 |
| 48 | 17.273 | 1609.3 | 204 | 69 | 41, 55, 79, 93, 107, 120, 133, 147, 161 | (Z)- *β*-Farnesene | 0.77 | – | – | – |
| 49 | 17.4195 | 1609.8 | 202 | 91 | 41, 53, 67, 79, 105, 119, 131, 145, 159, 187 | *β*-Atalantol | 0.77 | – | – | 0.43 |
| 50 | 17.6391 | 1617.5 | 222 | 95 | 43 ,55, 69, 81, 107, 119, 135, 150, 150, 161, 189, 204 | *Epi* Cedrol | 0.25 | – | 0.1 | 0.11 |
| 52 | 17.736 | 1669.9 | 222 | 93 | 41, 59, 67, 81, 107, 121, 136, 161, 175, 189 | *E*-Bisabol-11-ol | 0.11 | – | – | – |
| 54 | 17.7985 | 1675.6 | 220 | 41 | 55, 67, 81, 98, 109, 125, 161, 179, 207 | Z-*α*-Santalol | 24.44 | 13.73 | 29.19 | 22.2 |
| 56 | 17.8157 | 1688.0 | 222 | 43 | 55, 69, 79, 93, 109, 119, 134, 161, 189 | *α*-Bisabolol | – | – | – | 0.36 |
| 57 | 17.892 | 1687.7 | 222 | 69 | 41, 55, 81, 93, 107, 121, 136, 161, 191 | (2Z,6Z)-Farnesol | 3.72 | – | 1.35 | 3.54 |
| 58 | 18.001 | 1866.0 | 262 | 94 | 43, 55, 67, 79, 107, 122, 134, 159, 187 | E-*β*-Santalol acetate | – | – | – | 1.49 |
| 59 | 18.5500 | 1925.5 | 228 | 213 | 128, 141, 157, 185 | Seselin | 0.33 | – | 0.87 | 0.52 |
| | %Total identified compounds | | | | | | 95.93 | 89.82 | 90.79 | 87.27 |
| | % unidentified compounds | | | | | | 4.07 | 10.18 | 9.21 | 12.73 |
| | % of Hydrocarbons constituents | | | | | | 93.3 | 74.16 | 54.91 | 54.66 |
| | % of Oxygenated constituents | | | | | | 3.62 | 16 | 37.7 | 33.78 |

Rt= retention time; K.I. = kovat's index; M+= molecular weight; BP= Base peak.

compounds were 95.93, (89.82, 90.16 and 88.44% in autumn, spring, winter and summer respectively. The total number of constituents identified under the adopted conditions was 59 among which 15 were detected in the four oil samples under investigation. The rest of constituents appeared, however, unevenly distributed in the analyzed oils.

Hydrocarbons were the most abundant compounds detected, amounting the highest concentration in autumn (93.3%), followed by those collected in winter (74.16%). Nevertheless, spring and summer samples constituted the same amount of hydrocarbons; 54.91 and 54.66%, respectively. Monoterpene hydrocarbons constituted the most dominant chemical group among the four studied seasons; limonene was the most abundant in winter (35.5%), and amounted to 29.3% in summer, whereas, spring and autumn samples constituted 21.15 and 19.17% of limonene respectively. Next to monoterpenoids, sesquiterpenoids were the most detected compounds in the volatiles studied at different seasons from the leaves of *S. buxifolia*,

**Table 3.** Antibacterial and antifungal activity of the winter sample (essential oil of the leaves) of *S. buxifolia* (Poir.) Ten.

| Microorganism | Diameter of zone of inhibition (mm) | | |
|---|---|---|---|
| | Essential oil (4 µL / disc) | Ciprofloxacin | Amphotericin-B |
| *Bacillus Subtillus* (ATCC6051) | 16 | 31 | - |
| *Staphylococcus aureus* (ATCC 6538) | 17 | 30 | - |
| *MRSA* (Laboratory Collection) | 10 | 28 | - |
| *Listeria monocytogenes* (ATCC2180-1A) | 13 | - | - |
| *Escherichia Coli* (ATCC 8739) | 19 | 29 | - |
| *Pseudomonas aeruginosa* (ATCC 9027) | 12 | 29 | - |
| *Candida albicans (yeast)* (RCMB 05035) | 13 | - | 29 |

Values are average of three determinations.

with α-santalene accounting up to 20.87% in autumn sample followed by the winter sample (18.93%), then reaching 13.56% in the spring sample and finally, recorded the lowest concentration in the summer sample(8.1%) relative to the other samples.

Furthermore, γ- elemene was found but in a lesser extent than α-santalene amounting to (7.75%) in the oil collected in spring, (7.33%) in the autumn sample, (6.28%) in the winter sample and (5.54%) in the summer sample. Throughout our study in the four seasons; the number of oxygenated compounds ranged from 16 to 37.7%; spring sample recorded the highest content of oxygenated compounds (37.7%) with Z-α-santalol constituting the major sesquiterpene alcohol (29.19%), the oxygenated compounds amounted up to 3.62 % in autumn, where Z-α-santalol was the major compound (24.44%), followed by summer (33.78%) with same major sesquiterpene (22.2%), however winter showed the lowest oxygenated compounds recorded in all studied oil samples (16%), Z-α-santalol amounting to 13.37%.

These findings support the idea that the seasonal variation can influence the quantifier of oxygenated compounds present in the oil. Comparing the results to that reported (Scora and Ahmed, 1994), there is no great difference between the number of compounds identified; 59 components were also identified, however, the difference was clear in the detected compounds. The major reported components were α -santalene (24%), trans β -santalol (21%), germacrene-B (10%), and β-caryophyllene (7%) and the monoterpene limonene (19%) in one tested sample. On the other hand, another report showed variation (Dongxu et al., 2011) from our study as major identified components were isocyclocitral (41.598 %), guaiacol (15.234%), β-eudesmol (10.790%), thujopsene (2.581%), 1,7,7-trimethyl-bicyclohept-2-one (5.841%), santalol (3.702%), ferruginol (2.657%) at one sample. Herein, we have evidenced variation in the tested samples as regards to yield and components which were affected by the seasonal effect. Furthermore, there was an obvious impact on the climax of each season on the volatile constituents of each cultivated plant.

Previous reports evidenced that limonene possesses antifungal, bacteriostatic and bactericidal activities (Dorman and Deans, 2000; Dambolena et al., 2008; Jaroenkit et al., 2011; Chee et al., 2009). Moreover, it was also suggested to be used as a food preservative (Vuuren and Viljoen, 2007). Since winter sample recorded the maximum yield and constituted the highest percentage of limonene, therefore, winter sample was selected as a representative to undergo further *in the vitro* antimicrobial study.

There was a correlation between the inhibition zone diameter of the agar diffusion method (qualitative method) (Rashed and Butnariu, 2014a) and MIC (quantitative method) values of broth dilution method (Rashed and Butnariu, 2014b). The selected essential oil showed a maximum zone of inhibition with minimum MIC value among all tested microorganisms. The selected volatile oil sample of *S. buxifolia* leaves exhibited moderate antibacterial and antifungal activity at the given concentrations when compared to ciprofloxacin and amphotericin B respectively as a standard (Table 3). Moreover, the oil exhibited activity against most of the tested bacteria with a MIC of 1% v/v except for *L. monocytogenes.* This activity might be attributed to the high concentration of limonene in the sample; the mechanism of action for the antibacterial activity was previously reported; the inactivation of certain strain of *E. coli* by (+)-limonene, in a medium adjusted at certain pH, the effect of the synergistic lethal effect on combining (+)-limonene with heat and Pulse Electric field (PEF) treatments to inactivate *E. coli* (Espina et al., 2013). Referring to Table 4, the MIC of the volatile oil of the leaves of *S. buxifolia* against *L. monocytogenes* recorded 4 µl/ml, while 1 µl/ml against other bacterial strains. Therefore, this volatile oil could be considered as moderate antibacterial and antifungal agent.

## Conclusion

Our findings support the concept that collecting oil samples from the fresh leaves of *S. buxifolia* (Poir.)

**Table 4.** MIC of the winter sample (essential oil of the fresh leaves) of *S. buxifolia* (Poir.) Ten. on bacteria and fungi.

| Tested microorganism | Minimum inhibitory concentration (µl/ml )of essential oil |
|---|---|
| Gram-positive bacteria | |
| *Bacillus Subtillus* | 1 |
| *Staphylococcus aureus* | 1 |
| *MRSA* | 1 |
| *Listeria Monocytogenes* | 4 |
| **Gram-negative bacteria** | |
| *Escherichia Coli* | 1 |
| *Pseudomonas aeruginosa* | 1 |
| Fungi *Candida albicans (yeast)* | 1 |

Tenore at different seasons had an impact on their yield and composition. Winter sample was the best with the high content of limonene. There is variation in the antimicrobial activity of MIC of winter oil sample against the entire tested microorganism. The data indicated that the oil has antimicrobial activity against gram +ve and gram –ve bacteria and even Candida in close range.

## CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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*Full Length Research Paper*

# Ethnobotanical study of medicinal plants used to treat human ailment in Guduru District of Oromia Regional State, Ethiopia

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**This research was carried out to document ethnobotanical data and threats affecting medicinal plants. Semi-structured interviews, questionnaires, face to face discussion, and field visit was employed to gather the required data. A total of 92 informants 21 key and 71 randomly selected informants), of which 48 males and 44 females were used. The study documented 57 plants species which belongs to 55 genera and 41 families. Of these famlies, Asteraceae were represented by 4 species (7.123%), followed by Euphorbiaceae, Fabaceae and Rutaceae which is represented by 3 species each. The majority of the species 40 (70%) was gathered from natural habitats while 26% was cultivated and 4% collected from both. The most widely utilized plants are: Trees 19 (33.3%) species, followed by shrubs 18 (31.6%) species, herbs 16 (28.07%) species, and climbers with 3 (5.3%) species. The society also frequently uses plant parts such as fresh plant materials (68%) and leaves (33%). The most widely used route of medicine application was oral (58%), dermal (23%) and nasal (10.5%). The remaining remedies were taken with some other additives and solvents like water, butter, milk as well as honey. Traditional medicines were prepared by pounding (33.3%), and crushing (24.6%). *Carduus schimper* and *Ocimum forskolei* was medicinal plants with higher informant consensus. The disease classes with highest ICF rate (0.93) were fibril illness. The result reveals that there is high preference for *Ficus vasta* for healing Hemorrhoid disease whereas *Cissus cactiformis* was used for treatment of Rabies by traditional medicine practitioner. *Ekebergia capensis* was the highest multipurpose tree species.**

**Key words:** Ethnobotany, Guduru district, traditional practitioner, medicinal plants, ailment.

## INTRODUCTION

Ethnobotany is defined as the study of how people of a particular culture and religion make use of medicinal plants. From the beginning of humanity, indigenous community has developed their own local specific knowledge on plant utililization, protection and management (Cotton, 1996). The study of ethnobotany plays a vital role because of the direct contact that can be established with the authentic information on the uses of

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plants both wild and cultivated. These plants are used for purposes of food, fodder, medicine, clothing, shelter, agricultural implements, hunting, narcotics, poison, gums, dyes, energy, fiber, profits generation and the demand of cultural and spiritual needs throughout the world (Asfaw, 2013). In any case, ethnobotany is broadly defined as the study of interaction and interrelationships among humans and plants (Martin, 1995).

Traditional medicine has remained as the most important and available source of treatment in the primary healthcare system of communities (WHO, 2001). It is practiced throughout the world and depends on locally availabile natural resources and indigenous knowledge (Awas and Demissew, 2009). Particularly, traditional medicine has sustained its popularity in all regions of the developing world where modern medications are limited. Traditional medicine includes a holistic knowledge and practices, oral and written, functional and diagnosis, preventive and curative aspects of illness to promote total well-being (Behura, 2003).

It is estimated that more than 120 of the conventionally used pharmaceuticals were derived from higher plants. Indeed, well into the 20th century, the majority of the pharmacopoeia of scientific medicine was obtained from the herbal lore of indigenous community (Blumenthal, 2003; Toma et al., 2003). Starting from the ancient time, the utilization of traditional medicine has expanded globally and become popular. It has continued to be used for primary health care of the poor in developing countries and also has been used in countries where conventional medicine is predominant in the national health care system. According to WHO, herbal medicines serve the health needs about 80% of the world's population, particularly for millions of people in the immense rural areas of developing countries (Agisho et al., 2014).

It is obvious that the majority of countries in Africa, Asia and Latin America utilize traditional medicine to meet some of their primary health care demands. Particularly in Africa, up to 80% of the population uses traditional medicine for primary health care (WHO, 2001, 2003). Ethiopia is rich in biodiversity with different topographies, agro-ecology and various ethnic-cultures. Therefore, ethnobotanical study is crucial in prosperous biological resource areas for medicinal plants description, identification, documentation, ranking, protection, and sustainable usages (Abera, 2014). Ethiopian plants have shown extraordinary effective medicinal values for many disease treatments that affect people and livestock. About 80% of the Ethiopian peoples were thought to be dependent on traditional medicine for their healthcare system which is obtained from plants. However, indigenous knowledge of medicinal plants is hastily diminishing, because of the influence of western lifestyles, reduction in the number of traditional healers and unwillingness of the younger generations to work on the tradition and associated knowledge (Buwa, 2012) Ethiopia has high diversity of plant species (6500 to 7000 species of higher plants) making the country one of the most diverse floristic regions in the world. Most of these plant species are used in traditional medicine and 60% of plants are said to be indigenous with their healing potential (Edwards and Asfaw, 1992). The remaining medicinal plant of Ethiopia which mainly exists in explored areas still awaits further scientific investigations (Belayneh et al., 2012). In Ethiopia, traditional remedies represent integral components of the cultural beliefs, attitude of people as well as the struggle to fulfill their essential drug demands of people (Koduru et al., 2006).

The study was executed in Guduru district on culturally used medicinal plants, to document about medicinal plants and provide information for the society and other higher researchers about the part of the plants (structures) used as medicine, mode of preparation, dosage, amount used, and disease treated. In this district, cultural medicinal plants were used for many years, since there was shortage of health care service (clinics) and increasing price of commercial (synthetic) drugs people relieve from illness by using cultural medicine. Therefore, the objective this investigation was to file traditional medicinal plant species and associated indigenous knowledge used to treat human ailments in Guduru District, Horo Guduru, Wellega Zone, Western Oromia, Ethiopia.

## METHODOLOGY

### Description of the study area

The experiment was carried out in Guduru district, Horro guduru, Wellega Zone, Western Oromia, Ethiopia. Guduru district is located at about 372 km west of Addis Ababa, the capital city of Ethiopia and 67 km from the zonal administrative town (Shambu). Guduru district is absolutely located between 9°35' to 9°45' North and 37°24' to 37°44' East. It has an area coverage of 140869.069 hectar (CSA, 2007). The zone is characterized by various land forms like mountains (3%), plateau (57%), plain (40%), hills, and valley with altitudinal ranges between 1500 and 2350 m above sea level (GDLEPO, 2016). The study area is bordered by the Abbaya and Hababo Guduru and Fincha Damp North, Jimma South, Jimma Genet District West and East Gindeberet District of Western Shoa Zone (Figure 1). The district is classified into three agro-climate zones: Dega (18%), Woinadega (62%), and Kolla (20%). There is only one dominant soil type in the study area, commonly known as red soil and therefore, other types of soil are rare to be found. The study area receives about 1450 to 2500 mm rainfall annually. In addition, Guduru district is generally hot during winter seasons with the maximum temperature of 22°C and minimum temperature of 19°C (GDLEPO, 2016).

Eighty five percent of the whole land is used for agricultural activities from which the forest land supports 74.6% of the entire inhabitants of the district. Furthermore, the district was known by fast population growth, which accounts for about 2.9% annual growth rate. Currently, the entire population of the district is 128,041 having 63,765 male and 64,276 females (CSA, 2007). According to the report of Guduru District Health Office (2016), the first ten major diseases in the area are: internal parasites, malaria,

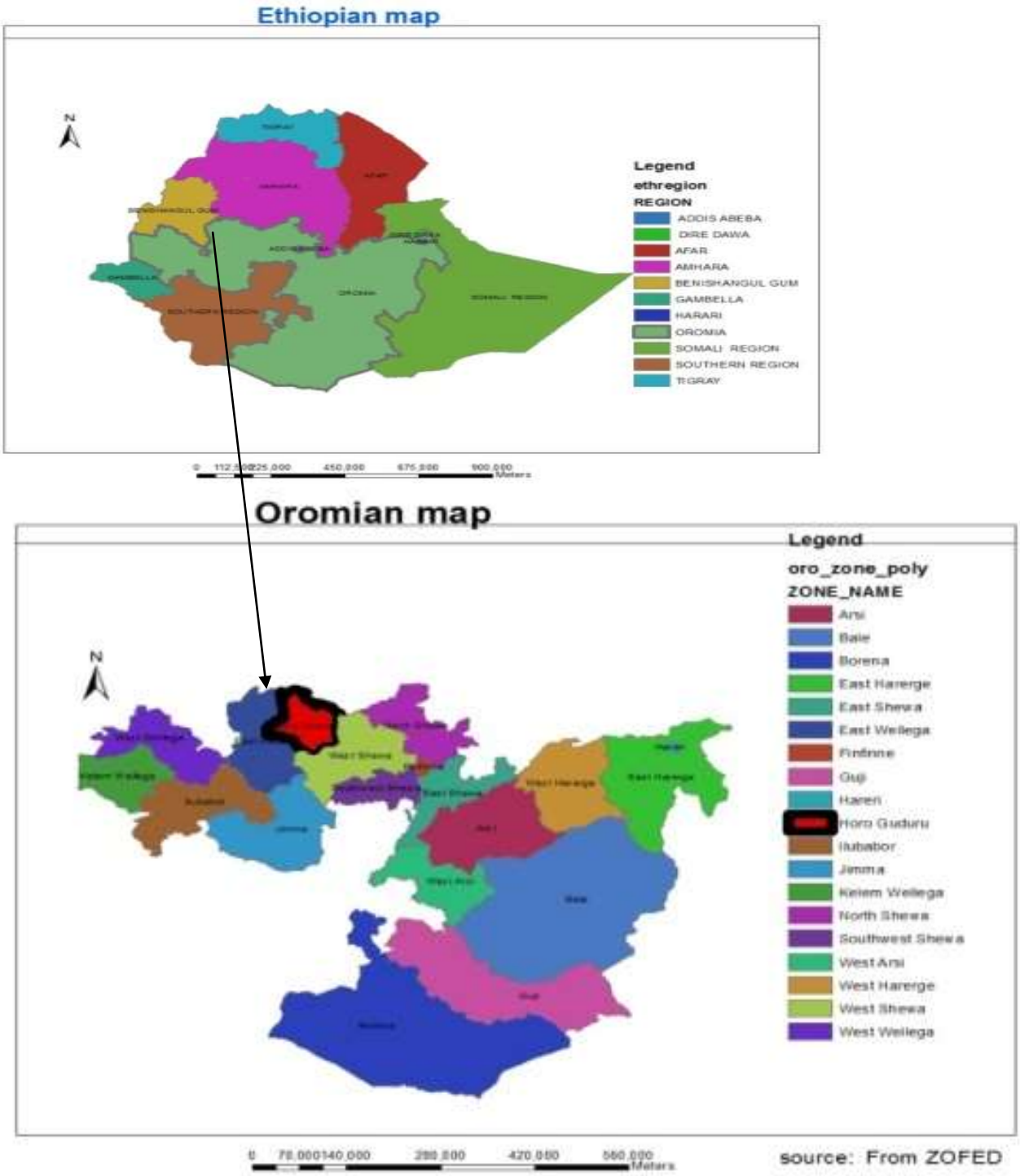


**Figure 1.** Map of Guduru District, Horo Guduru Zone, Oromia Regional State, Ethiopia.

diarrhea, eye disease, gastritis, wound, skin diseases, rheumatism, tonsillitis and sexually transmitted diseases (STDS). These diseases mostly affect people living in the rural areas where limited number of health services is available and the local communities are unable to afford the high cost of modern drugs and because of being far from health services. In the district, there are 7 health centers, 37 health stations, and 9 private clinics, but no hospital.

**Site selection**

Totally, there are thirty seven kebeles in Guduru district. From Dega, one kebele (20% out of seven kebele), weyinadega, five kebele (20% out of twenty kebele) and kola one kebele (20% out of six kebele) were selected based on altitudinal relative variation of the seven kebeles. Accordingly, Tokuma biya, Aga, Refgudane,

Walkituma, Gemechisa Bereji, Nubariye efe, and Gudane sonbo wako were considered from thirty seven kebeles. From these kebeles, Tokuma biya (Sonbo, Buru and Hula Ayele), Aga (Karu, Kajes and Gumja), Reef gudane (Agamsa Daso, Agamsa, and Lencha), Welkituma (Laaluu dogomaa, Birbirsa and Ciracha), Gemechisa Berji (Rare Amanuel, Gudane Gutu and Retane Yedal), Nubariye efe (Malka Naga Anchabbi and Kolba), and Gudane sonbo wako (Weljalechisa keru, Walin tane wabo and Gute ararame) were used due to their resourcefulness and altitudinal variations.

**Selection of informants**

A total of 92 informants' (that is, 48 men and 44 women) in the age group of 20 and above were selected from seven kebeles. Out of these, three key informants per kebele, a total of 21 key respondents (that is, from development agents, health post professionals and kebele leaders) were purposively chosen following Martin (1995). Seventy one informants were selected randomly by tossing a coin from the resident community of the district to understand their general indigenous knowledge on locally available medicinal plants. The residents can be considered as informer when numbered side of the coin was up and if he/she was showing interest to reveal their indigenous knowledge.

**Data collection**

Ethnobotanical data was collected from February 01 to 30, 2016 and March 1 to April 15, 2016, following Cotton (1996) and Martin (1995). Data collection tools such as questionnaires, semi-structured interview, observations, group discussion, and guided field walks had been employed to obtain indigenous knowledge of the local peoples on medicinal plants utilization, conservation status and threatening factors to the medicinal plants. Medicinal plants name mentioned by each informant both from wild and home gardens were gathered, pressed, dried, and taken to the National Herbarium of Addis Ababa University for identification. Finally, the collected data were summarized and analyzed using descriptive statistics. Data on local names of medicinal plants, parts used, methods of preparation, ailments treated, route of application, amount used, medicinal value, and management methods were recorded at the spot. Discussions were conducted with 28 (30%) of the respondents and local people seeking to understand the traditional medicinal plants use, management, and protection to know how indigenous knowledge is maintained and transferred to the younger generations.

**Data analysis**

Both qualitative and quantitative ethnobotanical data were collected and subjected to a descriptive statistical and spreadsheet 2007 to analyze and summarize the data on medicinal plants use and associated indigenous knowledge. Accordingly, multipurpose uses of traditional medicinal plants, proportions of variables like growth forms (habits), plant families, parts used, methods of preparation and administration were determined.

**Informant consent/agreement**

Respondents were contacted atleast two times for the same issue to check the validity of the information during the interview and finally, the validity of the information was proved and recorded. Following this, if the information is contradicting with the original information, it was rejected due to unreliability since only the applicable one was statistically analyzed (Alexiades, 1996). Likewise, factor of agreement (consent) was quantitatively analyzed for thirteen groups of medicinal plant uses reported by respondents. The Informant Censent Factor (ICF) was considered for each category to identify the agreements of the respondents on the reported cures for the ailments of both human and livestock. The ICF were calculated as NUR-NT/NUR (Kefalew et al., 2015), where NUR is a number of used citations in each category and NT is the number of species used. Always, the factor provides a range of 0 to 1, whereas the higher value acts as a good indicator for a high rate of respondents' agreement.

**Ethnobotanical preference ranking**

Ethnobotanical preference ranking was carried out following Martin (1995). Accordingly, the respondents recognized the six most important medicinal plants used in treating hemorrhoid, as traditional practitioners treat it frequently. Ten key traditional healers were chosen to distinguish the most-preferred medicinal plant species used for treatment of hemorrhoid. Each respondents was provided with six medicinal plants reported to cure this disease with each leaf of medicinal plant used being paper tagged name, and asked to assign the highest value six for plant species most preferred) and the lowest value one for the least preferred plant and in accordance of their order for the remaining ones. Finally, these values were collected and ranks were given to each plant species used against this illness.

**Ethnobotanical paired comparison**

According to Martin (1995), paired evaluation can be used to understand the degree of preferences or levels of importance of certain selected medicinal plants/parts of plants used. A pair of selected plant specimens with all possible combinations was presented to selected respondents and their responses were recorded and the value was summarized. To this effect, ten respondents were used to show the efficacy and status of five medicinal plants species used to treat the most frequent disease in the study area and rank was made based on the report of the respondents.

**Direct matrix ranking**

Multipurpose use of medicinal plants and the extent of its utilization versus dominance were done following Martin (1995). Based on information gathered from respondents, seven multi-use tree species were chosen from the total medicinal plants and eight use values of these plants were listed. These use-values include healing, forage, food, firewood, construction, charcoal, fencing, and furniture making. Fifteen key respondents were selected out to conduct this activity and each key informants was asked to assign the following use values (5=very high, 4=high 3=very good, 2=less used, 1=least used and 0=not used) for eight multipurpose medicinal plant species. Accordingly, the average value of each use-diversity for a species was taken and the values of each species was summarized and used for ranking.

**Fidelity level**

The fidelity levels (FL) were calculated for those frequently reported diseases by informants so as to identify the most important species. Two locations, different in altitude as well as in the prevalence of disease were selected to reveal the fidelity stage of the most frequently reported medicinal plants and the disease treated.

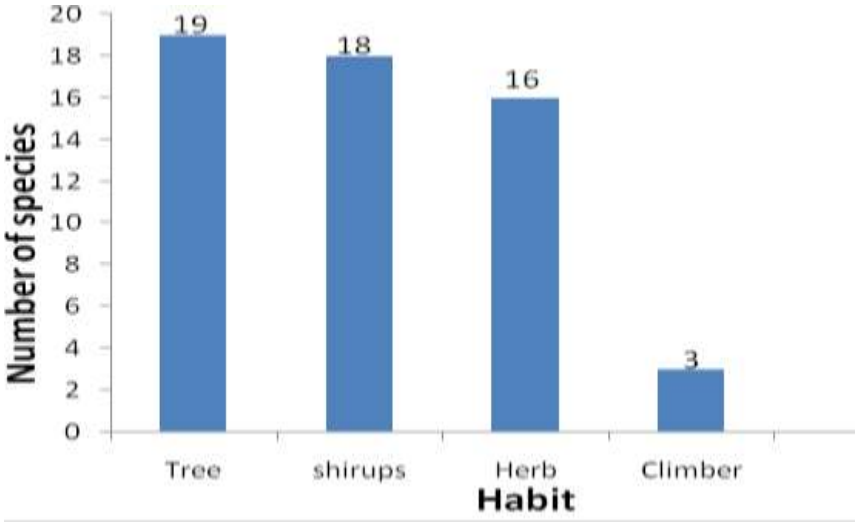


**Figure 2.** Types of medicinal plants used for human ailment treatment.

Therefore, seven study sites in which the disease is most and/or list frequent were selected and the fidelity level is calculated as: FL = Ni/N × 100, where Ni is the number of respondents who claims the use of a plant species to treat a particular disease whereas N is the number of respondents that use the plant as a medicine to treat any given disease. Therefore, fidelity level is designed to quantify the importance of the species for a particular purpose (Alexiades, 1996).

## RESULTS AND DISCUSSION

### Medicinal plants documented from the study area

Generally, a total of 57 medicinal plant species belonging to 55 genera and 41 families were documented from the study area. From these, the family Asteraceae was represented by the highest number of species 4 (7.12%), pursued by Euphorbiaceae, Fabaceae, Rutaceae (3 species, 5.26% each); Solanaceae, Rutaceae, Rubiaceae, Rosaceae, Oleaceae, myrsinaceae, Moraceae, and Lamiaceae (2 species, 3.51%) of each and the remaining families represented by one species each. The majority of the plants reported as medicinal plants in this study were also reported as having medicinal properties by different people from different parts of Ethiopia. For instance, Megersa et al. (2013) reported 29 species from similar studies in East Wollega, Meresha. Ashagere et al. (2016) reported 24 species from Lulekal et al. (2013) reported 37 species of medicinal plants from North Shewa Zone. The fact that the same plant species were reported from various parts of the country for medicinal properties may suggest that actual remedial potential of these medicinal plants and information flow between different localities of the country on some medicinal plants. As far as the habitat is concerned, the bulk of medicinal plant species were gained from wild vegetation 40 (70%), followed by cultivated medicinal plants 15 (26%) and for both 2(4%). The finding also quite agrees with that of Eshete et al. (2016), who studied medicinal plants of the Guji, Blue Hora District, Borana Zone, Oromia Regiona State, Ethiopia. This indicates that the community largely relies on wild plants; which as a result indicates the existence of higher threats on the wild medicinal plants.

### Plant habit, part(s) used and preparation

The present study result reveals that, the largest proportion of medicinal plants represented by trees 19 (33.3%) followed by shrubs 18 (31.6%), herbs 16 (28.07%), and climbers 3 (5.3%) (Figure 2). The same habit distribution of medicinal plants has been reported by Getaneh and Girma (2014). In contrast, Hunde et al. (2004) and Giday et al. (2003) reported the presence of higher number of medicinal plants as herbs. Furthermore, the Zay people obtain their medicine from herbs partly due to the fact that forests have been degraded and it takes much time as well as effort to harvest plant material from medicinal trees (Giday et al., 2003).

The present study finding also showed that leaves are the most commonly used plant parts accounting for 33% followed by root and seed (Figure 3). Similar studies also indicate leaves being the most widely used plant part for medicinal purpose (Awas and Demissew, 2009; Teklay, 2015; Seyum and Zerhun, 2014; Yineger and Yewhalaw, 2007). According to Seyum and Zerihun (2014), the ordinary use of leaves in the preparation of remedies could partly be because of the relative ease of finding this plant part. Moreover, it was known that over exportation of root, bark and whole plants might kill plants in harvest. Thus, practice of using leaves may help to minimize the

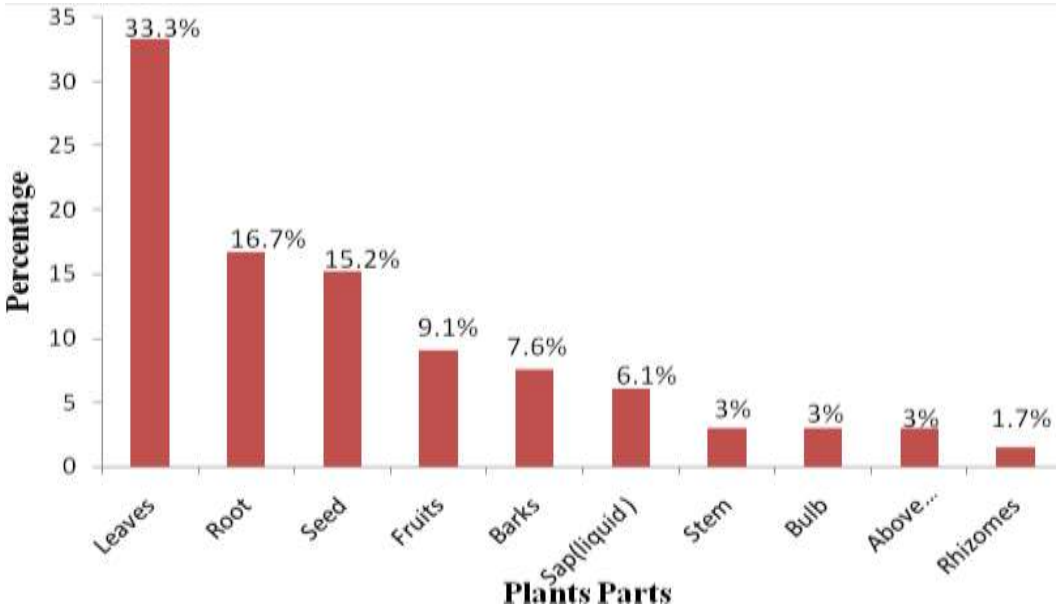


**Figure 3.** Parts of plants used for treatment of human ailment.

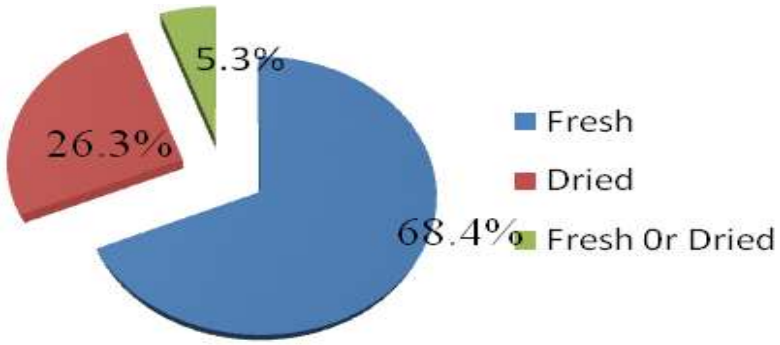


**Figure 4.** Condition of remedy preparation from plant in the study area.

rate of threat on plant species or helps for sustainable harvesting of plants and hence, the survival of the plant will be ensured (Giday et al., 2003). Abebe and Ayehu (1993) reported that, medicinal plants harvest which includes root, rhizomes, bulbs, barks and stems have serious effect on the survival of mother plants.

About 68.4% of the remedies were made from fresh leaves. Relatively, few medicinal plants (26.3%) were used in dried form whereas the remaining very few medicinal plants were used either fresh or dried (5.3%) (Figure 4). Many researcher also observed similar findings elsewhere (Enyew et al., 2014; Teklay et al., 2013; Abera, 2014; Belayneh et al., 2012; Awas and Demissew, 2009). The main reason for the favorite of fresh plants over dried ones may be due to the biologically active chemicals which are present in the leaves may decrease as a consequence of drying.

The majority of surveyed medicinal plants preparations include the use of solitary plant species or part (73.42%) whereas various plant parts (26.58%) were rarely used in the study area. Several study findings also reveals that, the use of multiple plants or plant parts for a single health problem was rare (Balemie et al., 2004; Megersa et al., 2013; Reddy et al., 2009). This result disagrees with the result of some studies which report as a preparation of traditional medicine drawn from mixtures of different plants or plant parts. For instance, local healers of Sokoru, mainly used more than one plant species or parts to prepare remedy for both human and livestock ailments (Yineger and Yewhalaw, 2007). The present study finding reveals that, most traditional practitioners use multiple plants or plant parts in order to increase strength and efficacy of the traditional medicine. For instance, rabies were treated by mixing the leaves of

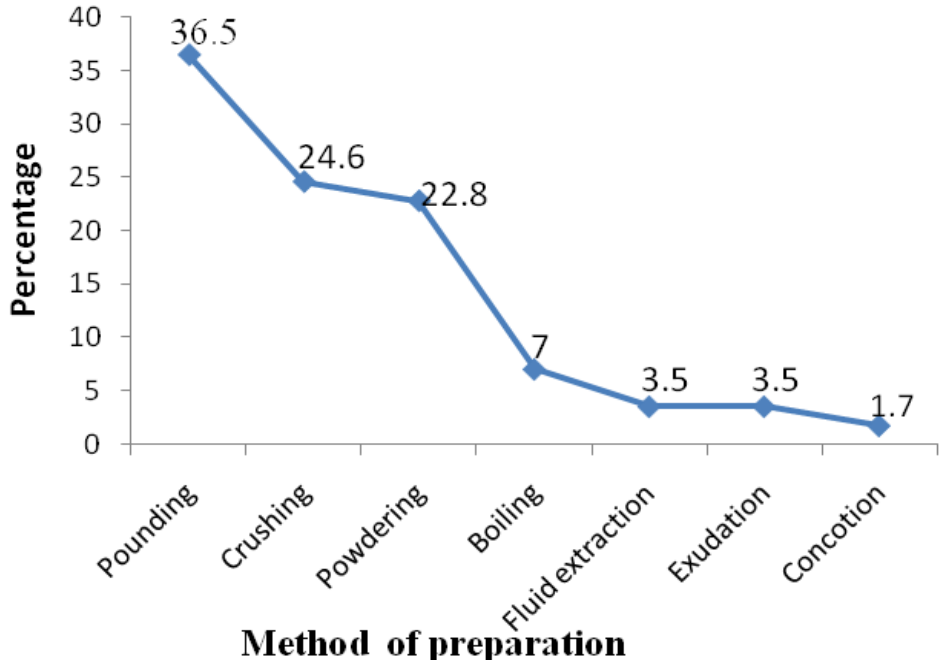


**Figure 5.** Method of remedy preparation from medicinal plants.

*Bersama abyssinica,* root of *Cissus cactiformis,* and root of *Clerodendrum myricoides* together.

Traditional practitioners in the study area also used different type of preparations which include pounding type 36.5%, crushing 24.6%, and powdering 23% (Figure 5). This may be due to the possibility of effective extraction of plant ingredients when pounding, crushing and powdering so that its curative potential would increase. This result was in line with Enyew et al. (2014), Megersa et al. (2013) and Eshete et al. (2016) work. But, disagrees with that of Lulekal et al. (2013) who indicates the dominant use of medicinal plants parts in Ankober district decoctions for various ailments might be related to their proven effectiveness over many years of trial and indigenous knowledge accumulated on efficacy of preparations whereas the frequent use of concoctions could be credited to the belief by many healers of synergic reactions (Dawit, 2001). Furthermore, very dominant method of remedy preparation in the Debre Libanose district was through crushing followed by squeezing (Getaneh and Girma, 2014).

The local people also use some additives in their preparations. For example honey, butter, tella, milk, coffee, sugar, yoghurt, white injera and some plant species such as *Allium sativum* are some of the additives that the local people reported to use them as additives. According to the respondents, these additive substances were assumed to increase the flavor and minimize the side effects of vomiting and diarrhea so that the efficacy of the traditional medicine (TM) would be increased. Similar additives have been reported previously by different studies (Seyum and Zerhun, 2014; Megersa et al., 2013; Abebe, 2011).

## Dosage and administration methods

The community of the study area mostly apply traditional medicine orally (57.9%), dermal application (23%), nasally alone (10.5%) and others (anal, optical, auditory and gum) accounting to 14% (Figure 6). Similar methods have been reported by previous studies (Lulekal et al., 2013; Alemayehu et al., 2015; Teklehaymanot and Giday, 2007). However, research carried out in Kilte Awulaelo district, Northern Ethiopia (Teklay et al., 2013) and Bench District, South-Western Ethiopia (Giday et al., 2009) revealed that, most external application were dermal creaming accounts for the highest ratio or proportion.

There were no uniformity regarding to the dosage of the medicine between the traditional healers, hence, all the traditional healers agree on the point that the dosage given for patient vary with age and physical strength. They also do agree that some medicines were not allowed to be taken by women when pregnant. Amounts to be administered will be estimated by the use of measurement such as length of a finger (that is, for bark, root and stem length), pinch (for powdered plant parts) and number count (for sap drops, leaves, seeds, fruits, bulb, and rhizomes).

The requirements of dosages vary with age, but not considered for gender variations. Dose of decoction is calculated in different ways which include coffee cup

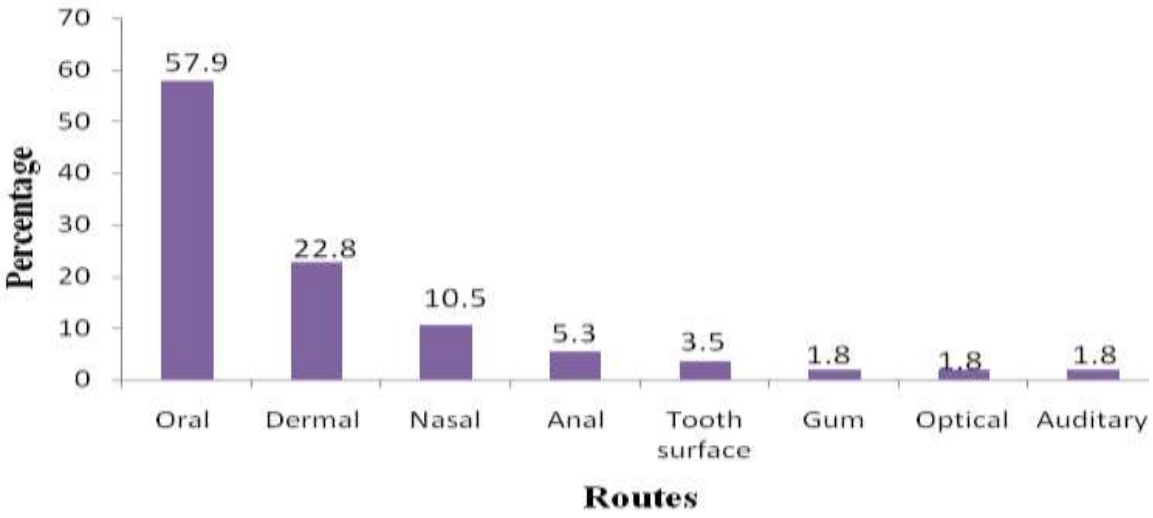


**Figure 6.** Route of administration of traditional medicine in the study area.

**Table 1.** Preference ranking of medicinal plants used for treating hemorrhoid disease.

| Species | Informant labeled from $R_1$-$R_{10}$ | | | | | | | | | | Total | Rank |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ | $R_7$ | $R_8$ | $R_9$ | $R_{10}$ | | |
| *Ficus vasta* | 5 | 6 | 6 | 5 | 5 | 6 | 4 | 5 | 4 | 5 | 51 | 1st |
| *Croton macrostachyus* | 6 | 3 | 5 | 4 | 3 | 5 | 6 | 4 | 6 | 4 | 46 | 2nd |
| *Ekebergia capensis* | 4 | 5 | 3 | 3 | 6 | 4 | 5 | 6 | 3 | 1 | 40 | 3rd |
| *Olea europaea* | 1 | 4 | 4 | 2 | 2 | 3 | 3 | 2 | 5 | 6 | 32 | 4th |
| *Rytigynia negelecta* | 2 | 1 | 1 | 6 | 4 | 2 | 1 | 1 | 2 | 2 | 22 | 5th |
| *Phytolacca dodecandra* | 3 | 2 | 2 | 1 | 1 | 1 | 2 | 3 | 1 | 3 | 19 | 6th |

*R: Represents respondents.

(locally 'Sini'). For instance, one coffee *Vernonia amygdalina* is taken orally for malaria treatment for five days, as well as a cup, alcohol (caticala-in Amharic) cup,' jok' (in Amharic) equal to a liter and a material which is made up of *Lagenaria siceraria* locally called 'wille'. But, these measurements are not accurate enough to determine the precise amount. For medicinal plants that are taken topically, they do not have clear cut dosage or standardized dosing in the application of traditional medicines in Ethiopia and elsewhere (Abebe and Ayehu, 1993; Bekele, 2007; Hizikias et al., 2011; Bekalo et al., 2009). The recovery from disease, which usually was determined by the disappearance of disease symptoms, was the criterion that the local people of this study area consider to determine the duration of using the medicine.

## Ethnobotanical preference ranking

People may show preference of one traditional medicine over the other when different medicines are prescribed for the same health problem. In this study, some cited human diseases were reported to be treated by multiple plant species. Table 1 indicates the preference ranking of six medicinal plants used to treat hemorrhoid disease. Accordingly, *Ficus vasta* scored 51 and leveled the first showing that, it is the most effective in treating hemorrhoid disease pursued by *Croton macrostachyus* and *Phytolacca dodecandra* which is the least effective (Table 1). This indicates that people have alternative plant species to treat a given disease; they do have preference to one over the other based on their long time experience on the relative curative power of the plants.

## Informant consent factor (ICF)

The ailment in the study area have been grouped into different categories based on the site of incidence of the disease, condition of the disease as well as treatment resemblance of the disease to the local people. The informant consensus factors have been calculated for

**Table 2.** Informants consensus factor (ICF) for more prevalent health problems of the study area.

| Categories of disease | Plant species | Percent species | Use citation | Percent use citation | ICF |
|---|---|---|---|---|---|
| Fibril illness, nerve illness | 2 | 3.1 | 15 | 9.3 | 0.93 |
| Evil eye, evil spirit | 2 | 3.1 | 10 | 6.2 | 0.89 |
| Tonsillitis, toothache | 3 | 4.7 | 14 | 8.7 | 0.85 |
| Respiratory infection, common cold | 6 | 9.4 | 26 | 16.1 | 0.80 |
| Ascaries, tape worm, stomachache | 10 | 15.6 | 31 | 19.3 | 0.70 |
| Skin rash (shife), fungal disease | 4 | 6.3 | 8 | 4.9 | 0.57 |
| Rabies, snake bite, bat poison | 8 | 12.5 | 16 | 9.9 | 0.53 |
| Wound, body swallowing | 7 | 10.9 | 13 | 8.1 | 0.50 |
| Hemorrhoids | 6 | 9.4 | 9 | 5.6 | 0.38 |
| Organ problem (Eye, Ear, and Liver) | 5 | 7.8 | 7 | 4.3 | 0.33 |
| Fire burn , bleeding | 4 | 6.3 | 5 | 3.1 | 0.25 |
| Sexually transmitted diseases | 3 | 4.7 | 3 | 1.9 | 0 |
| Malaria | 4 | 6.3 | 4 | 2.5 | 0 |

**Table 3.** Paired evaluations of five medicinal plant species used to treat Rabies.

| Plant species | Respondents (R1-8) | | | | | | | | | | Total | Rank |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 | | | | |
| *Cissus cactiformis* | 4 | 5 | 3 | 4 | 5 | 3 | 4 | 2 | 5 | 4 | 39 | 1st |
| *Brucea antidysenterica* | 3 | 2 | 2 | 5 | 4 | 5 | 3 | 1 | 4 | 5 | 34 | 2nd |
| *Bersama abyssinica* | 5 | 3 | 4 | 1 | 3 | 4 | 2 | 4 | 2 | 3 | 31 | 3rd |
| *Ricinus communis* | 2 | 1 | 5 | 2 | 2 | 2 | 1 | 5 | 3 | 2 | 25 | 4th |
| *Scandxus multiflorus* | 1 | 4 | 1 | 3 | 1 | 1 | 5 | 3 | 1 | 1 | 21 | 5th |

each category (Table 2). The results of the study revealed that, diseases that are common in the study area have higher informant consent factor. It is further shown that medicinal plants known by the local community members and assumed to be effective in curing certain diseases will have higher ICF values (Table 2) with the ICF values ranging from 0.93 to 0 per sickness category. Fibril illness had the highest ICF value of 0.93 due to the higher incidence of the disease in the study area whereas sexually transmitted diseases (STDs) and malaria had the lowest (0) may be due to the rare occurrence of these diseases and the fact that most are successfully treated by local healers.

## Paired comparison

A paired evaluation was made to decide the most favored medicinal plants among the five species that were used as remedies to treat rabies disease in the study area. The responses of 10 key respondents indicate that, *C. cactiformis* ranked primary followed by *Brucea antidysenterica*. Therefore, this result indicated that *C. cactiformis* is the most preferred while *Scandxus multiflorus* is the least privileged in treating rabies disease (Table 3).

## Ethnobotanical direct matrix ranking

Medicinal plants of the study area have been found to have several purposes other than medicinal uses. The key respondents of the study area identified seven medicinal plant species that were used by the local communities for extra function such as charcoal, firewood, buildings, fencing, forage, food, and medicine. The result of the matrix ranking revealed that, *Ekebergia capensis* ranked first followed by *Cordia africana, Eucalyptus globules, C. macrostachyus, Acacia abyssinica, Olea europaea* and *Carissa spinarum* (Table 4). This result indicates that *E. capensis* and *C. africana* appear to have more demand than the others as they were used for more diverse purposes. Although *E. globules* was known to relatively have diverse use next to *E. capensis* and *C. africana,* it is less threatened of over exploitation as it is regularly planted and managed by human. The direct matrix ranking result also shows that the local society harvest the seven multi-use plant

**Table 4.** Direct matrix ranking for seven plant species and main use in study area.

| Plant species | Use categories | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Charcoal | Construction | Fencing | Forage | Food | Fire wood | Furniture | Medicine | Total | Rank |
| *Ekebergia capensis* | 3 | 5 | 3 | 4 | 0 | 5 | 5 | 4 | 29 | 1 |
| *Cordia africana* | 4 | 5 | 1 | 2 | 1 | 4 | 5 | 3 | 25 | 2 |
| *Eucalyptus globules* | 3 | 5 | 5 | 1 | 0 | 4 | 2 | 3 | 23 | 3 |
| *Croton macrostachyus* | 5 | 1 | 3 | 1 | 0 | 4 | 3 | 5 | 22 | 4 |
| *Acacia abyssinica* | 1 | 1 | 3 | 3 | 1 | 4 | 2 | 4 | 19 | 5 |
| *Olea europaea* | 2 | 0 | 5 | 1 | 2 | 3 | 0 | 4 | 17 | 6 |
| *Carissa spinarum* | 1 | 1 | 4 | 1 | 1 | 5 | 0 | 3 | 16 | 7 |
| Total | 19 | 18 | 24 | 13 | 5 | 29 | 17 | 26 | 151 | - |
| Rank | 4th | 5th | 3rd | 7th | 8th | 1st | 6th | 2nd | - | - |

species mostly for fire wood followed by medicinal use, fencing, charcoal, construction, furniture, forage and food (Table 4).

## Fidelity level index (FLI)

Fidelity level is a crucial means to determine which plant species has more healing power. Accordingly, those species with high FLI are supposed to be more curative for the respective ailments. Fidelity level value in this study varied from 41.2 to 100% (Table 5). Therefore, traditionally used medicinal plants with high FLI value can be a focus for further pharmacological studies.

## Threats to medicinal plants and associated indigenous knowledge in the study area

The cause of threats to medicinal plants can be generally grouped into natural and human induced factors. However, anthropogenic factors such as deforestation due to over exploitation of plants for different uses including cutting and burning of plants to create new agricultural lands, charcoal making, fire wood collection, collection of construction woods, overgrazing, and climatic change were the most common treats for medicinal plants and associated indigenous knowledge. The respondents agree that, agricultural expansion was the primary threat to the medicinal plants pursued by firewood, charcoal collection and lower levels of threats by the other factors. Similar result was found in Blue Hora District of Borana Zone that fragmentation and destruction of their habitats due to agricultural expansion and overgrazing were the main threats (Eshete et al., 2016). Furthermore, the negative impact of deforestation on medicinal plants was also reported (Yirga, 2010).

On the other hand, except some plant species that are commonly known for their medicinal properties, knowledge on most medicinal plants, and known by healers were hidden from general public. Healers never show live plants and the name of traditional medicinal plants to their patients. Because of their believe that showing medicinal plants will incapacitate the healing power of the medicine as well as to avoid competitors in earning income from treating patients. Traditional healers earn income from their knowledge on medicinal plants.

The study indicates that introduction of modernization such as schooling and new religion influenced the acculturation and negligence of the present generation to acquire the knowledge and facilitated the threat to biological resources and associated indigenous knowledge in the study area. Similar finding was reported elsewhere (Hunde et al., 2004; Giday et al., 2003).

However, it was recognized that ethnobotanical knowledge on uses of some medicinal plants was transmitted to one or few family members to use in secrecy. They disclose their knowledge on medicinal plants at old age by the time when they most probably die before teaching the detail of medicinal plants. Ethnobotanical investigation done elsewhere in Ethiopia (Giday et al., 2003; Bekalo et al., 2009) shows that, elders are the owner of herbal remedies knowledge and modernization has a great influence to use traditional medicinal plants. Therefore, a number of combined factors mentioned earlier have

**Table 5.** Fidelity level value index of traditional medicinal plants to treat diseases.

| Medicinal plant | Treated diseases | Ni | N | FL (%) |
|---|---|---|---|---|
| *Rytigynia negelecta* | Hemorrhoid | 8 | 8 | 100 |
| *Aioa rivae* | Fire burned | 11 | 11 | 100 |
| *Solanum gigantum* | Wart | 5 | 5 | 100 |
| *Plantago lanceolata* | Bleeding | 13 | 13 | 100 |
| *Brucea antidysenterica* | Ascaris | 10 | 11 | 90.9 |
| *Phytolacca dodecandra* | Liver problem | 9 | 11 | 81.8 |
| *Vernonia amygdalina* | Gonorrhea | 3 | 4 | 75 |
| *Ricinus communis* | Tonsillitis | 4 | 6 | 66.7 |
| *Justicia schimperiana* | Skin infection | 9 | 17 | 52.9 |
| *Allium satvum* | Stomachache | 8 | 16 | 50 |
| *Croton macrostachyus* | Rabies | 7 | 17 | 41.2 |

resulted in loss of medicinal plant species which requires a great attention of government, NGOs and private sectors to rehabilitate and conserve the remaining vegetation in general and medicinal plants in particular with their associated indigenous knowledge.

## CONCLUSION

The medicinal plant species gathered and identified from the wild vegetation are 40 species, 15 species cultivated and 2 from wild or cultivated. Forty one human diseases were healed by traditional medicinal plants of the study area. Herbal remedies were prepared from fresh materials (68.4%), dried plant materials (26.3%) and fresh or dried (5.3%). Trees are highly utilized for medicinal purpose than herb and shrubs. Leaves (33.3%) were the most commonly used for medicinal purpose as compared to other plant parts for preparation of human remedies. The remedies were taken with different additive and solvents and water is more frequently used for this purpose. Most of the medicinal plants are administered orally (57.9%).

The major challenges to medicinal plants and associated indigenous knowledge in the study area include agricultural expansion, firewood collection, charcoal production, climatic change, construction and over grazing. Whereas threats that erode indigenous knowledge come from secrecy, oral based knowledge transfer, refusal of young next generation to gain the indigenous knowledge, unavailability of the species, influence of modern education and lack of awareness were the major ones.

Therefore, it is possible to conclude that, awareness creation activities should be needed to improve local community's knowledge on the importance, conservation and management of medicinal plants as well as among the healers to avoid erosion of the indigenous knowledge and to ensure its sustainable use.

## RECOMMENDATIONS

Founded on the findings of the present study, the following recommendations have been forwarded:
(1) Local people should be informed about the use value, management and conservation of medicinal plant of their locality.
(2) The district agricultural workers must involve in identifying medicinal plants and encouraging the local people to cultivate medicinal plants in their home gardens.
(3) Recognition and rational property right should be given to traditional practitioners, through certification to popularize their indigenous knowledge on medicinal plants.
(4) One way of preserving such important indigenous knowledge in the new generation is through integration to school about medicinal plants.
(5) The lesson of medicinal plants and conservation of indigenous knowledge should be included in school curriculum.
(6) Deforestation is still a problem in district of Guduru natural forests. So, the administrative body should take care of the natural habitats.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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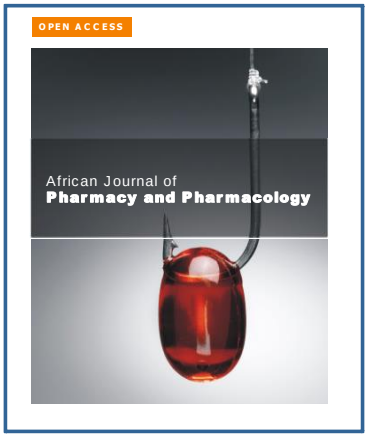







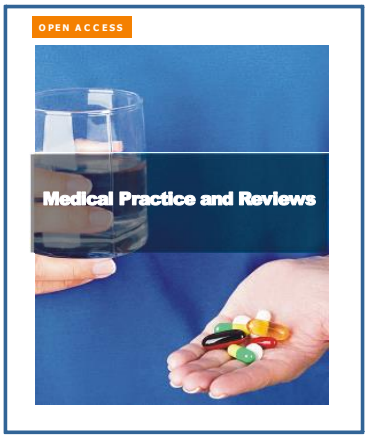


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