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ARTICLES

Secondary metabolites from endophytic fungus from *Lippia sidoides* Cham.  
Talita Pereira de Souza Ferreira, Gil Rodrigues dos Santos, Ilsamar Mendes Soares, 
Sergio Donizeti Ascêncio, Tarso da Costa Alvim, Chrystian de Assis Siqueira and 
Raimundo Wagner de Souza Aguiar  

307

Ethnobotanical study of ethnoveterinary plants in Kelem Wollega Zone, Oromia Region, 
Ethiopia  
Tolera Fufa Feyissa, Moa Melaku shigut, Tilahun Bekele Hailemariam, Tena Regassa and 
Nebiyu Kassa Kergano  

296
Secondary metabolites from endophytic fungus from *Lippia sidoides* Cham.

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**Lippia sidoides** Cham. (Verbenaceae) is a species native to the Brazilian northeast, widely used in popular medicine. Its leaves were used for the isolation of endophytic fungi and extraction of metabolites. Among them, three were selected according to fungitoxicity tests against the maize phytopathogenic fungus, *Curvularia lunata* (Wakker). However, the objective of this study was to identify the role of *L. sidoides* extracts associated with their endophytic fungi, necessary to reduce excess of fungicides applied on the maize crop. Metabolites were evaluated for antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH), phenols, total flavonoids and one of it endophytic fungus were evaluated for synergism (*Verticillium* sp. and plant extracts). The endophytic fungi and plant extracts evaluated for phenolic content ranged from 0.29 ± 0.05 to 96.94 ± 11.86 mgEAG/g, the content of flavonoids from 14.31 ± 1.56 to 192.33 ± 4.58 mgER/g, and antioxidant activity could only be observed for the plant extract with EC₅₀ 81 ± 0.3%. The secondary metabolites identified by HPLC in the plant extract were catechin, quercetin, gallic acid and naringin. Naringenin, catechin, epigallocatechin gallate and quercetin were identified in the extract of the fungi viz. *Verticillium* sp. and *Fusarium* sp. Synergistic analysis between a 1:1 proportion of plant and fungal extracts has shown more efficient (79.0%) inhibition of *C. lunata*. Thus, alternative control of phytopathogenic fungi can be accomplished using plant extracts associated with their endophytic fungi, reducing the excess of fungicides applied on the maize crop.

**Key words:** *Curvularia lunata*, endophytic fungal, HPLC, extract, *Verticillium* sp., *Fusarium* sp., *Colletrotrichum* sp.

**INTRODUCTION**

Endophytic fungi isolated from plants belonging to tropical regions produce more active secondary metabolites than those from temperate regions, because they are exposed to an environment of greater biodiversity (Bhardwaj et al., 2015). These fungi are recognized as important new sources of bioactive compounds for applications in agriculture, medicine, and the food industry (Yadav et al., 2014b; Sadananda et al., 2011). Over time, some endophytic fungi have developed the ability to produce the same or similar bioactive
substances to those produced by host plants (Sharma et al., 2016). This lends a great advantage to the study of the relationships between endophytes and their host plants (Fouda et al., 2015). Nevertheless, most of the time, these valuable bioactive compounds are scarce in nature; therefore, the development of a substitute approach for their efficient production is warranted (Kusari et al., 2013).

More recent estimates based on high-throughput sequencing methods suggest that as many as 5.1 million fungal species exist (Taylor et al., 2014). The estimate of known species has almost tripled in the period between 1943 (38000 described species) and the present, amounting to an increase of more than 60000 described species (Blackwell, 2011). Accordingly, endophytes are good sources of genetic diversity and new species belonging to a large group of fungi that colonize tissues of healthy plants without causing apparent symptoms (Fatima et al., 2016). They protect plants against herbivores, insect attack, or invading pathogens by entering this mutually beneficial relationship, according to host habitat conditions (Bhardwaj et al., 2015).

Bioactive products that can be produced by endophytes can be classified as saponins, phenols, flavonoids, steroids, tannins, alkaloids, anthraquinones, terpenoids, and others (Li et al., 2015). These compounds defend the plant against phytopathogenic fungi. For example, flavonoids are well-known substances, and their production involves a variety of processes such as cell signaling, plant growth, and reproduction (Garrido-Arandia et al., 2016).

*Lippia sidoides* Cham. (Verbenaceae), also known as “rosemary pepper” is an aromatic plant of popular medicinal use found in the Caatinga region of the Brazilian Northeast (Santos et al., 2016). It presents insecticidal activity against *Tenebrio molitor* (Lima et al., 2011) and larvae of *Aedes aegypti* (Lima et al., 2013) and acaricide against *Tetranychus urticae* Koch (Soares et al., 2016). When tested against different pathogenic bacteria, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* (Ratnaweera et al., 2015) and different fungi and yeasts, including *Candida albicans* (Premjana et al., 2016), it demonstrated a strong antimicrobial action. Thus, compounds produced by endophytic fungi of plants with recognized biological activity for biotechnological purposes can be applied in the alternative control of urban and agricultural pests (Dutta et al., 2014), in place of the toxic chemicals sold on the commercial market.

On the other hand, the *Curvularia* spot, a disease caused by the fungus *Curvularia lunata* (Wakker) Boed., has a high incidence in maize (Zeae mays L.). Since the 1990s, it has caused economic loss in maize crop productivity in China (Gao et al., 2015) and has been progressing in recent years in Brazil (Assunção et al., 2006). The pathogen mainly infects maize leaves, leaf sheaths, and husks and causes soaked or yellow necrotic spots in the early stages, which then expand to round, oval, spindle-shaped, or strip lesions (Hou et al., 2013). The green leaf area of maize is considered the main source of photoassimilates for the plant (Akram et al., 2014) and, according to Liu et al. (2016), a loss in this source may reflect the development of the plant and the production of grains.

In this paper, the objective was the isolation and identification of the endophytic fungus from *L. sidoides*. To this end, antioxidant activity, phenols and total flavonoids, and the evaluated metabolites were identified by HPLC. The extracts of endophytic fungi and plants were evaluated by the selective test of fungitoxicity and synergism against *C. lunata*.

**MATERIALS AND METHODS**

**Collection of the vegetal material and preparation of the vegetal extract**

*L. sidoides* from Ceará was collected in Gurupi (11°44′48″S latitude, 49°02′55″ Longitude O), Tocantins, Brazil. It was then identified and deposited at the Herbarium of the Federal University of São João Del Rei under the reference number 8303. The plant material was sent to the Laboratory Integrated Pest Management of the Gurupi Campus–UFT, where the extracts were dried and obtained according to methodology of Al-Marby et al. (2016). The leaves were dried in the shade at room temperature, subsequently cut and subjected to extraction with cold solvent. Thirty grams of plant were used for 1.5 L of methanol in each extraction for a period of 7 days. After this period, the mixture was filtered and evaporated under reduced pressure to obtain the extracts.

**Cultures of endophytic fungi**

For isolation, fresh leaves of *L. sidoides* were surface washed in tap water and soap to remove impurities. As described by Banhos et al. (2014), leaves were successively immersed in 70% alcohol (1 min), 2.5% sodium hypochlorite (4 min), and 70% alcohol (30 s), followed by washing with autoclaved distilled water (6 min) and drying on sterile filter paper. After washing, 50 μL of the distilled water used in the asepsis of the plant material was removed, then this volume was added (inoculated) in a Petri dish with potato–dextrose–agar medium. To verify the efficiency of the asepsis process. The leaves were fragmented and inoculated into 9-cm diameter Petri dishes containing PDA culture medium. To the culture medium were added 50 μg/mL tetracycline or 100 μg/mL chloramphenicol (antibiotics). For the isolation of pure cultures, sequential peaks were made in Petri dishes containing the same culture medium, using the streaking technique. Subsequently, they were stored in tubes for centrifugation with glycerol at -80°C for the...
Curvularia lunata strain

C. lunata fungus was obtained from Federal University of Tocantins, in our laboratory culture collection, previously identified based on morphological and molecular characters. The initial isolation of the fungus was obtained by growth on PDA medium (39 g L\(^{-1}\)) supplemented with ampicillin (500 mg L\(^{-1}\)) for 7 days. Petri dishes were monitored daily and fungal colonies that did not present contaminants were picked and transferred onto new plates with the same culture medium.

Extraction of secondary metabolites of endophytic fungi

Two 7-mm diameter mycelial agar discs of the endophytes were inoculated into 200 mL of Czapek fermentation medium to which was added 100 μg mL\(^{-1}\) chloramphenicol (Plotnikov et al., 2016). After 21 days of incubation under constant stirring (120 rpm) at 28 ± 1°C, the culture fluids were separated from the mycelial masses by vacuum filtration, and the extract was obtained following the methodology of Dhandkar et al. (2012). For extractions, organic solvents such as hexane and ethyl acetate were used in order to obtain the largest number of active substances.

Determination of total phenolic compounds

The content of phenolic compounds of both the plant extract and three extracts of the endophytic fungi with the best results for fungitoxic activity was determined by the Folin-Ciocalteu method, according to Sánchez-Rangel et al. (2013) with modifications, where gallic acid was used as the standard. Methanol solutions of the extracts were prepared at a concentration of 1 mg mL\(^{-1}\). Then, in 15 mL vials, 0.5 mL of the extract, 5 mL of Milli-Q water, 0.5 mL of Folin-Ciocalteu reagent (1 M) and 0.5 mL of sodium carbonate were added (25%, m/v). The blank was prepared by placing 0.5 mL of distilled water in place of the Folin-Ciocalteu reagent. An analytical curve was also prepared for the standard gallic acid reagent at concentrations from 10 to 100 μg mL\(^{-1}\). For the blank (reference solution) of the curve, 50 μL of distilled water was added instead of gallic acid. All tubes were homogenized with the aid of the test tube agitator. All reactions were performed in triplicate and kept under dark incubation for 1 h. The readings were performed in a spectrophotometer (BioSpectro model SP-220) at 765 nm (Zhou et al., 2009). The results obtained by spectrophotometric analyses of total phenols were expressed as milligram equivalents of gallic acid per gram of extract (mgEAG/g).

Determination of total flavonoids

The determination of the total flavonoid content was performed according to the methodology developed by Da Silva et al. (2015). The solutions of the plant extract and the three extracts of endophytic fungi with the best results in the fungitoxic activity were prepared using 1 mg mL\(^{-1}\) of methanol as the solvent. Then, in a 15-mL vial filled with aluminum foil, 0.5 mL of the extract, 0.5 mL of aqueous acetic acid (60%, v/v), 2 mL of pyridine methanolic solution (20%, v/v), 1 mL of aluminum chloride (5%, w/v) and 6 mL of Milli-Q water were combined. The blank (reference solution) was made with all the above-mentioned reagents replacing the aluminum chloride with methanol. All reactions were carried out in triplicate and homogenized in a tube shaker. The reactions were maintained for 1 h in the dark, and the absorbances were measured at 420 nm in a spectrophotometer (BioSpectro model SP-220). The total flavonoid contents were determined by means of an analytical curve containing standards at concentrations of 1 to 10 μg mL\(^{-1}\) of rutin, and the results were expressed in microgram equivalents of rutin per milligram of dry extract (μgER/mg).

Antioxidant activity

The antioxidant capacity was measured by the DPPH method, as described by Soares et al. (2014) with modifications. The extracts obtained were prepared using methanol as the solvent at concentrations of 20 to 180 μg mL\(^{-1}\), w/v. In triplicate, 0.5 mL of the extract was added to a methanolic solution of DPPH (3 mL at 40 μg mL\(^{-1}\), w/v). White (reference solution) was made by replacing the DPPH with methanol in each reaction. All test tubes containing the reactions were stirred and held in the dark for 30 min. An analytical curve was performed for this activity using standard (positive controls) ascorbic acid and rutin (20-180 μg mL\(^{-1}\), w/v) also adding it to a methanolic solution of DPPH. Absorbance readings were taken at 517 nm in a spectrophotometer (BioSpectro model SP-220). The absorbance of the DPPH solution at 40 μg mL\(^{-1}\) was also measured and used as a negative control. The antioxidant activity of free radical removal was expressed as the percentage of inhibition determined by the equation

\[
\%AA = \left( \frac{ABS_{nc} - (ABS_{sample} - ABS_{white})}{ABS_{nc}} \right) \times 100,
\]

Where %AA is the percentage of antioxidant activity; ABS\(_{nc}\) is the absorbance of the negative control; ABS\(_{sample}\) is the absorbance of the sample; and ABS\(_{white}\) is the absorbance of white.

Using calibration curves obtained by plotting the different concentrations in relation to the %AA, the efficient concentration, the amount of sample required to decrease the initial concentration of DPPH by 50% (EC 50), which was expressed in μg mL\(^{-1}\).

Analysis by high-performance liquid chromatography (HPLC)

High-performance liquid chromatography (HPLC) was developed in the Laboratory of Scientific Instrumentation, Federal University of Tocantins on a Shimadzu® LC-10 Series Awp Chromatograph, equipped with a pump (LC-10AD), degasser (DGU-14A), UV-VIS detector (SPD-10A) (CTO-10A), Rheodyne hand injector (20 μL loop), and CLASS integrator (LC-10A). Separation was performed by the gradient elution method using a Phenomenex Luna C18 5μ (2) (250 × 4.6 mm) reverse-phase column and pre-column Phenomenex C18 (4 × 3.0 mm) filled with material similar to that in the main column. Mobile phase A was 0.1% phosphoric acid in Milli-Q water and mobile phase B was 0.1% phosphoric acid in Milli-Q water/acetonitrile/methanol (54:32:11). Program gradient: 0 to 0.01 min, 0% B; 0.01-5 min, 0% B, 5-10 min, 30% B, 10-20 min 40% B, 20-29 min, 40% B, 29-30 min 50% B, 30-50 min 100% B, 50-80 min, 100% B. Flow rate: 1 ml/min; temperature: 22°C. UV detection was done at 280 nm. The compounds were identified by comparing the retention times of samples with the authentic standards, such as gallic acid, catechin, epigallocatechin gallate, naringin, quercetin, and naringenin (Sigma®). The quantities of the compounds were expressed in micrograms per milligram of extract (μg/mg) by correlating the area of the analyte with the calibration curve of standards built in concentrations of 4.5 to 18 μg mL\(^{-1}\).

Selection according to fungitoxicity against C. lunata

In order to select the endophytic fungi with the best antibiotic...
activity, the in vitro mycelial growth inhibition capacity of the phytopathogenic C. lunata fungus was previously isolated and identified. According to Carotenuto et al. (2015) with modifications, the evaluation of inhibition of mycelial growth of the phytopathogen was carried out by adding to the surface of the 9 cm diameter Petri dish containing approximately 20 ml of BDA culture medium already solidified, 100 μL of the extracts of the endophytic fungi. The extracts were diluted in Tween 80 and water. Discs 7 mm in diameter containing the mycelia of phytopathogenic fungus from pure colonies with approximately twelve days of growth in PDA were placed in the center of the plates.

Plates were incubated in biochemical oxygen demand (BOD) at 25 ± 1°C and a photoperiod of 12 h of light and 12 h of darkness. After growth, the presence or absence of zones of inhibition was observed.

The bioassays were performed in triplicate, and a plate containing only one disk of micelium agar of the phytopathogen without extract served as a positive control.

**Synergism evaluation**

After selection of endophytic fungi by the in vitro diffusible metabolite test according to the methodology to Mutawila et al. (2015), only one sample was chosen for synergism evaluation. The extract of endophytic fungus was used together with extract of L. sidoides. In accordance with the methodology of Tadtong et al. (2014), five different proportions of the two substances were used: 0:1; 1:3; 1:1; 3:1, and 1:0. Thus, the concentration of each substance was fixed at 7500 µg mL⁻¹. In Petri dishes with already solidified BDA culture medium were placed 100 μL of each proportion, scattered with a handle Drigalsky. Then, a mycelium-agar disk of C. lunata fungus was placed in the center of the plaque.

An evaluation was performed after 10 days of incubation, and the mycelial diameter was measured for comparison with the control (water and Tween 80).

**Table 1.** Mycelial inhibition screening of *Lippia sidoides* Cham. endophytic fungi against the plant pathogen *Curvularia lunata* Wakker.

<table>
<thead>
<tr>
<th>Fungus</th>
<th>Extract from ethyl acetate</th>
<th>Extract from hexane</th>
</tr>
</thead>
<tbody>
<tr>
<td>LS–1 (u.f.)</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Colletotrichum sp.</td>
<td>+</td>
<td>=</td>
</tr>
<tr>
<td>Verticillium sp.</td>
<td>+++</td>
<td>=</td>
</tr>
<tr>
<td>Alternaria sp.</td>
<td>+</td>
<td>=</td>
</tr>
<tr>
<td>LS–5 (u.f.)</td>
<td>+</td>
<td>=</td>
</tr>
<tr>
<td>LS–6 (u.f.)</td>
<td>+++</td>
<td>=</td>
</tr>
<tr>
<td>LS–7 (u.f.)</td>
<td>++</td>
<td>=</td>
</tr>
<tr>
<td>LS–8 (u.f.)</td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>Colletotrichum sp.</td>
<td>+++</td>
<td>=</td>
</tr>
<tr>
<td>Phomopsis sp.</td>
<td>=</td>
<td>+</td>
</tr>
<tr>
<td>Fusarium sp.</td>
<td>=</td>
<td>++</td>
</tr>
<tr>
<td>Fusarium sp. 1</td>
<td>+++</td>
<td>=</td>
</tr>
<tr>
<td>Fusarium sp. 2</td>
<td>+++</td>
<td>=</td>
</tr>
<tr>
<td>LS–14 (u.f.)</td>
<td>+++</td>
<td>=</td>
</tr>
<tr>
<td>LS–15 (u.f.)</td>
<td></td>
<td>++</td>
</tr>
</tbody>
</table>

LS, Initials of the *L. sidoides* plant to identify the endophytic fungus in the isolation. u.f., Morphologically unidentified fungus. They were visually classified as mycelial diameter equal to the control (=), smaller than the control (+), much smaller than the control (++), and much, much smaller than the control (+++).

**Statistical analysis**

The data obtained in the experiments were subjected to statistical analysis, such as analysis of variance (ANOVA) and Tukey’s test, with a significance level of α = 0.05, and linear regression. Calculations were performed using Sigmaplot® 12.0 and Assistat® software.

**RESULTS**

Fifteen morphologically distinct endophytic fungi were obtained from the fresh leaf fragments of *L. sidoides*. Biological tests were carried out using extracts made from both hexane and ethyl acetate against the phytopathogenic fungus of maize plants, *C. lunata*. The results of the selection according to fungitoxicity are presented in Table 1. Three extracts of different endophytes extracted with ethyl acetate were selected for the analysis of secondary metabolite synthesis. This analysis included the methanolic extract of *L. sidoides* leaves. These fungi were analyzed morphologically and identified at the genus level as *Verticillium* sp., *Colletotrichum* sp., and *Fusarium* sp. 1 (Table 1).

The three fungi with fungistatic capacity against *C. lunata* in this work were evaluated in order to identify the antioxidative profile of the respective fungal extracts. The results obtained by spectrophotometric analyzes of total phenols were expressed as milligrams of gallic acid equivalents per gram of extract (mgEAG/g). In this way, the total phenolic content for the plant extract, and extracts of the endophytic fungi *Verticillium* sp., *Colletotrichum* sp., and *Fusarium* sp. 1 were 96.94 ±
Table 2. Mean values and standard deviations of total phenol content and flavonoids found in Lippia sidoides Cham., and endophytic fungi Verticillium sp., Colletotrichum sp. and Fusarium sp. 1.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Total phenols (mgEAG/g)</th>
<th>Total flavonoids (mgER/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. sidoides</td>
<td>96.94 ± 11.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>192.33 ± 4.58&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Verticillium sp.</td>
<td>6.38 ± 1.27&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.56 ± 1.14&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Colletotrichum sp.</td>
<td>0.29 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>14.31 ± 1.56&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fusarium sp. 1</td>
<td>10.54 ± 1.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>64.59 ± 1.15&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>mgEAG/g, Milligram equivalents of gallic acid per gram of extract. <sup>b</sup>mgER/g, milligram equivalents of rutine per gram of extract. Means followed by the same letter in the column do not differ statistically from each other by the Tukey test (<i>p</i> > 0.05).

Figure 1. Percentage of antioxidant activity (%AA) of L. sidoides endophytes fungi Verticillium sp., Colletotrichum sp., and Fusarium sp. 1, and positive controls (rutine and ascorbic acid) by the DPPH method.

Figure 2. Extract concentrations (μg mL<sup>-1</sup>) vs. antioxidant activity EC<sub>50</sub> (%).

11.86, 6.38 ± 1.27, 0.299 ± 0.05 and 7.60 ± 1.69 mgEAG/g respectively (Table 2).

It was verified that the total flavonoid content of extracts of the fungi Verticillium sp., Colletotrichum sp., and Fusarium sp. 1 were 21.56 ± 1.14, 14.31 ± 1.56 and 64.59 ± 1.15 mgER/g, respectively (Table 2). These values are lower than that of the plant extract, which was 192.33 ± 4.58 mgER/g (Table 2).

The percentage of antioxidant activity (%AA) was also determined by the DPPH method. The curves are shown in Figure 1, indicating an increase only for the plant extract, with the increase of the amount of extract in the reaction medium. The results were higher than 65% from the concentration of 120 µg mL<sup>-1</sup>, reaching a maximum of 95% for the concentration of 200 µg mL<sup>-1</sup> for L. sidoides extract (Figure 1). The effective concentration (EC<sub>50</sub>), that is, the amount of sample required to decrease the initial concentration of DPPH by 50% was also expressed in µg mL<sup>-1</sup>. The EC<sub>50</sub> for the plant extract was 81.03 µg mL<sup>-1</sup> (Figure 1). The %AA for the highest concentration (200 µg mL<sup>-1</sup>) for the fungi Verticillium sp., Colletotrichum sp., and Fusarium sp. was 19, 17 and 21%, respectively. Therefore, the EC<sub>50</sub> calculation could not be performed.

From the HPLC analyses performed for detection and quantification, fingerprints were obtained (Figure 2), which revealed matrices of phenolic compounds and flavonoids. In Figure 2B, the phenolic compound gallic acid 0.37 µg mL<sup>-1</sup> (Rt 16.4 min) was detected, along with the flavonoids catechin 1.67 µg mL<sup>-1</sup> (Rt 23.7 min), naringin 44.77 µg mL<sup>-1</sup> (Rt 43.2), and quercetin 3.08 µg mL<sup>-1</sup> (Rt 52.2 min). A high concentration of the compound naringin in the methanolic extract of the plant is
Figure 2. High-performance liquid chromatography (HPLC) fingerprints of authentic standards of phenolic compounds mixture (A), methanolic extract of the leaves of *L. sidoides* (B) and isolated endophytic fungi: *Verticilium* sp. (C), *Colletotrichum* sp. (D), *Fusarium* sp. (E) detected at 280 nm, as described in material and methods. Peak 1: Gallic acid; peak 2: Catechin; peak 3: Epigallocatechin gallate; peak 4: Naringin; peak 5: Quercetin; peak 6: Naringenin.

It was also verified by HPLC in this study which flavonoids could be synthesized by the endophytic fungi and which could be identified and quantified according to the standards in the laboratory where the analysis was performed. According to Figure 2C, it can be seen that the extract of the endophytic fungus *Verticilium* sp. synthesized a compound identified as naringenin 0.61 μg mL⁻¹ (Rt 55.0 min) in addition to other compounds that could not be identified.

The fungus *Colletotrichum* sp. also produced secondary metabolites of the flavonoid class, yet it was not possible to compare any peak with laboratory standards. As shown in Figure 2D, the visualization of many uniform peaks indicated that many compounds were synthesized. The fungus *Fusarium* sp. synthesized (Figure 2E) three substances identified as catechin 0.31 μg mL⁻¹ (Rt 23.3), epigallocatechin gallate, and quercetin 19.73 μg mL⁻¹ (Rt 29.1 min).

A synergism test was also performed between *L. sidoides* extract and the endophytic extract of *Verticilium* sp. (Table 3). The curve was fitted to a 2nd degree equation with $R^2 = 0.9999$. The highest inhibitory effect was in the proportion of 50% essential oil and 50% fungal
extract, both of which were at a concentration of 7500 μg mL\(^{-1}\). Proportions of 0:1, 1:3, 1:1, 3:1 and 1:0 (v/v) inhibited 54.8, 43.5, 79.0, 71.3 and 22.4% of the mycelial growth of \(C. \text{lunata}\) in vitro (Table 3).

### DISCUSSION

Some previous works found the same endophytic fungi. You et al. (2009) isolated the endophytic fungus \(Verticillium\) sp. from the roots of \(Rehmannia glutinosa\) by identifying two compounds produced by this fungus: 2,6-dihydroxy-2-methyl-7-(prop-1-enyl)-1-benzofuran-3(2H)-one, reported for the first time, and the ergosterol peroxide, which inhibited the growth of pathogenic fungi. Suradkar et al. (2014) were able to isolate the endophyte \(Verticillium\) albo-atrum from \(Withania somnifera\) (L.) and \(Ocimum sanctum\) L.

The fungi \(Colletotrichum\) sp. and \(Fusarium\) sp. were also found in other studies isolating endophytic fungi. \(Alternaria alternata\), \(Colletotrichum\) gloeosporioides, \(Drechslera dematioides\), \(Guignardia bidwellii\), \(Fusarium lateritium\), and \(Phomopsis archeri\) were endophytic fungi isolated from \(L. \text{sidoides}\) and identified by Siqueira (2011). In this work, the author isolated 15 endophytic fungi from \(L. \text{sidoides}\), the same number of isolates in the present work. Similar results were verified, where the fungi \(Fusarium\), \(Colletotrichum\), \(Phomosis\), and \(Alternaria\) were also present in the work of Siqueira (2011).

Among the metabolites produced by \(Colletotrichum\) sp.: fusaretine 6,7-dimethyl ether, monocerin, and colletotrialide (Tianphanich et al., 2011), stigmasterol, sitostenone, squalene, ergosterol and ergosterol peroxide (Carvalho et al., 2016) were identified. \(Colletotrichum\) species have been identified to produce a variety of secondary metabolites genes, including flavones, peptides and terpenes (Crouch et al., 2014) and many other metabolites as Jayawardena et al. (2016) shows.

The genus \(Fusarium\) is also widely known as a producer of a variety of chemical compounds derived from its secondary metabolism (Nongalleima et al., 2013). Wang et al. (2011) reported anticancer activity against human PC-3 (prostate), PANC-1 (pancreas) and A549 (lung) cells from a secondary metabolite called beauvericin isolated from the endophytic \(Fusarium\) oxysporum of the \(Cinnamomum kanehira\) plant. This same endophyte, \(F. \text{oxysporum}\), nonpathogenic strains, isolated by Kundu et al. (2016), produced , bikaverin (1), 3-O-methyl-8-O-methyl fusarubin (2), 8-O-methyl fusarubin (3), anhydrofusarubin (4) and fusarubin (5).

In addition, the methanolic extract of \(Fusarium\) proliferatum prepared by Mohana et al. (2012), endophytic of \(Dysoxylum binectariferum\) demonstrated cytotoxic activity in HCT-116 (colon) and MCF-7 (breast) human cancer cells.

The results confirm that both the plant and endophytic fungi produce compounds of a phenolic nature. Some studies corroborate our results, even though some previously reported fungal extracts contained more total phenols than ours. Yadav et al. (2014a) verified the phenolic compound content of endophytic fungi extracts obtained from ethyl acetate. The highest concentration of phenols was observed in the extract of \(Chaetomium\) sp. (60.13 \(\pm\) 0.41 mgEAG/g), followed by that of \(Aspergillus niger\). The total phenol concentration values of the endophytic fungi studied ranged from 4.20 to 60.13 mgEAG/g. Srinivasan et al. (2010) obtained values of 18.33 \(\pm\) 0.68 mgEAG/g of total phenols from the extract of the endophytic fungus \(Phylllosticta\) sp.

In order to confirm the synthesis of total flavonoids by the endophytic fungi present in the \(L. \text{sidoides}\) and plant extract (Table 2), extracts were obtained with the solvent ethyl acetate (AcOEt) to extract compounds with recognized antimicrobial activity, such as phenols and flavonoids (Baba and Malik, 2015).

However, even if they are smaller, it can be verified that the endophytic fungi contribute to the plant in the production and synthesis of secondary metabolites for its protection. Hence, an appreciable amount of flavonoids production and synthesis of secondary metabolites genes, including flavones, peptides and terpenes (Crouch et al., 2014) and many other metabolites as Jayawardena et al. (2016) shows. They also clarified that some phenolic compounds are produced so...
that certain endophytic fungi survive along with their host plants. Qiu et al. (2010) found flavonoid contents of 0.01162 ± 0.0014 and 0.01256 ± 0.00378 mg ER/mL in extracts of Aspergillus nidulans, Aspergillus oryzae, and Ginkgo biloba endophytes, respectively. Their values were lower than those of the present work; however, different fungi were used.

The data obtained in the determination of this bioactivity is significant when compared with others obtained in the work of Almeida et al. (2010). At a concentration of 100 μg mL$^{-1}$ (or 1 mg mL$^{-1}$ as reported), there was an antioxidative activity of 99.5% and an EC$_{50}$ of 16.3 μg mL$^{-1}$ in the ethanolic extract of L. sidoides. The results obtained in the present study were lower; however, the solvent used for the extraction may have been the source of this difference. They also assayed the DPPH activity of plant-isolated substances such as tecomquinone and naringenin. At a concentration of 1 mg mL$^{-1}$, %AA was 64.7% and the EC$_{50}$ was 720 μg mL$^{-1}$ for the flavonoid naringenin. The compound tecomquinone did not display any activity.

As for the antioxidant activity of the extracts of endophytic fungi, they showed a low capacity to sequester free radicals, as there was no negative effect on the measured absorbance. Initial values remained essentially unchanged at all concentrations tested. It can be said that there was activity; however, compared with the results obtained from the plant extract, it was much lower than expected. It is believed that perhaps the cultivation conditions used did not promote the biosynthesis of antioxidant molecules that are detectable by this method. In addition, there was no change in the amount of free radicals as the concentration of fungal extract varied.

Observing the work of Devi and Singh (2015), they also did not obtain positive results of a significant amount of free radical sequestration by DPPH in extracts of Verticillium. However, they reported an antioxidative activity 95% in Alternaria sp. 2 made from ethyl acetate. Yadav et al. (2014a) carried out tests with extracts of endophytic fungi, including the genus Fusarium sp. They identified antioxidant activity by DPPH of Aspergillus peryonelli and Aspergillus niger of 71 and 72%, respectively, but identified low activity in extracts of Fusarium sp. Nath et al. (2014) also reported a strong activity in the sequestration of free radicals in ethanolic extracts of Colletotrichum gloeosporioides of 0.67 ± 0.05, 47.89 ± 0.06, 73.84 ± 0.08, 60.09 ± 0.08, and 52.77% ± 0.06% at concentrations of 10, 25, 50, 75 and 100 μg mL$^{-1}$, respectively.

Endophytic fungi and plants can produce flavonoids together, varying their biological activities, such as the genus Bauhinia variegata (cow’s foot), which had antidiabetic activity confirmed by the metabolic profiles of both the plant and the associated endophytic fungi (Costa, 2005).

These analyzes by HPLC allowed us to identify phenolic constituents described in the literature as important in several biological functions. Phenolic acid has a strong anti-inflammatory, antimutagenic, and antitumor action, inhibiting genes related to the cell cycle, metastasis, angiogenesis, and apoptosis (Verma et al., 2013). The detection of three types of flavonoids, catechin, naringin, and quercetin, in the L. sidoides plant (Figure 2B) also allows presenting and confirm the possibility of harnessing its leaves for biological applications. Several studies have already demonstrated the potential and ability of these metabolites to act to reduce the risks associated with pathologies such as intestinal inflammation, bacterial infections, diabetes, cardiovascular diseases, and cancer, among others (Clemensen et al., 2017; Macheleidt et al., 2016). In the present study, however, antifungal activities of the extracts are related to the antioxidative activity of flavonoids present in considerable amounts (Funari et al., 2012).

Many studies report the capacity of flavonoid production by plants, such as those found in the leaves of Euphorbia neriifolia (pencil-tree), where Sharma et al. (2014) identified a flavonoid as quercetin by HPLC. This substance and other flavonoids are credited with antioxidative and anticarcinogenic activity. Naringenin is a flavonoid of the flavanone subclass, found mainly in citrus fruits (Dou et al., 2013), with a beneficial antioxidative and neuroprotective effect (Raza et al., 2013).

Among these, the flavonoids catechin and quercetin were also found in plant extracts. Thus, it can be confirmed that these two metabolites were probably synthesized by the endophytic fungi in conformation with the plant-fungus mutualistic interactions. In addition, as endophytic fungi produce the same compounds present in the plant, it can be inferred that fungi produce these compounds for the plant as a way of assisting them in their defense. These substances contribute greatly to the antioxidative activities credited to L. sidoides plants (Funari et al., 2012).

Kumar et al. (2013) characterized the vinblastine alkaloid from the extract of the endophytic fungus F. oxysporum, isolated from Catharanthus roseus. By HPLC analyzes, they obtained 76 μg L$^{-1}$ of the compound. Also Zaiyou et al. (2015) found in the extract of the endophyte Fusarium sp the compound paclitaxel at the concentration of 0.0153 mg L$^{-1}$.

Besides, Chapla et al. (2014) also identified eight compounds synthesized by the endophyte Colletotrichum gloeosporioides, a new compound 2-phenylethyl 1H-indol-3-y acetate (1) and seven other known compounds: Uraclil (2), cyclo-(S+(S)-Pro-S*-Val) (4), 2-(2-aminophenyl) acetic acid (5), 2-(4-hydroxyphenyl) acetic acid (6), 4-hydroxy-benzamide (7), and 2-(2-hydroxyphenyl) acetic acid (8). Table 4 shows the compounds identified by HPLC from extracts of both the plant L. sidoides and the endophytic fungi isolated from it.
The production of compounds that possess biological activity by endophytic fungi can be stimulated in the plant, by the host plant extract, and in this case, by the extracts of fermented fungi without contact with any part or extract of the plant (Dos Santos et al., 2015). When grown in vitro, fungi continued to produce metabolites. Further studies must be carried out to find out if the fungi would cease production for some time or if, in the presence of the plant extract or any part of it, the synthesis would continue, that is, what factors could encourage this production. No reports were found in the scientific literature on the inhibition of mycelial growth of phytopathogenic fungi using extracts of endophytic fungi and of plants with synergistic effects. However, in the work of Oliveira et al. (2014), synergistic activity was found between a butanolic fraction of *Lippia alba* extract with commercial antifungal agents against *Candida glomerata*. The minimum inhibitory concentration was 0.062 μg mL$^{-1}$. This may be a potential alternative in the treatment for candidemia caused by yeast species.

The synergistic effect of the endophytic fungal extracts of *Aspergillus awamori*, *Penicillium* sp., and *C. gloeosporioides* with standard antibiotics was studied against the four test bacterial strains viz. *Streptococcus pyogenes*, *Escherichia coli*, *Enterococcus faecalis*, and *Salmonella enterica ser paratyphi*. A significant increase in the diameter of inhibition zones was observed when the fungal extracts in combination with the antibiotic Norfloxacin was used against the test pathogens. Crude extract of *A. awamori* in combination with Norfloxacin showed antimicrobial activity (25 and 31-mm inhibition zones) against *E. coli* and *E. faecalis*, respectively (Nath et al., 2013).

The secondary metabolites act on the fungus through cytoplasmic granulation, disorganization of cellular contents, and inactivation of enzymes, which inhibits germination, germinative tube elongation, and mycelial growth (Lo et al., 1996). Thus, it can be inferred that the best result of mycelial inhibition was determined by the joint action of the extract of *L. sidoides* and extract of the endophytic fungus *Verticillium* sp. However, it was only a preliminary test, and other tests should be performed in order to obtain results on the best concentration of the metabolites, test the efficiency of the other fungi along with the plant extract, and add another type of extract.

Recent discoveries, as exemplified in this work, have associated endophytic fungi with medicinal plants, important sources of secondary metabolites of pharmaceutical, agricultural, and industrial interest. Brazil has a wide potential source of plants, due to the varied biomes, and a study of plant-endophytic interactions is necessary. The discovery of metabolites with recognized bioactive action produced by endophytes can help to stop the excessive exploitation of plants and consequently, the whole set of endophytes associated with it (Pusztahelyi et al., 2015). Thus, the study of fungal endophytes is becoming a great necessity, because, as in the present work, they assist their host plants in the synthesis of metabolites with recognized bioactivity. This property of endophytes will help to diminish the exploitation of plant biodiversity for drug extraction. Consequently, conservation strategies will be more efficient.

### Conclusions

The analyses of the study showed the presence of phenols and flavonoids in all extracts and high antioxidant activity was only observed in the plant extract. Secondary metabolites were identified and quantified by HPLC. The best result for mycelial inhibition by the synergism test was determined by the joint action of the extract of *L. sidoides* and that of the endophytic fungus *Verticillium* sp. against *C. lunata*.

### CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.
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REFERENCES


Ethnobotanical study of ethnoveterinary plants in Kelem Wollega Zone, Oromia Region, Ethiopia

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Questionnaire based cross sectional study design was conducted from November 2015 to April 2016 in Dale Sadi district area, Kelem Wollega Zone of Oromia regional state of Ethiopia, to identify potential medicinal plants used for treatment of the livestock ailments. In this study 50 species of medicinal plant species were identified which were categorized under 32 different families. Among the medicinal plants 45(90%) were used for curative purpose, 2(4%) for only prophylactic purpose and 3(6%) for both curative and prophylactic activities. Shrubs 29(58%), herbs 10(20%) and tree 8(16%) were the main habitat of the herbal plants. The main routes of administration were oral and topical, 30(60%) and 9(18%) respectively. Leaves 18(36%) and roots 7(14%) were the main parts of the plant used as medicinal values. The results of this study play a significant role in encouraging further investigations by extracting and identifying bioactive constituents of those herbal medicines for the antimicrobial effect. It is recommended that further detailed examination should be conducted to investigate the medical principles and pharmaceutical activity found in these plants.

Key words: Livestock, traditional healers, medicinal plants.

INTRODUCTION

Medicinal plants have served through ages as a constant source of medicament for treatment of a variety of diseases (Okoli et al., 2007). The history of herbal medicine is almost as old as human civilization (Choudhary et al., 2015). In ancient cultures people developed their own herbal pharmacopoeias based on information gained through experience and in our today’s scientific pharmacopoeia much of the information on scientific medicine is derived from those herbal pharmacopoeias (Kim, 2005). Medicinal plants play a key role in the development and advancement of modern studies by serving as a starting point for the development of novelties in drug (Wright, 2005). Tropical plants have been used for medicinal purposes since the evolution of man. This knowledge is still alive and several hundred species are used in herbal remedies in indigenous system of medicines, where the whole plant or plant part or its extraction is used (Alawa et al., 2002; Okoli et al.,
Medicinal plants are the “backbone” of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis (Davidson-Hunt, 2000). Primitive people learned by trial and error to distinguish useful plants with beneficial effects from those that were toxic or non-active and also which combinations or processing methods had to be used to gain consistent and optimal results (Jagessar et al., 2008).

In comparison with modern medicine, herbal medicines cost less, are more often used to treat chronic diseases and the occurrence of undesirable side effects seems to be less frequent (Jagessar et al., 2007). Natural products have long been regarded as excellent sources for drug discovery, given their structural diversity and a wide variety of biological activities (Fu et al., 2008). Due to easy availability and low cost of ethno veterinary medicinal plants, the livestock owners of the remote areas use them as a first aid for their animals (Jabbar et al., 2006).

Ethno veterinary knowledge is acquired by communities over many years and passed between generations through oral tradition. Today, with rapid cultural changes, this knowledge is being lost, necessitating its scientific documentation (Mathias, 2001). Thus, saving the species, documenting and preserving indigenous knowledge is essential (Alam and Ali, 2010). There is a lack of ethno botanical survey carried out in most parts of the country. In view of these, documentation of the traditional use of medicinal plants is important to preserve the knowledge regarding the traditional plants. Once these local ethno medical preparations are scientifically evaluated and disseminated properly, people will be better informed regarding efficacious drug treatment and improved health status. Therefore, this study was attempted with objectives:

1. To identify and document potential medicinal plants for the livestock ailment treatments at the study area.
2. To investigate the scientific name of those medicinal plants used by the local traditional healers.

**MATERIALS AND METHODS**

**Study area and study population**

The study was conducted from November 2015 to April 2016 on some traditional medicinal plants found in Dale Sadi district. Dale Sadi is one of the districts in Kellom Wollega zone of Oromia Regional state. It is 510 km far from Addis Ababa capital city of Ethiopia. The area lies at average altitude of 1150 m above sea level. The area has temperature range of 33 of 35°C with more agricultural crops. The climatic condition alternates with long summer May to August and short rainy seasons from March to April. The winter dry seasons (November to February) with mean annual rain fall of 1200 mm (DDBOA, 2013). The target populations for this study were voluntary livestock’s owners such as farmers, traditional healers and veterinarians.

**Study design and sampling methods**

Cross sectional study design was conducted to identify traditional plants used in animals disease healing. Primary data were collected by questionnaire survey from purposively selected elders, especially traditional healer’s livelihoods which depend predominantly on traditional plants for curing their livestock and their own health. At the same time voluntary animal owners, animal health practitioners were interviewed using focus group discussions and field observation. Non-probability sampling method was carried out to collect information related to medicinal plants for livestock in the study area. All volunteer traditional healers selected based on recommendation from elders of the study area.

**Study methodology**

The structured questionnaire was used to collect information related to medicinal plants used to treat livestock and Information regarding local name of the plant, its habit, part(s) of plants used, route of administration, methods of preparation, local name of the animal diseases treated, dosage used and species of animals treated was recorded at spot. Based on ethno botanical information acquired from informants, the plants were collected from the surrounding forests and other parts of the study areas with the people who know the local name of the plants. Pictures (Figure 1) of voucher specimens were captured and their leaves were collected, pressed and dried to identify the scientific names of the collected medicinal plants at the National Herbarium of Biology Department of Natural Science Faculty, Wollega University, Ethiopia.

**Data analysis and management**

The information that was gathered through questionnaire survey was coded and entered into Microsoft Excel spreadsheet. Descriptive statics and chi-square were employed by SPSS version 20 software for analyzing of ethno botanical data.

**RESULTS**

The total numbers of species of plants collected during the study period were fifty (50). These were categorized under 32 different types of families. From the total 32 families of the plant-samples, 6(12%) plant species belong to family Asteraceae and 4(8%) plant species belong to family Solanaceae. Those family including Zygophyllaceae, Fabaceae, Rosaceae, Musaceae, Euphorbiaceae, Moraceae, Rutaceae, Malvaceae, Brassicaceae and Cucurbitaceae each contains 2(4%) species of plants. Each of the remaining twenty (20) families was represented by single species of plants (Table 1).

The identified traditional medicinal plants were used traditionally to cure livestock ailments by local communities. It was found that the medicinal plants were used used as anti-bacterial, anti-parasitic, anti-viral. At the same time those medicinal plants could be used for haemostatic, retention of fetal membrane, suturing the
physically damaged part, snake bites, fracture, snake allergy, poison consumed, free gas bloat, tympanic bloat, and others (Tables 2 to 6).

**Routes and dosage of the administered medicinal plants**

Numerous techniques of administrations routes were employed during administering the remedies. The highest numbers of medicinal plants were given orally 30(60%), followed by topical application 9(18%) (Table 7). According to study area much of recorded medicinal plants were found not to be toxic to animals even given at higher doses, except some plants like *Euphorbia Ampliphylla*, *Zehneriascabra*, *Capparistomentosa*, *Capsicum annuum* and *Eulophia* that were reported to be toxic at higher concentration (Table 7).

**Parts of plant used as medicinal values and their growth form**

The plants part used to treat animal disease varied from species to species and from diseases to diseases. Leaf, seed, bark, root, sap and latex were widely used for treating animal disease. Leaf 18(36%) were the most frequently used plant parts followed by root 7(14%) and seed 4(8%) and the minimum used part was pod (2%) (Table 8).

The investigated plants were fall under 5 groups. Shrubs are highest 29(58%), followed by herbs 10(20%), tree 8(16%) and epiphyte is the least 1(2%) (Table 9).

**DISCUSSION**

Medicinal plants play a key role in the development and advancement of modern studies by serving as a starting point for the development of novelties in drug. Herbal medicine has been widely formulated and used as an integral part of primary health care in Nigeria, China, Ethiopia and Argentina (Ogbuewu et al., 2015).

Among the plant families, Asteraceae was the dominant medicinal plant (12%) followed by family Solanaceae (8%). This finding was in line with Getaneh et al. (2014), in which Asteraceae was found to be the most dominant (11.2%) and Solanaceae was the second dominant (7.4%). This result is different with the finding of Zewdu et al. (2015), in which Euphorbiaceae was
Table 1. Lists of plant's family frequently used by traditional healers in the study area.

<table>
<thead>
<tr>
<th>Family name</th>
<th>No. of species of plants under the family</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asteraceae</td>
<td>6</td>
<td>12.0</td>
</tr>
<tr>
<td>Zygophyllaceae</td>
<td>2</td>
<td>4.0</td>
</tr>
<tr>
<td>Apocynaceae</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Scrophulariaceae</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Fabaceae</td>
<td>2</td>
<td>4.0</td>
</tr>
<tr>
<td>Urticaceae</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Combretaceae</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Solanaceae</td>
<td>4</td>
<td>8.0</td>
</tr>
<tr>
<td>Cactaceae</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Simaroubiaceae</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Scrophulariaceae</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Rosaceae</td>
<td>2</td>
<td>4.0</td>
</tr>
<tr>
<td>Musaceae</td>
<td>2</td>
<td>4.0</td>
</tr>
<tr>
<td>Capparaceae</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Loranthaceae</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Agavaceae</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Liliaceae</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Euphorbiaceae</td>
<td>2</td>
<td>4.0</td>
</tr>
<tr>
<td>Moraceae</td>
<td>2</td>
<td>4.0</td>
</tr>
<tr>
<td>Rutaceae</td>
<td>2</td>
<td>4.0</td>
</tr>
<tr>
<td>Phytolaccaceae</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Malvaceae</td>
<td>2</td>
<td>4.0</td>
</tr>
<tr>
<td>unknown</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>mucronata</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Brassicaceae</td>
<td>2</td>
<td>4.0</td>
</tr>
<tr>
<td>Curbetaceae</td>
<td>2</td>
<td>4.0</td>
</tr>
<tr>
<td>unknown</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Amaryllidaceae</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Francoaceae</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Acanthaceae</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Zingiberaceae</td>
<td>1</td>
<td>2.0</td>
</tr>
</tbody>
</table>

reported to have the highest number of species employed in the treatment of diseases followed by Cucurbitaceae, Lamiaceae, Solanaceae and Verbenaceae underscoring the significance of these species in the ethnomedicine of Gondar town.

**Citrus aurantifolia** is a traditional medicinal plants also identified in this study which have insecticidal property against lice infestation. Similar result was reported by Parle and Chaturvedi (2012), who indicated that orange peel oil produces lethal effect on fleas, fire ants, and houseflies due to its 90 to 95% limonene. Other than using as insecticides, it was reported that the juice of *C. aurantifolia* is considered as tonic for libido and as antidote for poison and the diluted form of the *C. aurantifolia* fruit juice is used for mouth wash to treat sore mouth, sore throat and useful to treat irritation, diarrhea and swelling due to mosquito bites (Aibinu et al., 2007; Khare., 2007; Akhtar., 2013). Similar to the reports of Firaol et al. (2013), *Euphorbia ampliphylla* plant was found to have the ability to cure the wart by topical application.

At the study area, *Phytolacca dodecandra* have medicinal values for internal parasites, abdominal aches and washing of the wound. However, the study results of Abebe et al. (2003) indicated that *P. dodecandra* used as taenicidal and molluscicidal activity and other study conducted in Hadya Zone, Ethiopia also revealed that this plant's root was useful for treating Anthrax and itchiness (Habtamu et al., 2014).

In this study finding the *Gossypium herbaceum* plant have medicinal values for treating of ocular problem. In contrast to this Dhamija et al. (2011) reported that aqueous extract of *G. herbaceum* showed significant antidepressant like effect due to activation of adenylyl cyclase
Table 2. Summary of general characteristics of the identified medicinal plants under family Asteraceae’s family.

<table>
<thead>
<tr>
<th>Plant local name</th>
<th>Scientific name</th>
<th>Plant type</th>
<th>Part used</th>
<th>Adm. Route</th>
<th>Method of preparation</th>
<th>Indication</th>
<th>Animal’s species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lomi simbira</td>
<td>Crassocephalum sarcobasis</td>
<td>H</td>
<td>Leaf</td>
<td>Oral, auricular and nasally</td>
<td>Its leaves are chopped and given with feed. It is also squeezed and the drop applied in both eyes and noses</td>
<td>For dulled and emaciated animals</td>
<td>Bovine</td>
</tr>
<tr>
<td>Kabaricho</td>
<td>Echinops</td>
<td>S</td>
<td>Root</td>
<td>Oral and nasal</td>
<td>The roots finely chopped and given with feed. Its root is also chopped and added to burning fire and the vapor of the smoke is inhaled</td>
<td>For abdominal aches, circling disease and dry coughing</td>
<td>All mammals</td>
</tr>
<tr>
<td>Ebicha</td>
<td>Vernonia amygdalina</td>
<td>S</td>
<td>Leaf</td>
<td>Oral and topical</td>
<td>Some of the leaves added to their feed with salt and wash the wound locally</td>
<td>For washing and healing the wound</td>
<td>All mammals</td>
</tr>
<tr>
<td>Ada</td>
<td>Guizotia cabra</td>
<td>S</td>
<td>Root</td>
<td>Topical</td>
<td>Its root are inserted into the needle for suturing of the wounded part</td>
<td>Suturing the wounded body</td>
<td>All domestic animals</td>
</tr>
<tr>
<td>Kasi</td>
<td>Ageratum coryzoides</td>
<td>S</td>
<td>Leaf</td>
<td>Topical</td>
<td>leaves are squeezed and dropped to the bleeding area</td>
<td>For haemostasis</td>
<td>All domestic Animals</td>
</tr>
<tr>
<td>Koricha ramo</td>
<td>Lactucinermis</td>
<td>S</td>
<td>Whole</td>
<td>Oral</td>
<td>After rooted off, whole parts are chopped together and given with their feed</td>
<td>For killing maggots found in open wound.</td>
<td>All domestic animals</td>
</tr>
</tbody>
</table>

S, Shrub; H, herb.

Table 3. Summary of general characteristics of the identified medicinal plants under family Solanaceae.

<table>
<thead>
<tr>
<th>Plant local name</th>
<th>Scientific name</th>
<th>Plant type</th>
<th>Part used</th>
<th>Adm. Route</th>
<th>Method of preparation</th>
<th>Indication</th>
<th>Animal’s species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mimita</td>
<td>Capsicum annuum</td>
<td>H</td>
<td>Pod</td>
<td>Oral &amp; Topical</td>
<td>2-3 pods are grinded with the bulb of Allium sativum then drenched. It also applied topically for leech treatment</td>
<td>For abdominal aches and removal of Leech from gum</td>
<td>All mammals</td>
</tr>
<tr>
<td>Asangira gibe</td>
<td>Datura arborea</td>
<td>H</td>
<td>Leaf</td>
<td>Oral</td>
<td>Leaves are given orally with the bulb of Zingiber officinalis for rumenants. But for equines it should be chopped and drench by bottle</td>
<td>For Black leg, dullness, ocular and nasal discharges</td>
<td>All mammals</td>
</tr>
<tr>
<td>Hidi warabesa</td>
<td>Solanum marginatum</td>
<td>S</td>
<td>Root</td>
<td>Oral</td>
<td>Some of its roots are chopped and added to their feed</td>
<td>Black leg</td>
<td>Ruminants</td>
</tr>
<tr>
<td>Tambo</td>
<td>Nicotiana tobacum</td>
<td>S</td>
<td>Leaf</td>
<td>Oral</td>
<td>The leaves are squeezed into throats of sheep and cattle</td>
<td>For coughing &amp; killing of Internal parasites</td>
<td>Ovine and bovine</td>
</tr>
</tbody>
</table>

cAMP pathway in signal transduction system and hence protecting the neurons from the lesion and Narasimha et al. (2008) also emphasized that G. herbaceum seeds to have antioxidant activity, anti-diarrheic, wound healing, anti-migraine, and diuretic activity. In addition, the *Allium cepa* is another type of traditional medicinal plants useful for treating Foot and mouth viral diseases. This result is different from the finding of Yusha’u et al. (2008), in which *A. cepa* is used in treatment of common ailments like cold, allergies, toothaches, laryngitis and cough.

In this study, the whole parts of *Bersama abyssinica* were identified having the ability to treat internal parasites. This is in agreement with
Table 4. Summary of general characteristics of the identified medicinal plants under families of Zygophyllaceae, Fabaceae, Rosaceae, Musaceae, Euphorbiaceae

<table>
<thead>
<tr>
<th>Plant local name</th>
<th>Scientific name</th>
<th>Family name</th>
<th>Plant types</th>
<th>Part used</th>
<th>Adm. route</th>
<th>Method of preparation</th>
<th>Indication</th>
<th>Animal's species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Koso</td>
<td>Hagenia abyssinica</td>
<td>Rosaceae</td>
<td>S</td>
<td>Seed</td>
<td>Oral</td>
<td>A cup of seed is grinded then the powder mixed with chopped form of A. sativum and given for taeniasis infected animals. It also grinded with ant's clustered on a tree then drench for donkey</td>
<td>For taeniasis, treat and prevention and from any kind of infections in donkey.</td>
<td>All mammals</td>
</tr>
<tr>
<td>Facah</td>
<td>NI</td>
<td>Zygophyllaceae</td>
<td>S</td>
<td>Root</td>
<td>Oral</td>
<td>Root are chopped and given with feed</td>
<td>For emaciation, coughing, black leg, trypanosomiasis</td>
<td>Ruminants</td>
</tr>
<tr>
<td>Ambalta</td>
<td>Entada abyssinica</td>
<td>Fabaceae</td>
<td>T</td>
<td>Barks and leaves</td>
<td>Topical</td>
<td>Leaves and bark are chopped together and applied topically to wound containing maggot's larvae</td>
<td>To kill maggot larvae found in the wound</td>
<td>All domestic animals</td>
</tr>
<tr>
<td>Bakanisa</td>
<td>Croton macrostachyus</td>
<td>Euphobiaceae</td>
<td>T</td>
<td>Latex</td>
<td>Oral and topical</td>
<td>The immature parts of leaves are given with its fruits for bloated animal. Its latex is also directly painted on the affected area</td>
<td>For bloats, dandruff</td>
<td>All mammals</td>
</tr>
<tr>
<td>Kacho</td>
<td>Ensete ventricosum</td>
<td>Musaceae</td>
<td>H</td>
<td>Stem</td>
<td>Oral</td>
<td>Its sap was collected to clean container and a few drops are drenched orally</td>
<td>For diarrhea and cough</td>
<td>Poultry</td>
</tr>
<tr>
<td>Adami</td>
<td>Euphorbia ampliphylia</td>
<td>Euphobiaceae</td>
<td>S</td>
<td>Latex</td>
<td>Oral and topical</td>
<td>The sap is collected and given with their feed and it is also applied to the local warts.</td>
<td>For any internal parasites, Lameness, wart.</td>
<td>All domestic animals</td>
</tr>
<tr>
<td>Warke</td>
<td>Musa sapientum</td>
<td>Musaceae</td>
<td>H</td>
<td>Stem</td>
<td>Topical</td>
<td>Its sap is collected to clean container and washing an animal body with a medicinal infusion</td>
<td>Mange mites, ticks and lice</td>
<td>All domestic animals</td>
</tr>
<tr>
<td>Tumjo</td>
<td>NI</td>
<td>Rosaceae</td>
<td>C</td>
<td>Leaf and stem</td>
<td>Oral</td>
<td>All stem and leaves are chopped together and given with their feed for 2 times per year</td>
<td>For prolonged emaciated animal, lameness, Anthrax, coughing, Black leg</td>
<td>All ruminants</td>
</tr>
<tr>
<td>Ceka</td>
<td>Calpurnia aurea</td>
<td>Fabaceae</td>
<td>S</td>
<td>Leaves</td>
<td>Topical</td>
<td>Some of the leaves juice are squeezed to their body and washed thoroughly</td>
<td>For all ecto-parasites, and alopecia</td>
<td>All domestic animals</td>
</tr>
<tr>
<td>Koricha bofa</td>
<td>Porteria hygrometra</td>
<td>Zygophaceae</td>
<td>S</td>
<td>Leaves</td>
<td>Oral</td>
<td>The leaves is chopped added to their feed</td>
<td>For snake bite</td>
<td>All domestic animal</td>
</tr>
</tbody>
</table>

S, Shrub; H, herb; T, tree; C, climber; NI, not identified.

The report of Mathewos et al. (2015) and Zewdu et al. (2015), that the extracts of B. abyssinica which could be administered orally for treating dysentery, stomach disorders such as abdominal pain, colic, diarrhoea, intestinal worms, amoebiasis. *Justicia schimperiana* have medicinal values for prevention rabies. The result was in line with other finding of Abiyu et al. (2014) that was conducted on Ethnobotanical study of traditional medicinal plants in and around Fiche District, Ethiopia. Anthelmintics use of H. abyssinica in ruminants has been in agreement with the finding of Abebe et al. (2000). However it was reported that *H. abyssinica* also used against tapeworms in humans in Ethiopia (Giday et al., 2003, Yayesh et al., 2015). According to local herbalists *Zingiber officinale* have medicinal values for treating any internal parasites. This is similar with the finding of Iqbal et al. (2006), who investigated the anthelmintic activity of crude powder and crude aqueous extract of dried Zinger (1 to 3 g/kg) in sheep naturally infected with mixed species of gastrointestinal nematodes. Contrary to this finding specific aspects of Zinger's actions has...
Table 5. Summary of general characteristics of the identified medicinal plants under families of Moraceae, Rutaceae, Malvaceae, Brassicaceae, Cucurbitaceae.

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Scientific name</th>
<th>Family name</th>
<th>Plant types</th>
<th>Part used</th>
<th>Adm. route</th>
<th>Method of preparation</th>
<th>Indication</th>
<th>Animal’s species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hida refa</td>
<td>Zehneriascabra</td>
<td>Cucurbitaceae</td>
<td>C</td>
<td>Whole parts</td>
<td>Oral</td>
<td>The whole plant is chopped and the powdered form is dissolved in water and drenched by bottle.</td>
<td>For dry cough, emaciated, lameness, alopecia, colic</td>
<td>Equine</td>
</tr>
<tr>
<td>Kiltu</td>
<td>Ficusvasta</td>
<td>Moraceae</td>
<td>T</td>
<td>Sap</td>
<td>Oral</td>
<td>Decocted leaf is collected and drunk by cup</td>
<td>For GIT parasites</td>
<td>All mammals</td>
</tr>
<tr>
<td>Lomi</td>
<td>Citrus aurantifolia</td>
<td>Rutaceae</td>
<td>S</td>
<td>Fruit</td>
<td>Oral</td>
<td>A few drops of fresh juice are squeezed orally to throat</td>
<td>Coughing and lice infestation</td>
<td>Poultry</td>
</tr>
<tr>
<td>Cildami</td>
<td>Ruta chalepensis</td>
<td>Rutaceae</td>
<td>H</td>
<td>Leaves</td>
<td>Oral</td>
<td>The leaf are chopped are squeezed to the bottle and drunk the poisoned animals</td>
<td>For poisoned animals</td>
<td>All domestic animal</td>
</tr>
<tr>
<td>Karabi</td>
<td>Sidarombifolia</td>
<td>Malvaceae</td>
<td>S</td>
<td>Leaves</td>
<td>Topical</td>
<td>The leaves are pinched and squeezed and dropped to wound topically.</td>
<td>Wound</td>
<td>All domestic animal</td>
</tr>
<tr>
<td>Sanaficha</td>
<td>Brassica nigra</td>
<td>Brassicaceae</td>
<td>H</td>
<td>Seed</td>
<td>Oral</td>
<td>A cup of its Seed are grinded and mixed with water and drunk by bottle</td>
<td>For frothy and free gas bloat</td>
<td>All mammal</td>
</tr>
<tr>
<td>Buke</td>
<td>Cucurbita Pepo</td>
<td>Cucurbitaceae</td>
<td>H</td>
<td>Leaves and fruit</td>
<td>Oral and topical</td>
<td>Some of its upper part are chopped and mixed with water then drench the neonates half of a bottle. Its fruit is roasted and applied topically to infection found on the nose</td>
<td>Prevention of disease from neonates like Orf</td>
<td>Ruminants</td>
</tr>
<tr>
<td>Jirbi</td>
<td>Gossypium herbaceum</td>
<td>Malvaceae</td>
<td>S</td>
<td>Seeds</td>
<td>Ocular</td>
<td>Some of its seed are grinded and the dust particles are applied to the infected eye only once</td>
<td>For ocular diseases</td>
<td>All animal</td>
</tr>
<tr>
<td>Shinfa</td>
<td>Lepidium salivum</td>
<td>Brassicaceae</td>
<td>S</td>
<td>Seed</td>
<td>Oral</td>
<td>A cup of its seed juices is mixed with water and drunk only once</td>
<td>For both productive &amp; dry coughing</td>
<td>All mammals</td>
</tr>
<tr>
<td>Odaa</td>
<td>Ficus sycomorus</td>
<td>Moraceae</td>
<td>T</td>
<td>Sap</td>
<td>Topical</td>
<td>A few drops of its latex are pasted with ash of the burned Aspilamossambicensis (Oliv.) and applied to the wound until it dry</td>
<td>For wound healing</td>
<td>All domestic animal</td>
</tr>
</tbody>
</table>

S, Shrub; H, herb; T, tree; C, climber; NI, not identified.

been practiced for centuries in Chinese, Ayurvedic and Tibb-Unani fields of study as herbal medicines for the treatment of catarrh, rheumatism, nervous diseases, gingivitis, tooth ache, asthma, stroke, constipation and diabetes (Wang and Wang, 2005; Tapsell et al., 2006). On the other hand (Grzanna et al., 2005) indicated the anti-inflammatory action of ginger, while Shukla and Singh (2007) dealt with the cancer prevention properties of the Zinger crude drug. *Echinops* is a well-known medicinal plant which is used for treating abdominal ache, dry cough and circling diseases. Similarly Hymete et al. (2007) reported that the root of *Echinops* is chewed and used to alleviate stomach ache. The anti-microbial activities of medicinal plants are varying; this is in agreement with (Yusha’u et al., 2008) who reported that antibacterial activity may vary from one plant to another. Generally herbal medicines collected during the study were found to have medicinal values in health care of livestock for treatment of multiple ailments. This was also reported in different countries (Deeba et al., 2009; Shinwari, 2010).

**Conclusion**

The results of the present study show presence of wide range of herbal medicine used for treating
Table 6. Summary of general characteristics of the remaining identified medicinal plants.

<table>
<thead>
<tr>
<th>Plant name</th>
<th>Scientific name</th>
<th>Local name</th>
<th>Family name</th>
<th>Plant types</th>
<th>Part used</th>
<th>Adm. route</th>
<th>Method of preparation</th>
<th>Indication</th>
<th>Animal's species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kulubi warabesa</td>
<td>Allium cepa</td>
<td>Allium sativum</td>
<td>Amaryllidaceae</td>
<td>S</td>
<td>Bulb</td>
<td>Oral</td>
<td>Roots are chopped and given with salt for 2 days</td>
<td>Foot and mouth disease</td>
<td>Ruminants</td>
</tr>
<tr>
<td>Jijimbila</td>
<td>Zingiber officinale</td>
<td>Zingiberaceae</td>
<td>S</td>
<td>Roots</td>
<td>Oral</td>
<td>Two bulbs are chopped with salt and given orally for 2 days</td>
<td>Ascariosis, stomach disorder</td>
<td>All domestic animal</td>
<td></td>
</tr>
<tr>
<td>Dumuga</td>
<td>Justicia schimperiana</td>
<td>Acanthaceae</td>
<td>S</td>
<td>Leaves</td>
<td>Oral</td>
<td>The juice of 2-5 leaves are squeezed to their feed and given for 3 days</td>
<td>Rabies</td>
<td>All mammals</td>
<td></td>
</tr>
<tr>
<td>Lolchisa</td>
<td>Bersama abyssinica</td>
<td>Francoaceae</td>
<td>T</td>
<td>Whole</td>
<td>Oral</td>
<td>All of its parts are grinded together and mixed with water and drench a bottle of the mixture</td>
<td>Black legs, chronically diseased, paralyzed animal that unable to stand</td>
<td>Ruminants</td>
<td></td>
</tr>
<tr>
<td>Asangira guracha</td>
<td>Eulophia spp.</td>
<td>S</td>
<td>Leaves and seed</td>
<td>Oral</td>
<td>Some of its leaves are chopped and grinded together with Justicia schimperiana's leaves and drench a bottle of the filtrate</td>
<td>Snake bite, for retained fetal membrane, abdominal aches</td>
<td>All domestic animal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aleltu</td>
<td>mucronata</td>
<td>mucronata</td>
<td>T</td>
<td>Leaves</td>
<td>Oral</td>
<td>Some of its leaves are chopped and grinded together with Justicia schimperiana's leaves and drench a bottle of the filtrate</td>
<td>Rabies prevention</td>
<td>All mammals</td>
<td></td>
</tr>
<tr>
<td>Tabanae</td>
<td>Acokanthera schimperi</td>
<td>Apocynaceae</td>
<td>T</td>
<td>Bark</td>
<td>Oral</td>
<td>Its bark grinded with leaf of Vernonia amygdalina and added to its feed</td>
<td>For healing of swelling</td>
<td>All mammals</td>
<td></td>
</tr>
<tr>
<td>Arangama</td>
<td>Capparistomentosa</td>
<td>Capparaceae</td>
<td>S</td>
<td>Root</td>
<td>Topical</td>
<td>Root is covered by clean cloth and applied locally to swelled area</td>
<td>For treat local swelling</td>
<td>All mammals</td>
<td></td>
</tr>
<tr>
<td>Dertu</td>
<td>Phragmantheramacr osolen</td>
<td>Loranthaceae</td>
<td>E</td>
<td>Leaves</td>
<td>Oral</td>
<td>The leaves are chopped and given with their feed</td>
<td>Clinical mastitis, prolonged emaciated animals</td>
<td>Ruminants</td>
<td></td>
</tr>
<tr>
<td>Kicu warabesa</td>
<td>Agave americana</td>
<td>H</td>
<td>Bulb</td>
<td>Oral</td>
<td>The bulbs are chopped and given with salt</td>
<td>For Black leg</td>
<td>Ruminants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basango</td>
<td>Canna indica</td>
<td>S</td>
<td>Root</td>
<td>Oral</td>
<td>The roots are given with the leaves of Datura arborea.</td>
<td>Black leg</td>
<td>Ruminants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kulubi adi</td>
<td>Allium sativum</td>
<td>H</td>
<td>Bulb</td>
<td>Oral</td>
<td>The bulb is chopped with salt and drenched orally</td>
<td>Abdominal aches and bloat</td>
<td>All domestic animal</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 6 cont’d

<table>
<thead>
<tr>
<th>Komogno</th>
<th><em>Brucea antidysenterica</em></th>
<th>Simaroubaceae</th>
<th>S</th>
<th>Leaves</th>
<th>Ocular and auricular</th>
<th>Some of its leaves are chopped and squeezed. Then its drops applied at the side of infected eye</th>
<th>For blinded and eye discharged animals</th>
<th>All domestic animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gura harre</td>
<td><em>Verbascumsinaoticum</em> Benth.</td>
<td>Scrophulariaceae</td>
<td>S</td>
<td>Leaves</td>
<td>Oral</td>
<td>Its leaves are chopped with the bulb of <em>Allium sativum</em> and drenched orally</td>
<td>For coughing</td>
<td>All mammals</td>
</tr>
<tr>
<td>Harkis</td>
<td><em>Opuntia ficus-indica</em></td>
<td>Cactaceae</td>
<td>S</td>
<td>Steam</td>
<td>Oral</td>
<td>Some of steam parts are chopped and given with salt</td>
<td>For retention of fetal membrane, and against bloat</td>
<td>All mammals</td>
</tr>
</tbody>
</table>

Table 7. Lists of administration route(s) for medicinal plant(s) in study area.

<table>
<thead>
<tr>
<th>Routes of administration of medicinal plant(s)</th>
<th>Number of responders</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>30</td>
<td>60.0</td>
</tr>
<tr>
<td>Topical</td>
<td>9</td>
<td>18.0</td>
</tr>
<tr>
<td>Ocular</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Oral and nasal</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Oral and topical</td>
<td>6</td>
<td>12.0</td>
</tr>
<tr>
<td>Oral, auricular and nasal</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Ocular and auricular</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Nasal and ocular</td>
<td>1</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Table 8. Summary of plant part(s) used as medicinal values.

<table>
<thead>
<tr>
<th>Parts of medicinal value(s)</th>
<th>Number of species</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>18</td>
<td>36.0</td>
</tr>
<tr>
<td>Root</td>
<td>7</td>
<td>14.0</td>
</tr>
<tr>
<td>Bark</td>
<td>2</td>
<td>4.0</td>
</tr>
<tr>
<td>Stem</td>
<td>3</td>
<td>6.0</td>
</tr>
<tr>
<td>Leaf and stem</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Bulb</td>
<td>3</td>
<td>6.0</td>
</tr>
<tr>
<td>Latex</td>
<td>2</td>
<td>4.0</td>
</tr>
<tr>
<td>Sap</td>
<td>2</td>
<td>4.0</td>
</tr>
<tr>
<td>Fruit</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Bark and leaf</td>
<td>1</td>
<td>2.0</td>
</tr>
</tbody>
</table>
Table 8. Cont’d

<table>
<thead>
<tr>
<th>Habit</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed</td>
<td>4</td>
<td>8.0</td>
</tr>
<tr>
<td>Pod</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Leaf and seed</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Leaf and fruit</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Whole parts</td>
<td>3</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Table 9. Distribution of the medicinal plants by their growth forms.

<table>
<thead>
<tr>
<th>Habit of the plants</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Herb</td>
<td>10</td>
<td>20.0</td>
</tr>
<tr>
<td>Shrubs</td>
<td>29</td>
<td>58.0</td>
</tr>
<tr>
<td>Tree</td>
<td>8</td>
<td>16.0</td>
</tr>
<tr>
<td>Climber</td>
<td>2</td>
<td>4.0</td>
</tr>
<tr>
<td>Epiphyte</td>
<td>1</td>
<td>2.0</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Abbreviations

AD, Anno Domini; CAMP, cyclic adenosine monophosphate; DDBOA, Dale District Bureau of Agriculture; EVM, ethno veterinary medicine; HSV, Herpes Simplex Virus; MDs, medicinal plants; TM, traditional medicine.

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REFERENCES


various animal ailments. The study area was endowed with plenty of medicinal plants fairly distributed throughout the region. The identified plants had broad spectrum of activities, the findings indicated that they are used for treatment of multiple ailments and have medical value against many diseases. The ethnomedical use of the plants is mainly for curative and prophylactic purposes. Oral and topical routes of administration are the main administration route for traditional herbal medicine. It is recommended that further investigations should be conducted to establish the medical principles and pharmaceutical activity found in these plants.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.


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