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Gas chromatography/mass spectrometry profiling of the costus plant *Saussurea lappa* (Decne.) C.B. Clarke root extracts and their anti-bacterial activity

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ABSTRACT

The costus *Saussurea lappa* is a well-known medicinal plant that has been widely used in the traditional medicines in many Asian countries. Here, we report on the chemistry of the ethanol and water extracts of *S. lappa* roots using gas chromatography mass spectrometry (GC/MS) analysis. We further tested their antimicrobial activities against *Staphylococcus aureus* (Gram +ve) and *Salmonella sp.* (Gram –ve) isolates. The GC/MS analysis indicate that the ethanol extract has more compounds (37) than does the aqueous extract (18) with sesquiterpene lactones as the most abundant compounds in both extracts. The results reveal that the ethanol extract has significant antimicrobial activity against *S. aureus* isolate with 18–20 mm zone of inhibition, while no effect was observed against *Salmonella* sp. In contrast, weak effects were found in the water extract against *S. aureus* and no effect against *Salmonella* sp. isolate. Together with the GC/MS analyses, we conclude that the ethanol extract contains active secondary metabolites that may have a specific activity likely in a synergy effects with other metabolites against Gram +ve bacteria, but not Gram –ve isolate. Further experiments are needed to clarify the specific effects of *S. lappa* ethanol extracts against Gram +ve bacteria.

INTRODUCTION

The importance of plants as future sources of natural antimicrobial agents holds great promise. Plants are inexpensive and proved to be effective medicines to cure myriad of diseases from mild microbial infections to complex diseases, including cancers and devastating infectious diseases, such as tuberculosis, caused by *Mycobacterium tuberculosis* with much less side effects when compared to the synthetic drugs (Abuzeid *et al*., 2014; Eklund *et al*., 2010; Koko *et al*., 2008). These advantages have encouraged scientists of different disciplines from chemistry to pharmacology to intensively screen for the plants as promising candidates for medicines to replace currently used synthetic drugs (Abdallah, 2011; Cragg and Newman, 2013; Zulkipli *et al*., 2015). One of the big problems that is greatly challenging the public health is the rapid growing resistance developed by infectious microorganisms against many synthetic drugs notably those frequently used against infectious diseases, such as food poisons, urinary tract, respiratory, and digestive systems (Cragg and Newman, 2013; Nascimento *et al*., 2000; Saga and Yamaguchi, 2009). Some of the most threatening multi-drug resistant (MDR) microorganisms are *Staphylococci*, *Streptococci*, *Salmonella*, and *M. tuberculosis* (MDR-TB) owing to the high rates of horiztubeontal gene transfer that confers an MDR in these microorganisms even between evolutionarily distant species (Munita *et al*., 2017; Ventola, 2015). Hence, there is an urgent demand for antimicrobial agents, especially those with narrow spectra to minimize the rapid spread of resistance among infectious

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microorganisms (Chandra *et al*., 2017; Ventola, 2015). To this end, researchers have turned eyes toward plants as a rich and ancient source of various active compounds of medicinal importance for producing next-generation drugs that could possibly overcome the MDR challenges, especially for problematic infectious agent, such as MDR-TB (Chandra *et al*., 2017).

One of the most intriguing medicinal plants that has been used widely in the traditional medicine is the Costus *Saussurea lappa* (Decne.) C. B. Clarke., which is widely used, along with other costus species, in many developing countries in Asia (Pandey *et al*., 2007; Waly, 2009). The *S. lappa* is a member of *Asteraceae* family, a family that is rich with well-known medicinal plants of highly economic importance. Many of which have been intensively studied for their phytochemical constituents and medicinal applications, including *S. lappa* (Pandey *et al*., 2007). The *S. lappa* root in particular has been widely prescribed for various infectious and physiological diseases, including jaundice, diarrhea, stomachache, respiratory tract infections, antispasmodic agents against spasms caused by asthma, rheumatism, leprosy, cholera, and chronic skin diseases among others (Gwari *et al*., 2013; Kala and Manjerkar, 1999; Kapoor, 2001). For example, many studies have also shown that different extracts of *S. lappa* possess various pharmacological activities, including hepatoprotective, anti-inflammatory, anti-cancer, and anti-ulcer properties (Chen *et al*., 1995; Cho *et al*., 2004; Li *et al*., 2005). This wide range of biological activities can be confidently attributed to the richness of *S. lappa* in pharmacologically active ingredients (Pandey *et al*., 2007). Phytochemical constituents, such as diosgenin, prosapogenin B of dioscin, diosgenone, cycloartanol, 25-en-cycloartenol, and octacosanoic acid, were isolated from the costus rhizomes (Qiao *et al*., 2002). The major constituents of *S. lappa* were found to be sesquiterpene lactones, such as saussurea lactone (Rao and Verma, 1951) and isodeydrocostus lactone (Kalsi *et al*., 1983). Active sesquiterpenes lactones, such as costunolide and dehydrocostus lactone, were previously isolated from the methanolic extracts of *S. lappa* (Shoji *et al*., 1986), and appeared to have an anti-inflammatory activity that causes an inhibition of the production of the tumor necrosis factor alpha in murine macrophage-like cells as well as possessing a strong anti-cancer activity when mixed together (Damre *et al*., 2003; Peng *et al*., 2017; Sunkara *et al*., 2010).

Different extracts of *S. lappa* roots, including hexane, chloroform, ethylacetate ether, methanol, and water extracts, have been reported to have various antibacterial and antifungal activities against several pathogenic bacteria, such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella typhi*, *Salmonella typhimurium*, *Streptococcus mutans*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* and fungi, such as *Trichophyton rubrum*, *T. mentagrophytes*, and *Magnaporthe grisea* (Negi *et al*., 2013; Ray and Mjumdar, 1977). For instance, the methanol extract of *S. lappa* was found to be effective against the growth and acid production activity of the *S. mutans* (Chang *et al*., 2012; Yu *et al*., 2007). It has also been demonstrated that the methanol extract of *S. lappa* can lower the adherence of *S. mutans* in a concentration dependent manner as well as inhibiting the biosynthesis of water-insoluble glucan, an extracellular polysaccharide substance produced by *S. mutans* that is implicated in dental caries (Yu *et al*., 2007).

To further our understanding on the phytochemistry and biological activities of *S. lappa* root, we applied gas chromatography/mass spectrometry (GC/MS) analyses on the essential oil composition of the aqueous and ethanol extracts and used these extracts to assess their antimicrobial activities against two bacterial isolates the *S. aureus* and *Salmonella* sp. representing Gram +ve and Gram –ve strains, respectively.

MATERIALS AND METHODS

Plant materials

Roots of *S. lappa* were purchased from general herbal market in Khartoum, Sudan. Taxonomical identification and authentication were taken place at the Herbarium of Medicinal and Aromatic Plants and Traditional Medicine Research Institute of the National Center for Research in Khartoum, Sudan. Voucher specimen was deposited to the same Herbarium of the National Center for Research in Khartoum, Sudan.

Microorganisms

To assess the antibacterial properties of the *S. lappa* root extracts two bacterial isolates of Gram +ve *S. aureus* and Gram –ve *Salmonella* sp. were kindly provided by the Department of Microbiology and Molecular Biology, Faculty of Science and Technology at Al Neelain University, Khartoum, Sudan. These bacterial strains were confirmed by applying conventional biochemical methods and prepared accordingly for further antibacterial tests.

Preparation of extracts

Root specimens were grounded into a fine powder and sample of 50 g was carefully loaded onto Soxhlet extractor (Pyrex, Germany) and extracted with 300 ml of 99.9% ethanol (248 Worli road, Mumbai-30) for 3 hours as described somewhere else (Pandey and Tripathi, 2014). The solvent was separated from the crude extract using rotary evaporator at 60°C for 30 minutes and then the extract was kept at the room temperature to allow drying gently. For aqueous extraction the same steps were followed by replacing the ethanol with water.

Gas chromatography mass spectrometry analyses

For qualitative and quantitative assessment of the essential oil components of the ethanol and aqueous extracts of the *S. lappa* roots, GC/MS analyses were performed using a Shimadzu GCMS-QP2010 Ultra (Shimadzu, Japan) equipped with a capillary column Rtx-5MS of 30 m × 0.25 mm ID with a film thickness of 0.25 µm according to a standard protocol (Pongpiachan *et al*., 2009; 2011). Before injection of a 1 µl of respective extract was dissolved in an equal volume of ethanol and ultrasonicated to homogeneity and mixed well with 0.1 g of anhydrous sodium sulfate before passing the mixture through syringe filter with 0.22 µm pore size. One microliter of the filtrate was injected directly into the capillary column to obtain chromatographic separation. Helium gas was used as a carrier in a flow rate of 1.69 ml/minute. The temperatures of the injector and the mass detector were maintained at 200°C and 250°C, respectively, and the column oven temperature was programed at 50°C–300°C with an initial increase of 7°C/minute until it reaches

180°C then the increment of temperature was raised to 10°C/ minute up to the final temperature for an optimal separation of peaks. Electron ionization was operated with 70 eV. Using scan mode, all the mass spectra were recorded in a range of 40–500 m/z (mass to charges ratio) for a total run time of 28 minutes and the identification of ethanol and water extracts components was conducted by comparing the obtained retention times and mass fragmentation with the documented retention indices available in the library of the National Institute of Standards and Technology.

Well-diffusion antibacterial assay

All the experiments were done in Mueller Hinton Agar medium (MHA) (Mumbai-400086, India) which was prepared according to the manufacturer protocol. To evaluate the antibacterial activities of the different extracts against *S. aureus* and *Salmonella* sp. a well-diffusion antibacterial assay method was used. In brief, freshly prepared MHA plates were cut with a sterile cork borer under aseptic environment to make four wells with a diameter of 10 mm per plate (Miller *et al*., 2002). Bacterial suspensions of 18 hours incubation were initially plated homogenously on MHA plates and three different concentrations of 50%, 100%, and 200% of 40 mg/ml ethanol or water extracts were used to fill the well with 50 µl/well from each concentration and plates were then kept at the room temperature for 2 hours to diffuse completely before incubated at 37°C for additional 18 hours. After incubation, the diameter of the growth inhibition zones was measured in millimeter (mm) and each extracts were assayed for at least three times against each bacterial isolate. For positive control, standard antibiotic azithromycin was used following the same protocol except that antibiotic discs were used instead of solution (i.e., disc-diffusion method).

RESULTS AND DISCUSSION

GC/MS profiling of Saussurea lappa aqueous and ethanol extracts

Saussurea lappa is a well-known medicinal plant that has been widely prescribed as whole plant, rhizome, or root in the traditional medicine in different developing countries in Asia (Pandey *et al*., 2007). The isolated compounds from *S. lappa*, such as costunolides and dehydrocostus lactones, were found to be effective against various microbial infections as well as exerting better anti-cancer properties against breast cancer (Kalsi *et al*., 1995; Kumar *et al*., 1995; Pandey *et al*., 2007; Peng *et al*., 2017). In addition to these compounds, cynaropicrin, a sesquiterpene lactone, was found to exhibit a range of diverse bioactivities, such as anti-inflammation and anti-cancer activities (Cho *et al*., 1998). In order to expand our knowledge in this important medicinal plant and further investigate the reasons behind the strong antibacterial activities of *S. lappa* that have been shown, in many previous studies, to have convincingly various pharmacological activities, such as anti-inflammatory, anti-ulcer, hepatoprotective, and antimicrobial activities (Chen *et al*., 1995; Damre *et al*., 2003; Li *et al*., 2005; Matsuda *et al*., 2000; Venkataranganna *et al*., 1998), we have chosen the *S. lappa* roots (Fig. 1) that is available commercially for medicinal purposes and analyzed, using GC/MS approach, the chemical constituents found in the aqueous and ethanol extracts of the plant roots. The results obtained from the GC/MS analysis

Figure 1. Sample of *Saussurea lappa* roots powder used for the GC-MS analysis and subsequent antimicrobial activity.

have led to the identification of a total of 37 and 18 compounds in the ethanol and aqueous extracts, respectively (Tables 1 and 2). As shown in the GC/MS chromatogram (Fig. S1), the ethanol extract of *S. lappa* has more phyto-constituents with almost double the number of compounds identified in the aqueous extract (Fig. S2). This is consistent with a previous study which reported that *S. lappa* and *S. costus* are rich in the phyto-constituents and that the GC/MS analysis of their ethanol extracts resulted in the identification of 39 and 41 compounds, respectively, with sesquiterpenes as major component (Gwari *et al*., 2013; Maurer and Grieder, 1997; Negi *et al*., 2013).

The ethanol extract in the present study as indicated from the GC/MS profile gave rise to several (8–10) compounds with significant peak areas when compared to that of the aqueous extract (5–7), indicating that ethanol solvent is superior in extracting GC/MS detectable phyto-constituents in comparison with that of water (Table 1; Figs. S1 and S2). Those compounds with significant peak areas (%) detected in the ethanol extract are; cyclodecacyclotetradecene,14,15-didehydro- (29a), 1,3-propanediol,2-(hydroxymethyl)-2-nitro- (15a), bufa-20,22-dienolide,14,15-epoxy-3,11-dihydroxy- (32a), bicycle(5.3.0)decane,2-methylene-5-(1-methylvinyl)-8- (23a), isosteviol methyl ester (31a), 2(3H)-benzofuranone,6-ethyenlhexahydro-6-methylene-7- (25a), 4,7,10,13,16,19-docosahexaenoic acid, methyl ester (28a), and androstan-17-one,3-ethyl-3-hydroxy-,(5.alpha)- (19a), which gave rise to peak areas (%) of 39.59, 11.05, 5.91, 4.36, 3.97, 3.88, 3.18, and 3.01, respectively (Fig. S1). In contrast, aqueous extract analyses by GC/MS has revealed that compounds cyclodecacyclotetradecene,14,15-didehydroxy (14b), cholest-7-en-3-ol,4-methyl-,(3.beta.,4.alpha)- (18b), 2(3H)-benzofuranone,6-ethenythexhydroxy- (12b), .Alpha.-Guaiene (10b), 3-oxatricyclo(20.8.0.0(7,16)) triaconta-1- (16b), 9,12,15-octadecatrienoic acid,(z,z,z)- (8b), and cyclohexane,1,2-diethenyl-4-(1-methyle-(9b) are dominant with peak areas (%) of 61.69, 5.10, 4.42, 4.17, 3.49, 3.47, and 3.12,

Table 1. GC/MS analyses of the ethanol extract of the *S. lappa* root indicating the percentages and the chemical constituents corresponding to the major peaks in the chromatogram.

No.	Retention time (minute)	Chemical constituents	Formula	Peak area (%)
1a	3.269	Glycerin	$C_3H_8O_3$	0.87
2a	3.346	Ethoxyacetaldehyde diethylacetal	$C_8H_{18}O_3$	1.28
3a	3.631	2-Furanmethanol	$C_5H_6O_2$	0.28
4a	4.234	Methanamine, N-hydroxy-N-methyl-	C_2H_7NO	2.31
5a	4.754	2-hydroxy-2-Cyclopenten-1-one	$C_5H_6O_2$	0.31
6a	5.715	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	$C_6H_8O_4$	0.29
7a	7.066	2,5-Hexanedione	$C_6H_{10}O_2$	0.21
8a	7.154	Pentanoic acid,4-oxo- (Levulinic acid)	$C_5H_8O_3$	0.11
9a	7.397	2,5-Dimethyl-4-hydroxy-3(2H)-furanone (Furaneol)	$C_6H_8O_3$	0.09
10a	7.845	Maltol	$C_6H_6O_3$	0.73
11a	9.209	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	1.14
12a	11.033	5-Hydroxymethylfurfural	$C_6H_6O_3$	2.15
13a	11.343	1,2,3-propanetriol,1-acetate	$C_5H_{10}O_4$	1.53
14a	12.297	Propanoic acid,3-(acetyloxy)-2-(hydroxymethyl)-,ethyl ester	$C_8H_{14}O_5$	1.04
15a	15.686	1,3-propanediol,2-(hydroxymethyl)-2-nitro-	$C_4H_9NO_5$	11.05
16a	17.997	11,11-Dimmethyl-spiro(2,9)dodeca-3,7-dien	$C_{14}H_{22}$	0.19
17a	18.585	1,2,3,5-Cyclohexanetetrol, (1.alpha.,2.beta.3.alpha.,5.beta.)-	$C_6H_{12}O_4$	0.76
18a	19.174	9,12,15-octadecatrienoic acid,(z,z,z)	$C_{18}H_{30}O_2$	2.49
19a	19.255	Androstan-17-one,3-ethyl-3-hydroxy-,(5.alpha)-	$C_{12}H_{34}O_2$	3.01
20a	20.604	2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydro-naphthalen-2-	$C_{15}H_{24}$	0.31
21a	20.677	Bicyclo(5.2.0)nonane,4-methylene-2,8,8-trimethyl-2-vinyl-	$C_{15}H_{24}$	0.32
22a	20.777	Andrographolide	$C_{20}H_{30}O_5$	0.17
23a	20.927	Bicyclo(5.3.0)decane,2-methylene-5-(1-methylvinyl)-8-	$C_{15}H_{24}$	4.36
24a	20.993	.Gamma.-guarjumenepoxide-(2)	$C_{15}H_{24}O$	1.77
25a	21.855	2(3H)-benzofuranone,6-ethenylhexahydro-6-methylene-7-	$C_{15}H_{20}O_2$	3.88
26a	22.237	Alloaromadendrene	$C_{15}H_{24}$	1.36
27a	23.156	Pentadecanoic acid	$C_{15}H_{30}O_2$	1.79
28a	23.227	4,7,10,13,16,19-Docosahexaenoic acid, methyl ester	$C_{23}H_{34}O_2$	3.18
29a	23.933	Cyclodecacyclotetradecene,14,15-didehydro-	$C_{22}H_{32}$	39.59
30a	25.734	.Beta.-Guaiene	$C_{15}H_{24}$	1.21
31a	25.863	Isosteviol methyl ester	$C_{21}H_{32}O_3$	3.97
32a	26.180	Bufa-20,22-dienolide,14,15-epoxy-3,11-dihydroxy-	$C_{24}H_{34}O_2$	5.91
33a	26.997	30-Norlupan-28-oic acid,3-hyroxy-21-	$C_{29}H_{46}O_4$	1.05
34a	28.511	Octadecanoic acid ,2,3-dihydroxypropyl-	$C_{21}H_{42}O_4$	0.41
35a	29.991	9,12-Octadecadienoic acid (z,z)-	$C_{18}H_{32}O_2$	0.34
36a	30.631	Pregnennolone	$C_{21}H_{32}O_2$	0.21
37a	31.009	Spiro(5.5)undeca-1,8-diene,1,5,5,-	$C_{15}H_{24}$	0.33

respectively (Fig. S2). Figure 2 shows the deduced structures of some of these compounds detected in significant levels in both ethanol and aqueous extracts. Compound 29a (14b), for example, was found to be the major component of both aqueous (61.69%) and ethanol (39.59%) extracts (Fig. S1 and S2). Interestingly, compound 25a (12b), commonly known as dehydrosaussurea lactone with a molecular structure of $C_{15}H_{20}O_2$ (Fig. 2B), was found in reasonable amounts in both extracts with a slightly higher concentration in the aqueous extract than the ethanol extract. This sesquiterpene lactone is very similar structurally to another sesquiterpene lactone called costunolide, was previously isolated from the methanol extract of *S. lappa* and found to be a major component in the root volatile oil (Govindan and Bhattacharaya, 1977; Gwari *et al*., 2013; Maurer and Grieder, 1997; Shoji *et al*., 1986). This compound, in a combination with constunolide ($C_{15}H_{20}O_2$), has been recently shown to exhibit a better anti-breast cancer activity by inducing cell cycle arrest and apoptotic action against cancerous cell indicating the significance medicinal properties of this compound (Peng *et al*., 2017). Compound 31a, Isosteviol methyl ester ($C_{21}H_{32}O_3$), was found only in the ethanol extract with significant amount (3.97%) (Table 1). This compound is regarded as a bio-active agent and has been shown to reduce the oxidative stress and ar-

Table 2. GC/MS analyses if the aqueous extract of the *S. lappa* root indicating the percentage and chemical constituents corresponding to the major peaks in the chromatogram.

No.	Retention time (minute)	Chemical constituents	Formula	Peak area (%)
1b	4.772	2-Cyclopenten-1-one,2-hydroxy-	$C_5H_6O_2$	0.61
2b	10.071	1-Dodecanol	$C_{12}H_{26}O$	0.36
3b	11.401	1,2,3-propanetriol,1-acetate	$C_5H_{10}O_4$	1.16
4b	14.314	Cyclohexane,1-ethenyl-1-methyl-2,4-bis-	$C_{15}H_{24}$	0.17
5b	14.918	Caryophyllene	$C_{15}H_{24}$	0.15
6b	15.000	3-Buten-2-one,4-(2,6,6-trimethyl-2-cyclo-	$C_{13}H_{20}O$	0.18
7b	15.220	1,3-propanediol,2-(hydroxymethyl)-2-	$C_4H_9NO_5$	2.88
8b	19.178	9,12,15-octadecatrienoic acid,(z,z,z)-	$C_{18}H_{30}O_2$	3.47
9b	19.256	Cyclohexane,1,2-diethenyl-4-(1-methyle-	$C_{13}H_{20}$	3.12
10b	20.928	.Alpha.-Guaiene	$C_{15}H_{24}$	4.17
11b	20.993	.delta.4-androstene-3.beta.,17.beta.-diol	$C_{19}H_{30}O_2$	1.88
12b	21.842	2(3H)-benzofuranone,6-ethenythexhydroxy-	$C_{15}H_{20}O_2$	4.42
13b	23.227	4,7,10,13,16,19-Docosahexaenoic acid, methyl ester	$C_{22}H_{34}O_2$	2.96
14b	23.889	Cyclodecacyclotetradecene,14,15-didehydroxy	$C_{22}H_{32}$	61.69
15b	25.863	1,4-Methanocycloocta(d)pyridazine,1,4,4a-	$C_{12}H_{20}N_2$	2.73
16b	26.178	3-oxatricyclo(20.8.0.0(7,16))triaconta-1-	$C_{29}H_{42}O$	3.49
17b	28.520	Octadecanoic acid,2,3-dihydroxypropyl-	$C_{21}H_{42}O_4$	1.44
18b	31.285	Cholest-7-en-3-ol,4-methyl-,(3.beta.,4.alpha-	$C_{28}H_{48}O$	5.10

senic-DNA induced damage beside its capability to induce apoptosis against cancerous cells (Das *et al*., 2013). In addition, compound 28a, a fatty acid methyl ester, was only found in the ethanol extracts with somewhat increased level (3.18%). Such fatty acid methyl ester compounds were found to possess potential medicinal activities against various microorganisms (Chandrasekaran *et al*., 2008).

Other compounds of significant medicinal and biological interests were identified in trace amounts in the aqueous and ethanol extracts (Tables 1 and 2). Intriguingly, compounds 2a (dodecan-1-ol), and 5a (caryophyllene), a sesquiterpene, which were identified only in the aqueous extract (Table 2), whereas, compounds andrographolide (22a), gamma-gurjunenepoxide-(2) (24a), and alloaromadendrene (26a) were detected only in the ethanol extract (Table 1). These compounds were found to have various biological activities as demonstrated in many previous studies (Banerjee *et al*., 2017; Dahham *et al*., 2015; De Lima *et al*., 2016; Sonboli *et al*., 2006; Tyagi *et al*., 2013).

Anti-bacterial activities of ethanol and aqueous extracts

The anti-bacterial activities of the *S. lappa* of both the ethanol and aqueous extracts were evaluated using a well-diffusion method against *S. aureus* (G +ve) and *Salmonella* sp. (G –ve) isolates with azithromycin as a positive control (Figs. 3–5). The results showed that both the ethanol and aqueous extracts exhibited antibacterial activity against *S. aureus*, whereas no antibacterial effect was noticed against *Salmonella* sp. isolate (Figs. 4 and 5). This result may be due to a developing resistance of this strain against anti-microbial agents found in the *S. lappa* root or a lack of effective agents against Gram –ve bacteria as indicated by its resistance to the azithromycin control which has somewhat shallow antibacterial activity (Fig. 3). This is

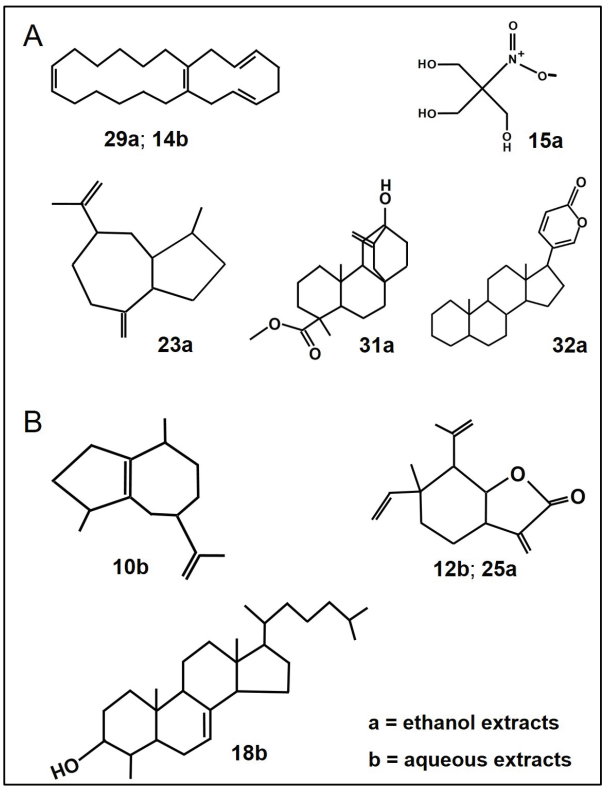


Figure 2. Chemical structures of some of the phyto-constituents detected in the ethanol and aqueous extracts. These compounds are **29a(14b)**, Cyclohexane, 1,2-diethenyl-4-(1-methyle-); **15a**, 1,3-prpanediol,2-(hydroxymethyl)-2-; **23a**, Bicyclo(5.3.0)decane,2-methylene-5-(1-methylvinyl)-8-; **31a**, Isosteviol methyl ester; **32a**, Bufa-20,22-dienolide,14,15-epoxy-3,11-dihydroxy-; **10b**, alpha.-Guaiene; 12b(25a), 2(3H)-benzofuranoze,6-ethenythexhydroxy-; and **18b**, Cholest-7en-3-ol,4-methyl-,(3.beta.,4.alpha-).

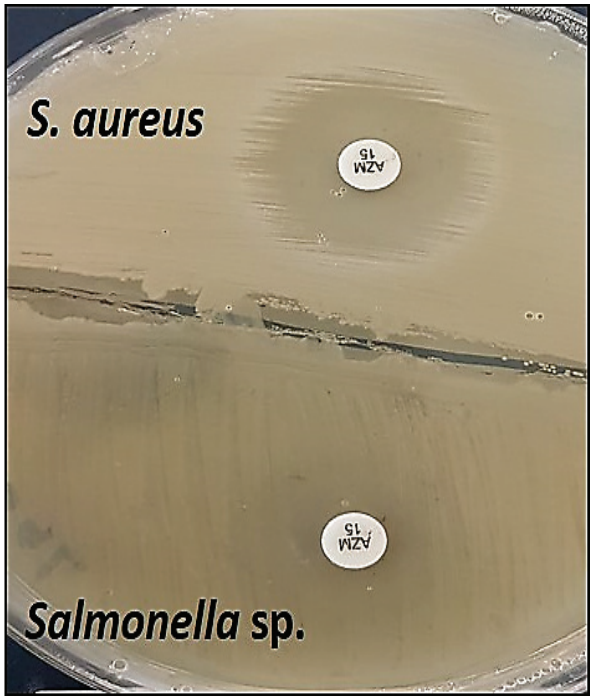
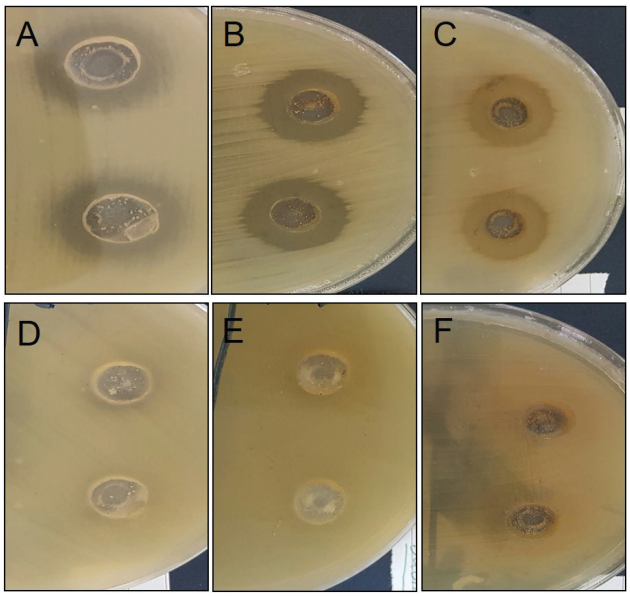


Figure 3. Antibacterial sensitivity test of Azithromycin (control) against *S. aureus* and *Salmonella* sp isolates.

plausible since *Salmonella* isolates is known for its ability to develop rapid resistance against available antibiotics as reported in many previous studies (Skov *et al*., 2007; Su *et al*., 2004). In accordance with the present study, Parekh and Chanda (2007) reported that the aqueous extract of *S. lappa* is inactive against some isolated bacteria and has some activities against others, including a standard *S. typhimurium* (ATCC 23564). A previous study has attributed the antibacterial activities of *S. costus* rhizome extracts against G +ve *S. aureus*, *S. epidermidis* and G –ve *E. coli* and *S. typhimurium* to the presence of diosgenin, a bioactive phytosteroid sapogenin compound ($C_{27}H_{42}O_3$) (Ariharan *et al*., 2012). Interestingly, similar compounds were found in detectable amounts in both extracts, i.e., compounds 32a and 18b (Table 2). Therefore, the results showing that ethanol and aqueous extracts have exclusive activities against *S. aureus* but not *Salmonella* sp. may be attributed to these compounds and that such compounds have no apparent antimicrobial properties against our *Salmonella* sp. isolate or this isolate has already developed a resistance against these compounds or similar ones. Similarly, the ethanol extracts of *S. lappa* was found to be effective against caries-causing bacteria *S. mutans* which significantly lower the adherence of the microbe as well as inhibiting its growth and acid production (Yu *et al*., 2007). Nonetheless, the mechanism behind such activity against G +ve bacteria and resistance to our *Salmonella* isolate is not clear in the present study and further study is request to draw a decisive conclusion.

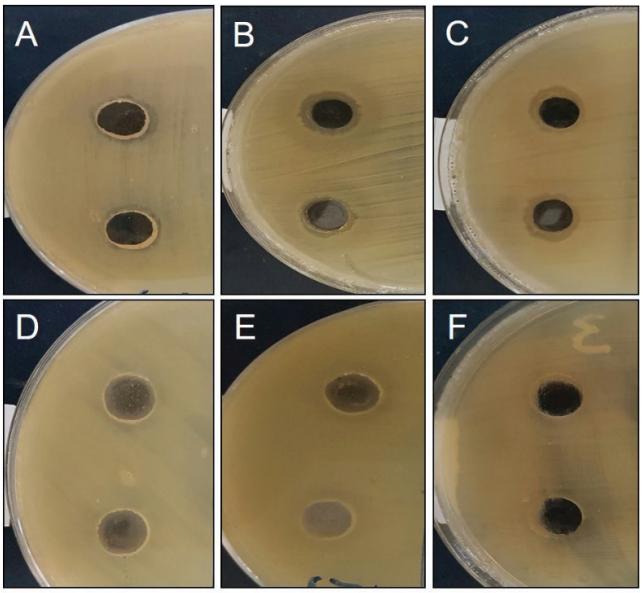
Generally, the ethanol extract was more effective against *S. aureus* with inhibition zones ranging between 18 and 20 mm than the aqueous extracts which showed mean inhibition zones around 15 mm at higher concentrations (>20 mg/ml)



Saussurea lappa roots	Mean of Inhibition Zone Diameter, IZD (mm)	
Ethanol extract concentrations, (%)*	*S. aureus*	*Salmonella* sp.
50	18	0.0
100	20	0.0
200	20	0.0
Azithromycin	20	12

Figure 4. The antibacterial activities of the ethanol extracts from *S. lappa* roots against *S. aureus* and *Salmonella* sp. at different concentrations. (A) The effect of 50% of 40 mg/l crude ethanol extract against Gram +ve *S. aureus*, (B) 100% of 40 mg/l crude, and (C) 200% of 40 mg/l. (D) The effect of 50% of 40 mg/l crude, (E) 100% of 40 mg/l, and (F) 200% of 40 mg/l crude against the Gram −ve *Salmonella* sp.

(Figs. 4 and 5). This can be confidently attributed to the richness of ethanol extracts of *S. lappa* root with bio-active compounds and possibly that a synergetic action of two or more of such active compounds might have occurred to inhibit the growth of *S. aureus* in our MHA media. Compounds 22a, 28a, and 31a that have been known for their potential medicinal activities and found exclusively in the ethanol extracts (Chandrasekaran *et al*., 2008; Das *et al*., 2013). In support of this interpretation, *S. aureus* strain was found to be more sensitive to compound 22a, andrographolide, with a minimal inhibitory concentration of 100 µg/ml, which is comparable to the amount detected in the ethanol extract found in the present study (Banerjee *et al*., 2017). All together, the significant antimicrobial activity of the ethanol extract against *S. aureus* may be due to the action of these unique compounds found only in the ethanol and/or the presence of a highly active compound such as 22a in the ethanol extract and its absence from the aqueous extract.



Saussurea lappa roots	Means of inhibition zone diameter, MIZD (mm)	
Aqueous extract concentrations, (%)*	*S. aureus*	*Salmonella* sp.
50	0.0	0.0
100	14	0.0
200	15	0.0
Azithromycin	20	12

Figure 5. The antibacterial activities of the aqueous extracts from *S. lappa* roots against the Gram +ve *S. aureus* and Gram −ve *Salmonella* sp at different. (A) The effect of 50%, (B) 100%, and (C) 200% aqueous extracts against S. *aureus*. (D) The effects of 50%, (E) 100%, and (F) 200% of the aqueous extracts against *Salmonella* sp.

CONCLUSION

In conclusion, we demonstrate that *S. lappa* root is rich in various bioactive compounds as indicated in the GC/MS profiles of the ethanol and aqueous extracts. The study also revealed that the ethanol extract is more effective as antibacterial agent against G +ve *S. aureus* owing to the richness of this extract with several bioactive compounds which were not detected in the aqueous extracts. We also conclude, based on the condition followed in this study, that the *S. lappa* root has no effects against G –ve strain *Salmonella* sp. Hence, further studies are required to investigate the antibacterial effects of *S. lappa* extracts and understand their mode of actions to help found an antibacterial agent that would compete the conventional antibiotics.

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CONFLICT OF INTEREST

All authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

AMM conceived and supervised the overall work; OREE and OIMA performed all of the experiments; FHMK analyzed data, and drafted the manuscript; AMM and FHMK commented and discussed the final manuscript with input from other authors.

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Supplementary Materials

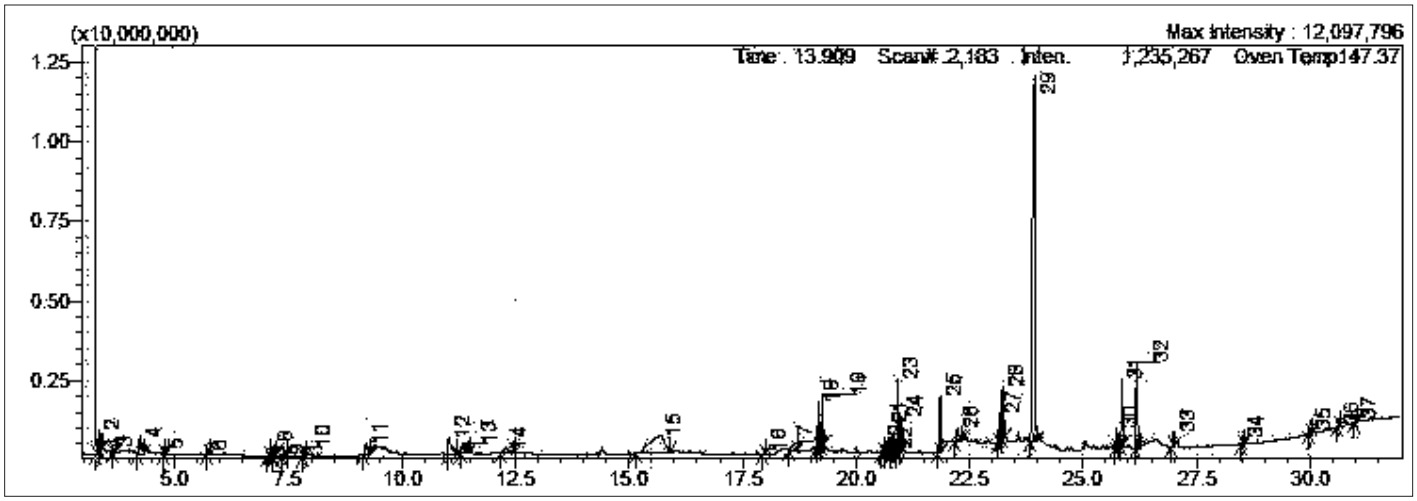

Figure S1. GC/MS representative chromatogram from the analyses of the ethanol extracts of the essential oil of the *S. lappa* roots.

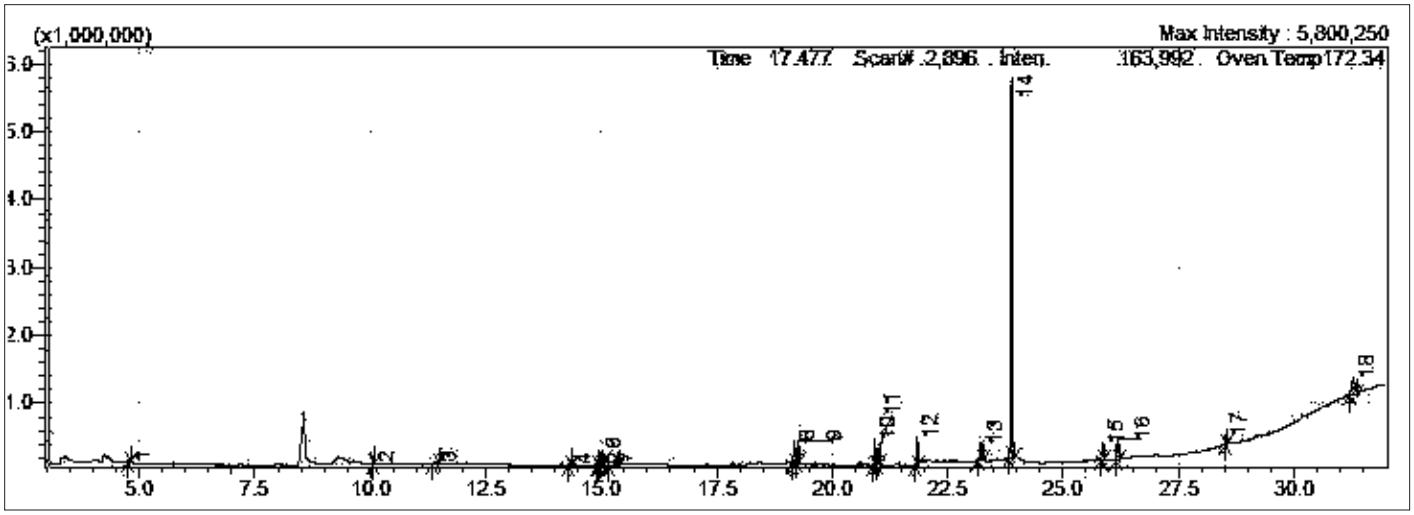

Figure S2. GC/MS representative chromatogram from the analyses of the aqueous extracts of the essential oil extracted from the *S. lappa* roots in this study.