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Original article

Evaluation of GC × GC-TOF-MS untargeted metabolomics, cytotoxicity and antimicrobial activity of leaf extracts of *Artemisia afra* (Jacq.) purchased from three local vendors



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ABSTRACT

Objectives: As microorganisms are developing resistance to therapies, the search for potent antimicrobial compounds continues. The potential of detecting new compound(s) with antimicrobial activities from extracts of *Artemisia afra*, a South African plant frequently used for the treatment of diseases and widely traded in the market was explored. *A. afra* was purchased from three vendors. **Methods:** The dried leaf powder extracted separately with butanol, chloroform, and water were evaluated for cytotoxicity and antimicrobial activity using standard methods, and characterized for antimicrobial compounds using untargeted metabolomics. **Results and Conclusion:** All the extracts exhibited antibacterial activity. Butanol extract of vendor B sample was the most active against *P. aeruginosa* while the antibacterial activity of water extract of vendor A against *S. aureus*, *E. coli*, and *E. faecalis* was similar to butanol extract of vendor B. All other extracts were less cytotoxic than the chloroform extract of vendor B (LC₅₀ value = 414.56 ± 4.81 µg/mL). GC × GC-TOF-MS detected 436 compounds, 280 identifications were confirmed with 34 having known antimicrobial properties. The concentrations of compounds varied among the vendors' samples with the highest in all samples and particularly in samples from vendor C (14.85 µg/mg) being Bicyclo[2.2.1]heptan-2-one 1,7,7-trimethyl-(1S)-, an antimicrobial compound. While two compounds were undetectable in vendor A sample, four were undetected in vendor C sample and eleven compounds in vendor B sample. The antibacterial activity of extracts could not be related to the metabolites variations and concentration. The *A. afra* species purchased from vendors from the traditional medicinal market showed antibacterial potential. To our knowledge, this was the first study to report the metabolomics of *A. afra* in respect to antimicrobial activities.

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1. Introduction

Emerging infectious diseases are increasing probably due to travel, overcrowding caused by urbanization and inadequate health-care systems leading to new interactions between human beings, and animals as well as other diseases (Alirol et al., 2011; Muleya et al., 2014). Therefore, resolving the challenges associated with infectious diseases is of utmost importance. Man has been able to utilize the natural resources in the environment (the flora and

fauna) to alleviate pain and improve health (Patil et al., 2011). The exploration of natural resources has led to the discovery of medicinal plants with diverse health benefits. Consequently, medicinal plants are the target for investigation in drug discovery as they are the primary sources of bioactive phytochemicals (Agbor and Ngogang, 2005; McGaw et al., 2008; Singh et al., 2016). An estimated 80% of the population of Asian and African as well as an estimated 65% of the world's population depend on traditional herbal remedy to cure and prevent infectious ailments, and chronic diseases (Patil et al., 2011). Several studies in South Africa have documented traditional plants with therapeutic benefits against conditions associated with coughing, respiratory ailments and fever (McGaw et al., 2008; Van Wyk, 2011; Mahomoodally, 2013). Therefore, *Artemisia afra* (Jacq.), a commonly used plant is explored as a possible source of phytochemicals with high potency against microorganism associated infectious diseases.

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A. afra (Jacq.) commonly called African wormwood is a popular medicinal plant with wide application in many parts of Africa (Van Wyk, 2011). It is used traditionally to cure various ailments or assuage the pain caused by sicknesses such as common colds, coughs, sore throat, influenza, asthma (van Wyk and Wink, 2004; Patil et al., 2011; More et al., 2012; Hübsch et al., 2014). The traditional use of *A. afra* as an anti-infectious remedy was studied as potential antimicrobial activities (McGaw et al., 2008; More et al., 2012; Martini et al., 2020). *A. afra* was reported to have a broad spectrum of inhibitory activity against microorganisms (Muyima et al., 2002). It exhibited antifungal properties (Abad et al., 2012) as well as antibacterial potency against a range of Gram-positive and Gram-negative bacteria (Jäger, 2003; More et al., 2012; Martini et al., 2020). Other pharmacological investigations demonstrated *A. afra* to possess spasmolytic, antidepressant, cardiovascular and antioxidant activity (More et al., 2012).

Although many rural communities rely on commercially sold medicinal plants for their primary health care, investigations have often focused on extracts of medicinal plants collected from known sources and processed in accordance with standard guidelines. There is a dearth of information on the therapeutic effectiveness of *A. afra* sold in the traditional herbal markets. To our knowledge, this is the first study to investigate the activity of *A. afra* purchased from the traditional market. The challenge associated with extracts of medicinal plants of unknown sources includes quality of the plant materials and post-harvest handling. This may compromise the secondary metabolite constituents and invariably the efficacy, and safety. Metabolomics is the metabolic profiling of metabolites in a biological system including plants under specific conditions (Satheeshkumar et al., 2012; Mukherjee et al., 2016). This approach has been utilized in comprehensive profiling of secondary metabolites of plant extracts and applied for quality control of plant materials, and discovery of lead compound(s) (Satheeshkumar et al., 2012; Mukherjee et al., 2016). Untargeted metabolomics attempt to profile all the known and unknown metabolites of plant extracts (Cai et al., 2015). The techniques used for metabolic profiling of extracts of plants includes spectral (nuclear magnetic resonance (NMR), infrared (IR), ultraviolet-visible (UV), mass spectrometry (MS)), chromatographic techniques (gas chromatography (GC), high-performance liquid chromatography (HPLC), capillary electrophoresis (CE)) or their hyphenated techniques (GC-MS) or (LC-NMR) (Satheeshkumar et al., 2012). Therefore the first objective of this research was to investigate the antimicrobial and cytotoxic activity of the extracts of *A. afra* purchased from different vendors in the traditional medicinal plant market. The second was to characterize and compare the phytochemicals, and antimicrobial compounds of the plant extracts using 2-dimensional untargeted GC × GC-TOFMS (gas chromatography/time-of-flight mass spectrometry) metabolomics analysis.

2. Material and methods

2.1. Reagent and chemical

Chloroform and butanol were from Merck (South Africa), Gentamycin (Virbac, South Africa), doxorubicin (Pfizer, South Africa), Mueller-Hinton agar, *p*-iodonitrotetrazolium violet, dimethyl sulfoxide, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Minimum essential medium growth medium, phosphate buffered saline, 3-Phenylbutyric acid, methoxyamine hydrochloride N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA), trimethylchlorosilane (TMCS) were from Sigma Aldrich (South Africa). All organic solvents used for the GC × GC analysis were ultra-pure Burdick & Jackson brands (Honeywell International Inc., Muskegon, MI, USA).

2.2. Collection of plants

Samples of the plant *Artemisia afra* (Jacq.) were purchased at the Soshanguve Muthi-Market, Pretoria (GPS coordinates $-25^{\circ} 31' 22.19''$ S and $28^{\circ} 06' 19.20''$ E), South Africa from three different vendors designated as vendor A, B and C. These were identified and authenticated at SANBI (South African National Biodiversity Institute). Sample specimens for the plants were deposited to the Department of Biology, Sefako Makgatho Health Sciences University under the code: vendor A (SMU-MMC001), vendor B (SMU-MMC002), and vendor C (SMU-MMC003). The dried leaves were ground to powder and stored in air amber bottles until needed for the extraction process.

2.3. Extraction of plants

The powdered leaves (3 g) from each vendor were separately extracted with 30 mL each of solvent (chloroform, butanol and water) in a 50 mL polyester centrifuge tube on a platform shaker for 30 min. The tubes were centrifuged for 10 min at 70 rpm and the tubes were left overnight. The supernatants were filtered using Whatman No. 1 filter papers into pre-weighed glass containers and were allowed to evaporate to a constant weight to calculate the yield.

2.4. Antibacterial activity assay

Four bacterial strains available during the period of the study were used; two Gram-positive bacteria [*Staphylococcus aureus* (*S. aureus*) (ATCC 29213) and *Enterococcus faecalis* (*E. faecalis*) (ATCC 29212)] and two Gram-negative bacteria [*Escherichia coli* (*E. coli*) (ATCC 25922) and *Pseudomonas aeruginosa* (*P. aeruginosa*) (ATCC27853)]. The bacterial strains were maintained on Mueller-Hinton (MH) agar. The minimum inhibitory concentrations (MIC) of plant extracts were determined using the broth microdilution method in 96-well microtitre plates (Jadaun et al., 2007; Makhafola et al., 2019). The lowest concentration containing clear wells was measured as the MIC values.

2.5. Cytotoxicity assay using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)

Cultured Vero cells were harvested, counted and seeded at a density of 1×10^6 cells/mL Minimum essential medium growth medium (100 μ l) into each well of 96-well plates and treated using the method described by Olaokun et al. (2017). The extracts of *A. afra* were tested for cytotoxicity, using the Vero cell lines by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Mosmann, 1983). The lethal concentration values (LC₅₀) calculated from a plot of the log of concentration versus average absorbance represents the concentration of plant extract that resulted in a 50% reduction of absorbance in comparison to the untreated cells.

2.6. GC × GC-TOFMS (gas chromatography/time-of-flight mass spectrometry)

The analysis was performed using the comprehensive two-dimensional gas chromatography (GC × GC) (Pegasus 4D GC × GC-TOFMS Leco Corporation, St. Joseph, MI, USA) consisting of a GC system (7890A series, Agilent, Atlanta, GA) coupled to a time of flight mass spectrometer (TOFMS) (Leco Corporation, St. Joseph, MI, USA). The system is equipped with a Gerstel Multi-Purpose Sampler (MPS) (Gerstel GmbH & co. KG, Eberhard-Gerstel- Platz 1, D-45473 Mülheim an der Ruhr) and a cryogenic cooler. The temperature of the primary GC oven was automated

to start at 70 °C for 2 min, thereafter to increase at 4 °C/min and finally held at 300 °C for 2 min. The temperature of the secondary GC oven programmed at 85 °C for 2 min was to increase at 4.5 °C/min and finally to 300 °C, at which it was held for 4.5 min. The modulator automated with a starting temperature of 100 °C for 2 min was increased by 4.0 °C/min to a final temperature of 310 °C where it was sustained for 12 min.

2.6.1. Metabolome extraction method

A single-phase extraction method was utilized for the test samples and the two quality control (QC) samples (approx. 50 mg each). To each sample was added 50 µl of internal standard (3-Phenylbutyric acid 50 ppm). Thereafter the extraction solution mixture (1 mL) comprising of chloroform, methanol and water (1:3:1), and 3 mm tungsten carbide bead was added. The sample mixtures were shaken in a vibration mill at 30 Hz for 10 min and centrifuged at 12,000 × g for 5 min. Thereafter, 800 µl of the supernatant was dispensed into a GC tube and evaporated to dryness under a stream of nitrogen at 37 °C. For derivatization purposes, each dried sample extract was incubated at 60 °C for 60 min after the addition of 50 µl methoxyamine HCl (150 mg/10 mL), and again for 60 min at 40 °C after adding 40 µl BSTFA (N,O-Bis(trimethylsilyl)trifluoroacetamide) + 1% TMCS (trimethylchlorosilane). The derivatized sample was then centrifuged for 5 min at 2000 × g and the supernatant was transferred to a 0.1 mL insert in a GC-MS sample vial which was capped prior to GC × GC-TOFMS analysis.

2.6.2. Data processing information and peak identification

ChromaTOF software version 4.71 (Leco Corporation, St. Joseph, MI, USA) was utilized for data processing; for peak finding and mass spectral deconvolution. By employing the mass fragmentation patterns produced by the MS, combined with their corresponding GC retention times, the identities of these peaks were resolved by matching it with commercially available NIST spectral libraries (mainlib, replib).

2.7. Statistical analysis

All the assays were executed in three replicates and the results were expressed as mean ± standard deviation (SD). Data were analysed by one-way analysis of variance (ANOVA) and by a two-tailed *t*-test after Bonferroni where significant difference was found using the Microsoft Excel 2010 statistical package. The *p* values less than 0.05 were considered statistically significant.

3. Result

3.1. Extraction of plants

The average percentage yield of extractable materials from the dried powdered leaves of *A. afra* is presented in Table 1. Compared to other solvents chloroform was an efficient solvent for the extraction of phytochemicals from *A. afra*. The chloroform extract of Vendor B's sample had the highest yield (2.65 ± 0.34%). The per-

Table 1
Percentage (%) yield of extracts of *A. afra* purchased from three vendors.

<i>Artemisia afra</i>	Solvents		
	Water	Butanol	Chloroform
Vendor A	1.02 ± 1.65	1.06 ± 0.5	2.10 ± 0.25
Vendor B	1.08 ± 0.45	1.13 ± 0.04	2.65 ± 0.34
Vendor C	1.05 ± 0.82	1.35 ± 0.02	2.09 ± 0.46

Yield calculated as mean ± standard deviation of mean of three sets of extractions.

centage extraction yield of the water extracts was not different from those of butanol extracts.

3.2. Antimicrobial activity assays

The inhibition of the activity of selected bacterial strains by the chloroform, butanol, and water extracts of *A. afra* reported as MIC values is presented in Table 2. All the extracts exhibited antibacterial activity. There were no trends in the antibacterial activity of all the extracts against the four bacteria strains. The butanol extracts of plants of all three vendors strongly inhibited *P. aeruginosa* having the lowest MIC values and the sample from vendor B (4.82 ± 0.39 µg/mL) was the strongest. No significant difference (*p* < 0.05) was found between the antibacterial activity of the water extract of vendor A and butanol extracts from vendor B against *S. aureus*, *E. coli*, and *E. faecalis*.

3.3. Cytotoxicity assay against Vero cells

The chloroform, butanol, and water extracts of *A. afra* from all the vendors were less cytotoxic against Vero monkey kidney cells than doxorubicin, the positive control (Fig. 1). All the extracts exhibited relatively low cytotoxic effect against the Vero cells with LC₅₀ value >500 µg/mL with the exception of the chloroform extract of *A. afra* purchased from vendor B having the LC₅₀ value of 414.56 ± 4.81 µg/mL. The positive control doxorubicin was very cytotoxic exhibiting a high LC₅₀ value of 57.83 ± 3.02 µg/mL.

3.4. Untargeted GC × GC-TOFMS metabolites profiles analysis of butanol extracts

GC × GC-TOFMS analysis was conducted to identify and quantify all the metabolites composition of the butanol extracts of *A. afra*. The classical total ion chromatograms of the extracts of *A. afra* from each of the vendors measured by GC × GC-TOFMS are presented in Fig. 2. In total, 22,293 ion features were measured across all three samples and translating to the identification of 436 compounds (Supplementary Table) using the automated data processing software (Progenesis QI). The identity of 280 compounds were confirmed with 34 of them already demonstrated to exhibit antimicrobial properties, using the in-house database containing authenticated standards (Supplementary Table and Table 3). The extracts of *A. afra* samples from the three vendors (A, B, C) showed variations of compounds in respect to constituents and quantity. The most abundant compounds were Bicyclo[2.2.1]heptan-2-one 1,7,7-trimethyl-(1S)- (14.85 µg/mg, 13.38 µg/mg, and 12.01 µg/mg) and 3-Penten-2-one 3,4-dimethyl- (4.23 µg/mg, 4.13 µg/mg, and 3.60 µg/mg) respectively for vendor C, vendor A, and vendor B samples in decreasing order (Table 3). The concentration of Myo-Inositol 4-C-methyl- was 3.26 µg/mg, 2.56 µg/mg, and 2.48 µg/mg for vendor B, vendor C, and vendor A samples in decreasing order. The sample from vendor A contained more compounds while those from vendor B contained the least amount of compounds (including identified and unidentified compounds) (Supplementary Table). While two compounds were below detectable limits for vendor A sample, four were undetected in vendor C sample and eleven compounds were below detectable limits in vendor B samples. Glycerine and 2H-Tetrazole -2-methyl- were among the compounds not detected in the samples from vendors B and C.

4. Discussion

Despite advances in technology and science in the battle against diseases, there is an increased emergence of threatening infectious diseases, as result of an increase in the transient attribute of viral,

Table 2
Antibacterial activity (MIC values µg/mL) of *A. afra* extracts purchased from vendors.

Antibacterial activity (MIC)					
Plant extracts	Bacterial strains				
	<i>E. coli</i>	<i>E. faecalis</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	
Vendor A					
Water	4.42 ± 1.16	4.42 ± 0.16	9.88 ± 0.43	4.49 ± 0.38	
Butanol	5.21 ± 1.80	8.85 ± 1.81	6.25 ± 0.01	5.21 ± 0.82	
Chloroform	8.33 ± 0.95	4.69 ± 1.61	6.25 ± 0.00	4.69 ± 0.71	
Vendor B					
Water	5.20 ± 0.02	4.89 ± 0.13	6.07 ± 0.56	6.92 ± 0.22	
Butanol	4.03 ± 1.03	4.09 ± 1.04	4.82 ± 0.39	4.95 ± 0.45	
Chloroform	5.30 ± 0.06	4.29 ± 1.01	8.80 ± 0.32	6.34 ± 0.86	
Vendor C					
Water	8.28 ± 1.07	4.75 ± 0.12	8.96 ± 0.46	6.39 ± 0.75	
Butanol	5.82 ± 0.58	6.32 ± 1.56	7.28 ± 0.23	6.22 ± 0.82	
Chloroform	5.29 ± 1.06	6.28 ± 1.07	8.25 ± 0.45	9.05 ± 0.45	

Gentamicin (positive control) MIC = 0.01 ± 0.00 µg/mL.

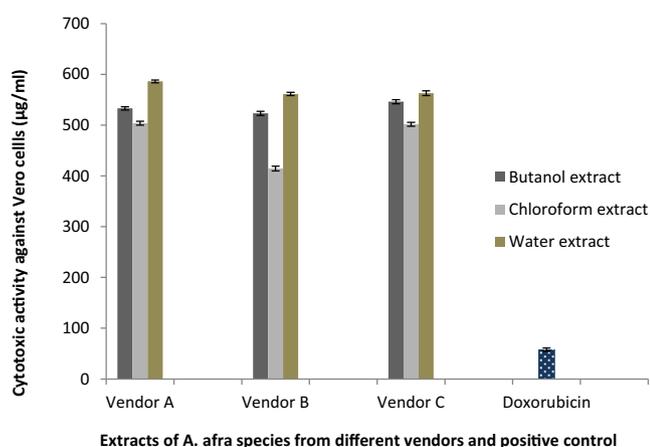


Fig. 1. Cytotoxic effect of *A. afra* extracts purchased from three vendors (A, B, C) and positive control (doxorubicin) against Vero monkey kidney cell lines expressed in comparison to the untreated control cells ± standard error of mean.

fungus, bacterial, and parasitic diseases (Cohen, 1998). The search for potent antimicrobial compounds continues and medicinal plants are seen an alternative for new chemical entity because they contain variety of secondary metabolites. Medicinal plants such as *A. afra* utilized in folk medicine for curing and preventing diverse diseases related to infections was selected for investigation and

there is a dearth of study on the efficacy of *A. afra* traded in the traditional market. The scientific basis of evidence-based medicine for herbal remedies is still very poor, especially for those traded in the traditional medicinal plant market. Investigation of parameters such as the quality of plant materials, standardization of secondary metabolites, safety and efficacy is vital for plants from unidentifiable sources. Thus, this study explored metabolomics analysis to assess the extracts of *A. afra* purchased from three vendors in South Africa.

Untargeted GC × GC-TOFMS metabolomics analysis comprehensively highlighted the variations of chemical constituents of the extracts of *A. afra* from the three vendors. The differences may be due to either the environmental influence on the chemical profile or genetic mutations which increase genetic diversity or geographic influences (Viljoen et al., 2006). The environment influence is minimal on chemical composition of plants except when treated with insecticide (Arundale et al., 2015). While a total of 436 compounds were detected, none of the *A. afra* extracts possessed all the compounds. The extract of sample from vendor A had the highest amount of the chemical composition while extract from sample from vendor B had the lowest amount of chemicals composition. Some authors using NMR spectroscopic analysis to identify the secondary metabolites of *Rhodiola rosea* rhizome extracts from three geographic areas, found differences in the sample composition of the plants (Satheeshkumar et al., 2012). Also, Chatterjee et al. (2010) with NMR, HPLC and GC-MS, identified 62 metabolites from the leaves and 48 from the roots of *Withania*

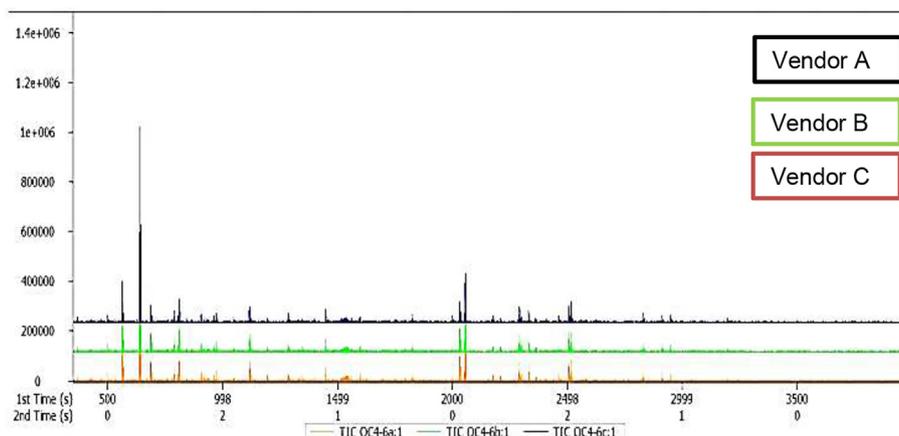


Fig. 2. Total ion chromatograms (TIC) of the butanol extracts of *A. afra* species from three vendors (A, B, C) measured by GC × GC-TOFMS.

Table 3
Antimicrobial compounds identified and concentration ($\mu\text{g}/\text{mg}$) in butanol *Artemisia afra* extracts from three vendors by GC \times GC-TOF-MS.

S/N	Sample name Metabolite name	Mass	Mean RT (s)	A. <i>afra</i> from vendor A ($\mu\text{g}/\text{mg}$ sample)	A. <i>afra</i> from vendor B ($\mu\text{g}/\text{mg}$ sample)	A. <i>afra</i> from vendor C ($\mu\text{g}/\text{mg}$ sample)
1	3,3,6-Trimethyl-1,4-heptadien-6-ol	59	365	0.368	0.320	0.383
2	Glycerin	61	371	0.288	0	0
3	2H-Tetrazole, 2-methyl-	56	398	0.049	0	0
4	o-Cymene	119	419	0.303	0.187	0.189
5	Pyridine*	52	417.5	0	2.041	1.698
6	3-Penten-2-one, 3,4-dimethyl-	69	560	4.126	3.597	4.234
7	Bicyclo[3.1.0]hexan-3-one, 4-methyl-1-(1-methylethyl)-*	81	560	0.888	0	1.062
8	Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-, [1S-(1 α ,3 α ,5 α)]-	92	626	0.076	0.062	0.080
9	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-***	95	638	13.381	12.009	14.853
10	7-Octen-2-ol, 2-methyl-6-methylene-	59	680	0.067	0	0.072
11	2-(1-Cyclopent-1-enyl-1-methylethyl)cyclopentanone	109	683	0.033	0	0.063
12	p-Mentha-1,5-dien-8-ol	59	731	0.046	0	0.042
13	2-Hexanone, 3-methyl-	72	761	0.236	0.241	0.261
14	Borneol, trimethylsilyl ether*	95	785	1.056	0.943	1.148
15	Butane, 1,1-dibutoxy-	57	839	0.410	0.298	0.372
16	Glycerol, tris(trimethylsilyl) ether	73	884	0.360	0.319	0.321
17	Bicyclo[2.2.1]heptane-2-carboxylic acid, 3,3-dimethyl-	67	959	0.320	0.314	0.309
18	Ethanedioic acid, dibutyl ester	57	1043	0.278	0.175	0.257
19	5-Hepten-2-one, 6-methyl-*	108	1118	0.926	0.861	0.941
20	Bicyclo[3.2.0]heptan-2-one, 5-formylmethyl-6-hydroxy-3,3-dimethyl-6-vinyl-	107	1127	0.019	0.018	0.028
21	Binapacryl	83	1136	0.187	0.168	0.183
22	Acetophenone, 4'-hydroxy-	121	1196	0.750	0.759	0.727
23	D-Allose*	60	1280	1.844	2.076	1.842
24	trans-calamenene	159	1349	0.282	0.222	0.241
25	(-)-Spathulenol	91	1445	0.298	0.325	0.304
26	Benzoic acid, 4-[(trimethylsilyloxy)-, trimethylsilyl ester	73	1526	0.134	0.100	0.128
27	Myo-Inositol, 4-C-methyl-**	87	1531	2.487	3.261	2.563
28	Isoaromadendrene epoxide	71	1820	0.164	0.185	0.186
29	cis-Z- α -Bisabolene epoxide	55	1829	0.059	0.029	0
30	Scopoletin*	192	2031	1.690	1.865	1.743
31	n-Hexadecanoic acid**	60	2053	2.456	2.467	2.418
32	5-Benzofuranacetic acid, 6-ethenyl-2,4,5,6,7a-hexahydro-3,6-dimethyl- α -methylene-2-oxo-, methyl ester*	120	2516	1.151	1.344	1.330
33	Ingol 12-acetate	111	2831	0.539	0.530	0.558
34	1-Cyclohexyl-5-(4-methoxy-benzyl)-pyrimidine-2,4,6(1H,3H,5H)-trione	121	3098	0.250	0.260	0.255

Internal standard added: 50 μl (50 $\mu\text{g}/\text{mL}$); Samples: 2.5 μg added per sample. Relative concentrations of the metabolites were calculated as: detected area of metabolite/detected area of internal standards \times 2.5 μg . Compound name were identified using in-house mass spectral libraries (NIST). Key: RT: Retention Time, s: seconds, ***Compounds with highest concentration (12–15 $\mu\text{g}/\text{mg}$ sample), **Compounds with second highest concentration (2.4–5.0 $\mu\text{g}/\text{mg}$ sample), *Compound with third highest concentration (0.85–2.0 $\mu\text{g}/\text{mg}$ sample)

somnifera (Ashwagandha) and found only twenty-nine compounds were common to the two tissues. Therefore metabolomics fingerprinting may be valuable for the evaluation of quality of medicinal plants (Satheeshkumar et al., 2012).

The safety of medicinal plants is very critical because plant extracts have been shown to have low lethal concentration ($\text{LC}_{50} \leq 20 \mu\text{g}/\text{mL}$) which is very cytotoxic (Zirih et al., 2005). All the extracts of *A. afra* were relatively non-cytotoxic against Vero cell ($\text{LC}_{50} \geq 414.56 \pm 4.81 \mu\text{g}/\text{mL}$) than doxorubicin, the positive control. This result was different from those of authors (Mativandlela et al., 2008), who showed that the crude ethanol extract of *A. afra* (LC_{50} value of $113.0 \pm 2.05 \mu\text{g}/\text{mL}$) was cytotoxic against Vero cells. The solvent of extraction may be responsible for this because it had been demonstrated that a sesquiterpene lactone isolated from *A. afra* ethanol extract was cytotoxic (Jenett-Siems et al., 2002).

Based on the acceptable MIC values $\leq 1.0 \text{ mg}/\text{mL}$ (Ndhlala et al., 2009); all the extracts of *A. afra* were potent inhibitors against all the bacterial strains. All the butanol extracts of the plants strongly inhibited *P. aeruginosa* while the antibacterial activity of water extract of vendor A was not significantly different from those of butanol extract of vendor B for the other bacterial strains examined. This indicates that the activity of the *A. afra* extracts probably resides in the polar solvent. Although metabolomics analysis detected more antimicrobial compounds in the sample from vendor A (33) than those from vendor B (28) and C (31), the lack of sig-

nificant difference between the antimicrobial activity of water extract of vendor A and butanol extract of vendor B cannot be clearly understood with the metabolomics profiling. Neither can the superior antimicrobial activity of butanol extract of vendor B sample against *P. aeruginosa* be explained. However, about eight antimicrobial compounds in relatively high concentrations were identified in all the vendors' samples (Table 3) including Bicyclo [2.2.1]heptan-2-one 1,7,7-trimethyl-(1S)- (Al-Marzoqi et al., 2015) with the highest concentration in the three samples, followed by Myo-Inositol, 4-C-methyl (Gaitonde and Ramesh, 2018), n-Hexadecanoic acid (Mehdi, et al., 2021) and Scopoletin (More et al., 2012). Probably these antimicrobial compounds and perhaps some other compounds are exhibiting numerous modes of actions which have synergistic antimicrobial potentials irrespective of compound composition, and quantity within the plant extract.

5. Conclusion

The study has shown that metabolomics analyses are vital for the quality evaluation of medicinal plants for the therapeutic benefit of the consumers. The chemical composition of medicinal plants, efficacy and safety may be dependent on the plants' growth, the climate and soil, the collection time, and method of drying. The profiling of metabolites is also dependent of the sensitivity of the

analytical methods used for empirical and numerical assessment of the numerous plants phytochemicals. All the *A. afra* species purchased from the three vendors in the traditional medicine market has been shown to possess potential antibacterial activity. Although no relationship was found between the chemical constituents of *A. afra* extracts purchased from the vendors and the antibacterial activity, none of the extracts were cytotoxic.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jksus.2021.101422>.

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