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DNA Fingerprinting, Phytochemical screening, Proximate analysis, chemical investigation of lipoidal matter and Antimicrobial activities of *Sansevieria trifasciata* Prain. and *Sansevieria suffruticosa* N.E.Br.

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Sansevieria trifasciata Prain. and *Sansevieria suffruticosa* N.E.Br. are ornamental plants in Asparagaceae family. This study aimed to authenticate the two *Sansevieria* species growing in Egypt by using random amplified polymorphic DNA (RAPD) technique, which helps in the identification of these *Sansevieria* species showing great morphological similarity. In addition, phytochemical screening and proximate analysis were conducted for the two *Sansevieria* species showing presence of carbohydrates and/or glycosides, tannins, flavonoids, sterols and/or triterpenes, saponins, proteins and amino acids. Proximate analysis of fresh aerial non-flowering parts of each of the two *Sansevieria* species yielded a moisture content of 80.77 and 89.06%, total ash of 1.06 and 2.21%, acid-insoluble ash of 2.97 and 0.14%, water-insoluble ash of 2.93 and 1.18% and crude fiber content of 12.5 and 22.05% for *S. trifasciata* Prain. and *S. suffruticosa* N.E.Br. respectively. Lipoidal matter content was analyzed by GCMS. Regarding unsaponifiable matter, phytol was the major compound in the two species representing 48.15% and 30.61% in *S. trifasciata* Prain. and *S. suffruticosa* N.E.Br., respectively. While, linoleic acid methyl ester was major compound in saponifiable matter analysis of *S. trifasciata* Prain. (33.11%) and *S. suffruticosa* N.E.Br. (39.83%). Furthermore, antimicrobial activity of ethanolic extracts of the aerial non-flowering parts of both *Sansevieria* was evaluated against many micro-organisms showing strong antifungal activity against *Aspergillus flavus* showing diameters of inhibition zones 26.93 and 24.93 mm for *S. trifasciata* Prain. and *S. suffruticosa* N.E.Br., respectively, compared to ketoconazole.

Keywords: *Sansevieria trifasciata*, *Sansevieria suffruticosa*, Asparagaceae, RAPD, antimicrobial, lipoidal matter

INTRODUCTION

Asparagaceae consists of about 153 genera and 2,500 species of flowering plants; it is widely distributed all over the world. The family members are combined primarily by evolutionary relationships and genetic rather than morphological resemblances (Qasim et al., 2020). Genus *Sansevieria* is native to tropical and

subtropical areas. *Sansevieria* is an aggressive invasive plant that grow in a wide variety of habitats. It is of high economic value for having high fiber content that can be used as raw material for textiles (Spellenberg et al., 2014; Tallei et al., 2016). *Sansevieria* species are primarily known worldwide for their cultivation in gardens or in pots as ornamentals due to their succulent leaves, the variation of leaf shapes, sizes and color that ranges from plain green to variegated and mottled leaves and their ability to tolerate drought and neglect (Takawira-Nyenya and Stedje, 2011). Different therapeutic effects were attributed to certain *Sansevieria* species. *Sansevieria trifasciata* Prain. is an ornamental plant that is easy to grow without much care.

It has been used traditionally for the treatment of colds, ear pain, swellings, boils and fever, diarrhea, cough, bronchitis, ulcers and bites of snakes and insects (Andhare et al., 2012; Febriani et al., 2019; Sunilson et al., 2009). *Sansevieria* species are reported to have a variety of medicinal purposes such as antidiabetic (Haldar et al., 2010), antimicrobial (Philip et al., 2011), antifungal (David and Afolayan, 2013), hepatoprotective (Ikewuchi et al., 2011), analgesic and anticancer activities (Roy et al., 2012). Certain *Sansevieria* species were reported to contain alkaloids, terpenoids, flavonoids, saponins, steroids, phenols and tannins (Kumar and Kumari, 2015). Several steroidal sapogenins and saponins were isolated from certain *Sansevieria* species (Gamboa-Angulo et al., 1996; Mimaki et al., 1997). *Sansevieria suffruticosa* N.E.Br. has no review of literature till the time of this work. Both *S. trifasciata* Prain. and *S. suffruticosa* N.E.Br. are cultivated in Egypt for ornamental purposes.

The main aim of the present study was to carry out DNA fingerprinting using RAPD technique as a method of authentication, proximate analysis, phytochemical screening, lipid content analysis using gas chromatography coupled with mass spectroscopy (GC/MS) to identify compounds of saponifiable and unsaponifiable content and antimicrobial evaluation of the two plants

MATERIALS AND METHODS

2.1. Plant material

The aerial non-flowering part of the two *Sansevieria* species (Figure 1), *Sansevieria trifasciata* Prain. and *Sansevieria suffruticosa* N.E.Br. were collected from El Orman Botanical Garden, Giza, Egypt on September 2016. They were kindly identified by Dr. Reem Samir Hamdy, Associate Professor in Taxonomy Department, Faculty of Science, Cairo University and vouched specimens were deposited within the herbarium of Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt with numbers: 8-9-2016 I and 8-9-2016 II, respectively.

Figure 1. Photos of the two *Sansevieria* species, A: *S. trifasciata* Prain.; B: *S. suffruticosa* N.E.Br.

Fresh samples of the two species were kept, separately, at -70^{o}C, freeze - dried, and ground to a fine powder under liquid nitrogen prior to DNA isolation. All the selected leaf samples were normal and free from any pathogenic symptoms. Bulked DNA extraction was performed using DNeasy plant Mini Kit (QIAGEN). DNA analysis was done at the Food Technology Research Institute, Agriculture Research Center, Ministry of

Agriculture and Land Reclamation, Giza, Egypt in 2018.

2.1.2. Plant material for lipoidal matter

The lipoidal matter was obtained by extraction of 500 g fresh aerial non-flowering parts of the two investigated species of *Sansevieria* with 95% ethanol on cold by maceration till exhaustion. The ethanolic extracts were completely dried under reduced pressure at 45°C using rotary evaporator (Buchi®R- 300, USA) and stored at 20°C until use. The yield was 35 g and 40 g extracts of *S. trifasciata* Prain. and *S. suffruticosa* N.E.Br., respectively. Part of the ethanolic extract (20 g) of both species was suspended in distilled water, separately, and successively extracted with *n*-hexane till exhaustion. The n-hexane fractions were evaporated under reduced pressure till dryness to yield 1.85 g and 2.25 g of *S. trifasciata* Prain. and *S. suffruticosa* N.E.Br., respectively. The residue was kept in tightly closed containers for analysis.

2.1.3. Plant material for preliminary phytochemical screening

The aerial non-flowering parts of plants under investigation were used fresh, reduced to small pieces then screened qualitatively for the presence of various groups of phytoconstituents using different chemical tests (Harborne, 1973).

2.1.4. Plant material for proximate analysis

Fresh aerial non-flowering parts of plants under investigation were reduced to small pieces then percentages of moisture content, total ash, water-insoluble ash, acid-insoluble ash, and crude fiber values were assessed according to the official methods (General Organization for Government Printing Affairs, 2005).

2.1.5. Plant material for antimicrobial evaluation

The ethanolic extract previously prepared for lipoidal matter was used for antimicrobial evaluation.

2.2. Material for DNA

2.2.1. Kits

DNA isolation kit: QUIAGEN DNeasy kit (Germany); including Cell lysis buffer (AP1), RNAse Protein-depleting buffer (AP2), AP3/E Buffer, AW Buffer, AE Buffer, TBE Buffer (Quiagen Santa Clara, CA).

2.2.2. Reagents

Ethidium bromide: The stock solution was prepared by dissolving 1g of ethidium bromide in 100 ml distilled water and mixed well with magnetic stirrer. Then transferred to a dark bottle and stored at room temperature, 5x Trix-borate (TBE): 5.4 g Tris-base, 2.75 g boric acid, 0.29 g EDTA 500mM (PH 8) and distilled water up to 100 ml, Sample loading dye (5x): 2 ml Na-EDTA, 500Mm (PH 8), 5 ml Glycerol (100%), 0.75 ml Bromophenol blue (2%) and 1.5 ml distilled water, PCR reagent: Amplification reaction was performed in 25 µl volume tubes according to method reported by (Williams et al., 1990) that contained: 2.50 µl dNTPs (2.5 mM), 2.50 µl $MgCl_2$ (25 mM), 2.50 µl Buffer (10x), 3.00 µl primer (10 pmol), 1.00 µl *Taq* DNA polymerase (10 µl), 3.00 µl Template DNA (25 ng) and 10.50 µl steile double distilled water.

2.2.3. Columns

QIAshredder mini spin column for removal of cell debris and DNeasy mini spin column for binding DNA to get it pure.

2.2.4. Primers

A total of 5 random decamer oligonucleotide primers (synthesized by Operon biotechnologies, Inc. Germany) were used to amplify the genomic DNA with the following sequences; OP-A18 [5`AGGTGACCGT3`], OP-C05 [5' GATGACCGCC 3'], OP- C15 [5' GACGGATCAG 3'], OP-D16 [5'AGGGCGTAAG3'] and OP-Z19 [5' GTGCGAGCAA 3'].

2.2.5. Molecular weight marker

DNA ladder (100 bp) mix was used as standard DNA with molecular weights of 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp. (Promega Corporation).

2.2.6. Apparatus

A DNA thermocycler (model Techno 512) was used for the amplification of DNA, an agarose gel electrophoresis tool (Bioard wide Mini Sub Cell) was used for the separation of RAPD fragments according to size and a UV Polarid camera was used for visualization of the RAPD fragments.

2.3. Methods and technique

2.3.1. Molecular investigation

2.3.1.1. DNA extraction

DNA extraction was performed using DNeasy plant Mini Kit (QIAGEN) following the original procedure of the kit supplemented by the manufacture DNeasy® Plant Mini Kit and DNeasy Plant Maxi Kit Handbook for DNA isolation 2006. Plant tissues were ground using liquid nitrogen to a fine powder, then, the powder was then transferred to an appropriately sized tube.

Four hundred µl of buffer AP1 and 4 µl of RNase a stock solution (100 mg/ml) were added to a maximum of 100 mg of ground plant tissues and vortexed vigorously. Then, the mixture was incubated for 10 min at 65^{o}C and mixed 2-3 times during incubation by inverting tube.

One hundred and thirty µl of buffer AP2 were then added to the lysate, mixed and incubated for 5 min on ice. The lysate was added to QIA shredder spin column sitting in a 2 ml collection tube and centrifuged for 2 min at maximum speed (10.000 rpm). The supernatant was transferred to a new tube without disturbing the cell-debris pellet. Typically, 450 µl of lysate was recovered.

One half volume of buffer AP3 and 1 volume of ethanol (96-100%) were then added to the cleared lysate and mixed by pipetting. Six hundred and fifty µl of this mixture were applied through DNeasy Mini spin column setting in a 2 ml collection tube, then, centrifuged for 1 min at 8000 rpm and flow-through was then discarded. DNeasy column was then placed in a new 2 ml collection tube, then, 500 µl buffer AW were added onto the DNeasy column and centrifuged for 1 min at 8000 rpm to dry the column membrane. DNeasy column was then transferred to a 1.5 ml microfuge tube and 100 µl of preheated (65^{o}C) buffer AE were pipetted directly onto the DNeasy column membrane, then, incubated for 5 min at room temperature and centrifuged for 1 min at 8000 rpm to elute. Elution was repeated once as described. A new microfuge can be used for first elute. The microfuge tube is reused for the second elution step to combine the elutes.

2.3.1.2. Polymerase chain reaction (PCR) program:

Amplification was conducted in 25µl reaction volume containing the following reagents: 2.5µl of dNTPs (2.5mM), 2.5µl MgCl2 (2.5mM), 2.5µl of 10 x buffer, 3.0 µl of primer (10 pmol), 3.0µl of template DNA (25 ng/µl), 1µl of Taq polymerase (10/µl) and 10.5µl of sterile double distilled H_2O. The DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 94^{o}C for 4 min followed by 45 cycles of 1 min at 94^{o}C, 1 min at 36^{o}C and 2 min at 72^{o}C. The reaction was finally stored at 72^{o}C for 10 min.

2.3.1.3. Gel Electrophoresis and Staining

Amplified products were size-fractioned using ladder marker (100 bp) Fermentas.co by electrophoresis in 1.5 % agarose gels in TBE buffer at 120 V for 1 h. The bands were visualized by ethidium bromide under UV florescence and photographed.

2.3.1.4. Data analysis

RAPD bands were marked as absent (0) or present (1) and treat each of which was as an independent character regardless of its intensity. Only consider prominent and reproducible bands obtained for each RAPD primer. Species bands were identified by comparing the banding patterns of species for a primer. Faint or unclear bands were not treated or considered.

2.3.2. Lipoidal matter analysis

2.3.2.1. Preparation of the unsaponifiable and saponifiable matter

The unsaponifiable matter and saponifiable matter were prepared according to (Ackman et al., 1973) method with some modifications.

2.3.2.2. Preparation of the unsaponifiable matte*r*

About one gram of the *n*-hexane extract of the leaves of the two *Sansevieria* species was, separately, saponified by refluxing with alcoholic KOH (10%) for 3 hours. The mixture was cooled then transferred to a separating funnel and extracted with ether (4x20ml). The combined ethereal extracts were washed with water till free from alkalinity, dried over anhydrous Na_2SO_4 and evaporated under vacuum at 40˚C yielding yellow residue (0.23 g) and (0.37 g) of *S. trifasciata* Prain. and *S. suffruticosa* N.E.Br., respectively. The unsaponifiable fraction was ready for analysis using GC/MS technique.

2.3.2.3. Preparation of the saponifiable matter

The aqueous fraction prepared in the previous step was acidified with 2N HCl, then

extracted with ether (4x20ml). The combined ethereal extracts; containing free fatty acids, were washed with distilled water till free from acids, then dehydrated over anhydrous Na_2SO_4. The resulting free fatty acids were methylated by refluxing with acidified methanol (with drops of sulphuric acid) for 2 hours. The methylated fatty acids were extracted with ether (4x20ml), then ether was evaporated under vacuum at 40˚C. The residue (0.77 g) and (0.65 g) *S. trifasciata* Prain. and *S. suffruticosa* N.E.Br., respectively, was the saponifiable fraction ready to be analyzed using GC/MS technique.

2.3.2.4. Gas Chromatography-Mass Spectrometry

The saponifiable and unsaponifiable fractions of each species were separately subjected to GC/MS analysis on an Agilent system consisting of a model 6890 gas chromatograph, equipped with a model 5973 mass selective detector (EIMS, electron energy, 70 eV), and an Agilent ChemStation data system. The GC column was an HP- 5ms fused silica capillary with a DB-5 (5% phenyl methyl polysiloxane) stationary phase, film thickness of 0.25 µm, a length of 30 m, and an internal diameter of 0.25 mm. Helium was the carrier gas with a flow rate of 1.0 ml/min. Inlet temperature was 200°C and MSD detector temperature was 270°C. The mass spectrometer was operated in electron impact ionization (EI) mode with 70eV energy. The mass range was 50-700 Da and the ion source temperature was 200 °C. The GC oven temperature program was used as follows: 80°C initial temperature, for 2 min.; then programmed at 15°C/min to 270°C and held for 10 min. Each sample was dissolved in acetone to give a 1% w/v solution; 1 µL injections using a splitless injection technique were used.

Identification of components was achieved based on their retention indices (RI, determined with reference to a homologous series of normal alkanes), and by comparison of their mass spectral fragmentation patterns with those reported in the literature and stored on the MS libraries [NIST 05; Mass Finder database (G1036A, revision D.01.00 and Wiley7 Mass Finder].

2.3.3. Antimicrobial Screening

2.3.3.1. Standards

Gentamycin (10µg, Oxoid, UK, Antibacterial agent), Ketoconazol (5µg, Sigma Chemical Co., St. Louis, Mo., Antifungal agent)

2.3.3.2. Bacterial test strain and growth conditions

For this study, filamentous fungi as *Aspergillus flavus* (RCMB 002002), Gram-positive bacteria as *Staphylococcus aureus* (RCMB010010) and *Bacillus subtilis* (RCMB 015 (1)NRRL B-543); Gram-negative bacteria *Escherichia coli* ((RCMB 010052) ATCC 25955) and *Salmonella typhimurium* (RCMB 006 (1)ATCC 14028) and yeast as *Candida albicans* (RCMB 005003 (1) ATCC 10231).

One hundred µl of the test bacteria/fungi were grown in 10 ml of fresh media (Mueller-Hinton agar plates (HiMedia) for bacteria and Potato Dextrose Agar plates for fungi (HiMedia)) until they reached approximately 108 cells/ml for bacteria and 105 cells/ml for fungi. 100 µl of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained. Isolated colonies of each organism that might be playing a pathogenic role should be selected from primary agar plates and tested for susceptibility by disc diffusion method.

Plates inoculated with filamentous fungi as *Aspergillus flavus* at 25°C for 48-72 hours; Gram-positive bacteria as *Staphylococcus aureus* and *Bacillus subtilis*; Gram-negative bacteria *Escherichia coli* and *Salmonella typhimurium*, they were incubated at 35-37° C for 24-48 hours and yeast as *Candida albicans* incubated at 30°C for 24-48 hours. Then the diameters of the inhibition zones were measured in millimeters.

2.3.3.3. Determination of antibacterial activity of plant extracts by the disk diffusion method

Antimicrobial activity of the ethanolic extracts of the two *Sansevieria* species was screened using (Bauer et al., 1966) disc diffusion method with slight modifications. DMSO with a concentration up to 2% was used to dissolve the plant extracts. Filter paper disks (6 mm in diameter) saturated with 20µL of the tested extracts or DMSO (solvent control) were placed on the surface of the inoculated plates. To evaluate the efficiency of the methodology; 50µL of each extract was inserted simultaneously in a hole made in new plates. The plates were incubated at 37°C for 24 h. The diameter of the inhibition zone was measured in millimeter with slipping calipers of the National Committee for Clinical Laboratory Standards, and was recorded as mean ± SD of a triplicate experiment. Standard disks of Gentamycin (10µg), Ketoconazol (5µg) served as positive controls for antimicrobial

activity but filter disks impregnated with 10 µl of solvent (DMSO) were used as a negative control.

Determination of the Minimal Inhibitory Concentration (MIC) was carried out by a serial broth dilution method described by (NCCLS, 1993; Sahm, D.H. & Washington, 1991). The extracts were diluted in DMSO and were added to 5 ml sterile MHB tubes to give different concentrations (1.0 - 50.0 µL/ml). Then, 0.5 ml of the exponentially growing microbial broth culture of the strains that were sensitive by disc diffusion test was inoculated into respective test tubes. Another set of tubes containing only the growth medium without DMSO (control) and with DMSO (solvent control) and each of the test strains was set up separately. In 96-well plates organisms, at a concentration of approximately 1.5 x 108 colony forming units (CFU)/ml, were added to each well. The tubes were incubated at 37°C for 24 h and the growth was measured by measuring optical density at 520 nm using spectrophotometer comparing the sample readout with the non-inoculated nutrient broth. The MIC was regarded as the lowest concentration (without turbidity) of the extract that inhibited the growth of bacteria or fungi. DMSO to 2% was used to dissolve the extracts in the culture media when necessary. The control was the solvent used for extracts and it showed no inhibitions in preliminary studies. DMSO to 2% was used to dissolve the extracts when necessary and as a negative control whereas Gentamicin was used as a positive control for bacterial strains, Ketoconazol was used as a positive control for fungi. The plates were done in triplicate.

Statistical analysis

Statistical analysis was performed using SPSS (statistical package of social sciences, version 22), SPSS Inc., Chicago, IL, USA. Statistical significance was acceptable to a level of $p < 0.05$.

RESULTS AND DISCUSSION

DNA fingerprinting

The banding profile produced by five decamer primers (OP-A18, OP-C05, OP-C15, OP-D16, OP-Z19) used in the RAPD analysis of the two *Sansevieria* species is illustrated in figure 2.

The amplified fragments, the percentage of polymorphic fragments and similarity coefficient for the two *Sansevieria* species based on RAPD-PCR analysis using five primers is shown in table 1

The two plants were subjected to RAPD assay; this was performed using five different primers. In this study, the presence of same bands in DNA of different *Sansevieria* species indicates degree of taxonomical relationship; also the presence of characteristic bands in DNA of each plant may help for differentiation between these plants.
The OP-Z19 primer was found to be the most effective in generating polymorphic bands on application of RAPD technique as compared to the total number of RAPD fragments it generates high level of polymorphism (85%). From the previous findings, it can be concluded that OP-Z19 primer can be used to discriminate between the two *Sansevieria* species depending on their low values of similarity coefficient and high level of polymorphism. However, the other estimated RAPD-primers, which produce high values of similarity coefficient and low levels of polymorphism, could be used in the identification of these plants.

Preliminary phytochemical screening

The preliminary phytochemical screening revealed the presence of carbohydrates and/or glycosides, tannins, flavonoids (free and combined), sterols and/or triterpenes, saponins, proteins and amino acids in the aerial non-flowering parts of the two *Sansevieria* species under investigation. Anthraquinones (free and combined), volatile substances, alkaloids and cardiac glycosides are absent in the aerial non-flowering parts of the two *Sansevieria* species.

Proximate analysis

Percentages of moisture content, total ash, acid-insoluble ash, water-insoluble ash and crude fiber of each of the two plants were estimated and the results are presented in table 2

Proximate analysis plays an important role in quality control and standardization of the plant. *S. suffruticosa* N.E.Br. showed higher moisture content (89.06%) than *S. trifasciata* Prain. (80.77%). Total ash is a measure of the total amount of inorganic materials and/or minerals remaining after the water and organic matter have been removed by heating. It is obvious that *S. suffruticosa* N.E.Br. showed higher total ash percentage (2.21 %) followed by *S. trifasciata* Prain. (1.06 %).

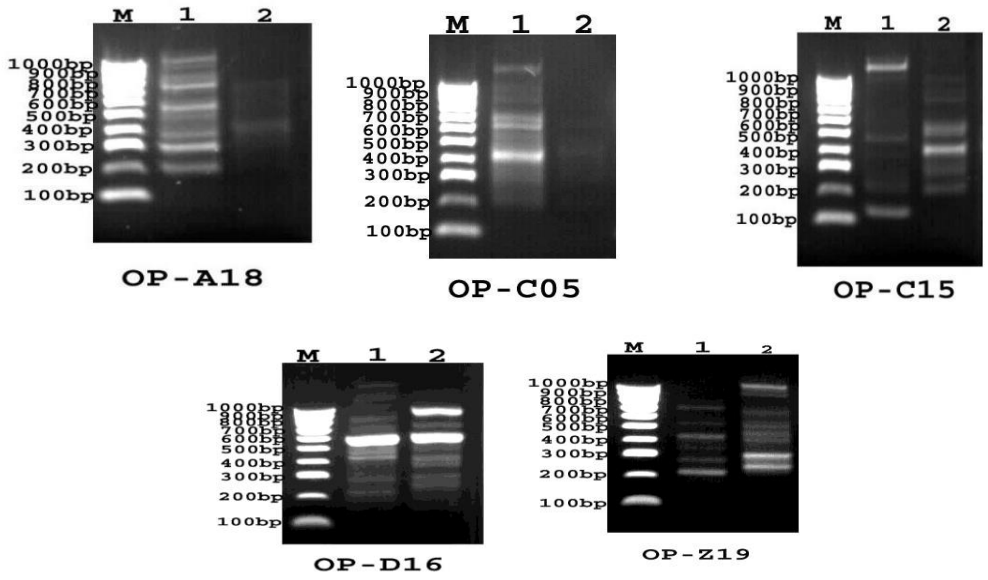

Figure 2. RAPD-PCR analysis of the two *Sansevieria* species [1. *S. trifasciata* Prain.; 2. *S. suffruticosa* N.E.Br.]

Table 1. RAPD-PCR analysis of the two *Sansevieria* species

Primer code	TAF	PB	Polymorphic fragments %	Similarity coefficient %
OP-A18	10	6	60	40
OP-C05	9	1	11	89
OP-C15	11	4	36	64
OP-D16	14	8	57	43
OP-Z19	13	11	85	15

TAF: Total Amplified Fragments, **PB:** Polymorphic Bands for each primer

Table 2. Pharmacoepial constants of the aerial non-flowering parts of the two *Sansevieria* species

Item (%)	*S. trifasciata* Prain.	*S. suffruticosa* N.E.Br.
Moisture	80.77	89.06
Total ash	1.06	2.21
Acid-insoluble ash	2.97	0.14
Water-insoluble ash	2.93	1.18
Crude fibre	12.5	22.05

While, *S. trifasciata* Prain. showed higher percentage of acid-insoluble ash and water-insoluble ash (2.97 % and 2.93 %, respectively), than *S. suffruticosa* N.E.Br. (0.14 % and 1.18 %, respectively).

Identification of lipoidal content

From the GC/MS analysis of the unsaponifiable matter of the aerial non-flowering parts of *S. trifasciata* Prain. (table 3), 20 compounds were identified representing 97.87% of the unsaponifiable matter. Major components were phytol (48.15%), γ - sitosterol (13.39%), stigmasterol (11.01%) and β-sitosterol (10.55%).

Regarding the GC/MS analysis of the unsaponifiable matter of the aerial non-flowering parts of *S. suffruticosa* N.E.Br. (table 3), 54 components were identified representing 98.55% of the unsaponifiable matter. Major components were phytol (30.61%), 2,4-dimethyl-2,3-pentanediol (22.25%), stigmasterol (15.71%) and β-sitosterol (12.21%).

Collective % of different groups in

unsaponifiable matter of the aerial non-flowering parts of the two *Sansevieria* species is illustrated in figure 3.

RT, Retention Time; RTT, Relative Retention Time to 37.698 (retention time phytol which is the major compound in the two species).

The results of the GC/MS analysis of the saponifiable matter of the aerial non-flowering parts of *S. trifasciata* Prain. in table 4 revealed that the percentage of the identified components (96.27%), the percentage of the identified saturated fatty acids (34.15%) , where hexadecanoic acid methyl ester (palmitic acid methyl ester) (23.38%) was the major compound. While, the percentage of the identified unsaturated fatty acids (62.12%), where 9, 12-octadecadienoic acid methyl ester (linoleic acid methyl ester) (33.11%) was the major compound.

The results of the GC/MS analysis of the saponifiable matter of the aerial non-flowering parts of *S. suffruticosa* N.E.Br. in table 4 revealed that the percentage of the identified components (96.73%), the percentage of the identified saturated fatty acids (34.15%), where hexadecanoic acid methyl ester (palmitic acid methyl ester) (27.08%) was the major compound. While, the percentage of the identified unsaturated fatty acids (62.58%), where where 9, 12-octadecadienoic acid methyl ester (linoleic acid methyl ester) (39.83%) was the major compound.

Concerning the results of the unsaponifiable fraction, phytol was found to be the major component in both *S. trifasciata* Prain. and *S. suffruticosa* N.E.Br. (48.15%) and (30.61%), respectively. Phytol has many biological activities such as antimicrobial, anti-inflammatory, diuretic, cytotoxic, anti-anxiety, antioxidant, and anti-inflammatory effects (Gajalakshmi et al., 2012; Islam et al., 2018), so**,** extraction of phytol from these plants should be done. While, 9,12-Octadecadienoic acid methyl ester (linoleic acid methyl ester) contributed the highest percentage in saponifiable matter analysis of *S. trifasciata* Prain. and *S. suffruticosa* N.E.Br. with a value of 33.11 and 39.83%, respectively. This compound reported to have anti-inflammatory, neuroprotective and antidepressant activities (Blondeau et al., 2015). So, trials for its isolation should be done for its medical importance.

The percentage of unsaturated fatty acids is greater than that of saturated fatty acids in the two *Sansevieria* species. Saturated fatty acids, when compared to unsaturated fatty acids, raise plasma LDL; a causal risk factor for coronary heart diseases. Unsaturated fats help lower a person's levels of LDL cholesterol, reduce inflammation and build stronger cell membranes in the body (Nettleton et al., 2017)**.** Therefore, trials should be done to isolate unsaturated fatty acids from both plant species.

Antimicrobial activity

In the present study, antimicrobial activity of the ethanolic extracts of the two *Sansevieria* species was done. Results of the mean diameter of inhibition zone in mm (6mm) of antifungal activity are shown in figure 4 and of antibacterial activity are shown in figure 5. MIC of the ethanolic extracts of the two *Sansevieria* species are shown in figure 6.

This study revealed that ethanolic extracts of both *Sansevieria* species exhibited strong antifungal activity against *Aspergillus flavus* diameters of inhibition zones were 26.93 and 24.93 mm for *S. trifasciata* Prain. and *S. suffruticosa* N.E.Br., respectively compared to ketoconazole (16 mm) and moderate effect against *Candida albicans* with higher activity of *S. trifasciata* Prain. (22.50 mm) than *S. suffruticosa* N.E.Br. (16.83 mm) compared to ketoconazole (23.03 mm).

Regarding Gram-positive bacteria, the extracts from *S. suffruticosa* N.E.Br. give higher activity than *S. trifasciata* Prain. against *Staphylococcus aureus* with diameters of inhibition zones were 22.73 and 15.43 mm, respectively. *Bacillus subtilis* isolate was resistant to both extracts.

Finally, concerning Gram-negative bacteria, both extracts showed moderate activity against *Escherichia coli* with 16 and 19.83 mm for *S. trifasciata* Prain. and *S. suffruticosa* N.E.Br., respectively, compared to gentamycin (27.09 mm). *Salmonella typhimurium* was resistant to both extracts.

In agreement with the results obtained from the present study, previous research papers documented the noticeable antimicrobial potency of the ethanolic extracts from *S. trifasciata* Prain. against *Escherichia coli* (Tkachenko et al., 2017).

According to MIC values, the activity was more pronounced on *Aspergillus flavus* with an MIC of 4.88 µg/ml and 19.53 µg/ml for ethanolic extract of *S. trifasciata* Prain. and *S. suffruticosa* N.E.Br., respectively. While, the ethanolic extracts showed activities in the concentrations (19.53 µg/ml and 78.13 µg/ml) for *S. trifasciata* Prain. and *S. suffruticosa* N.E.Br., respectively, on *Candida albicans.*.

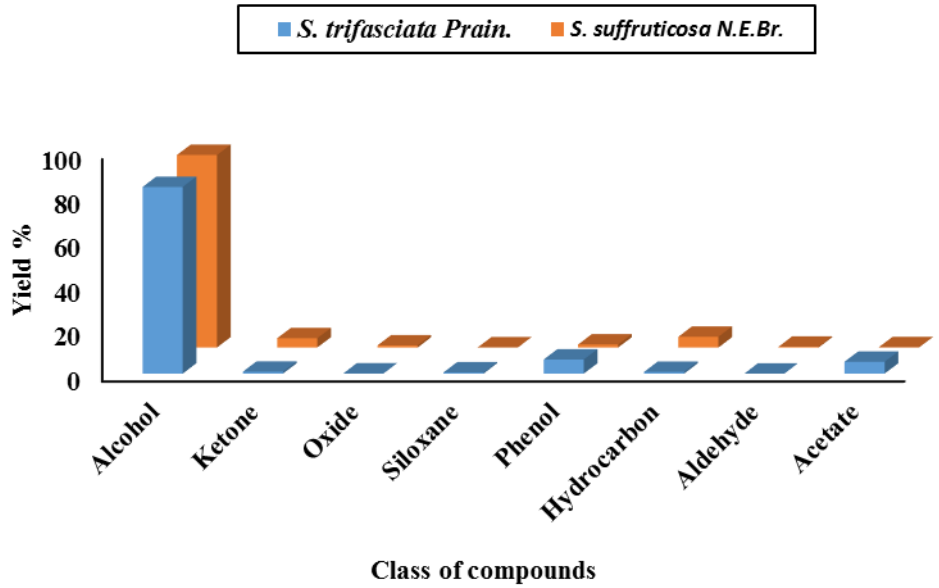

Figure 3: Collective percentages of different groups in unsaponifiable matter of the aerial non-flowering parts of the two *Sansevieria* species under study

Table 3. Results of GC/MS analysis of the unsaponifiable fraction of the aerial non-flowering parts of the two *Sansevieria* species

No	RT	RRT	Area %		Compound name	Base m/z
			S. trifasciata Prain.	*S. suffruticosa* N.E.Br.		
1	3.368	0.089	0.03	0.04	Tetrahydro-2-furanmethanol	70.95
2	4.601	0.122	0.03	0.04	2-methyl-2-hexanol	59
3	6.167	0.164	0.55	0.07	2-heptanone	43
4	7.92	0.21	0.05	0.07	1-ethylbutyl-hydroperoxide	43.05
5	14.475	0.384	0	22.25	2,4-dimethyl-2,3-pentanediol	56
6	14.908	0.395	0.05	0.07	2,2,4-trimethyl-1,3-pentanediol	56
7	23.046	0.611	0.3	0.06	6,10-dimethyl-5,9-undecadien-2-one	43
8	23.572	0.625	0	0.13	7,9-di-tert-butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione	57
9	24.146	0.641	0.54	0.06	3-butoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	73
10	24.648	0.654	5.74	1.14	Butylated hydroxytoluene	205.05
11	25.229	0.669	0	0.24	5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone	111
12	26.475	0.702	0.06	0.06	1-pentadecene	55
13	26.556	0.704	0	0.87	Caryophyllene oxide	41
14	27.838	0.738	0.63	0.14	trans-longipinocarveol	41
15	28.279	0.75	0	0.83	Eudesma-4(14),11-diene	81
16	28.639	0.759	0	0.16	Andrographolide	91
17	28.934	0.768	0	0.11	2-octylcyclopropene-1-heptanol	41.05
18	29.417	0.78	0	0.11	Heptadecanal	43.05
19	29.53	0.783	0.07	0.09	6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	57
20	29.752	0.789	0	0.16	[S-(E,Z,E,E)]-3,7,11-trimethyl-14-(1-methylethyl)-1,3,6,10-cyclotetradecatetraene	41.05
21	29.838	0.792	0	0.1	5α-3-ethyl-3-hydroxy-androstan-17-one	41
22	31.106	0.825	0.04	0.07	1-octadecene	43.05
23	31.795	0.843	0	0.34	Unidentified	41
24	32.13	0.852	0.71	0.05	Unidentified	68
25	32.29	0.857	0	1.77	6,10,14-trimethyl-2-pentadecanone	43
26	32.477	0.862	0	0.17	(Z)-14-methyl-8-hexadecenal	70
27	32.535	0.863	0.83	0.07	Unidentified	43.95
28	33.239	0.882	0	0.3	Unidentified	41.05
29	33.385	0.886	0	0.13	Nonadecane	57

30	33.904	0.899	0	1.07	(E,E)-6,10,14-trimethyl-5,9,13-pentadecatrien-2-one	43
31	33.98	0.901	0.06	0.07	Unidentified	73.95
32	36.129	0.958	0	0.11	(2E,6E,10E)-3,7,11,15-tetramethyl-2,6,10,14-hexadecatetraenyl acetate	43
33	36.298	0.963	0	0.11	Farnesyl acetone	43
34	37.121	0.985	0	0.09	1-heptadecanol	55.05
35	37.365	0.991	0	0.11	2-methyltetracosane	57.05
36	37.698	1	48.15	30.61	Phytol	43
37	37.945	1.007	0.53	0.02	Unidentified	43
38	39.41	1.045	0	0.31	Unidentified	43
39	41.009	1.088	0	0.08	tetratriacontane	57
40	41.189	1.093	0	0.5	1,2-epoxynonadecane	82
41	42.109	1.117	0	0.47	4,8,12,16-tetramethylheptadecan-4-olide	99
42	43.778	1.161	0	0.2	1,54-dibromo-tetrapentacontane	57
43	44.114	1.17	0	0.22	1-hentetracontanol	57
44	44.29	1.175	0.07	0.06	Pentadecanol	55
45	44.366	1.177	0.66	0.09	Tetratriacontane	57
46	45.324	1.202	0	0.11	Unidentified	149
47	47.476	1.259	0	0.19	2-methyloctacosane	57.05
48	50.366	1.336	0	0.58	Tetratetracontane	57.05
49	51.289	1.361	0.61	0.22	δ-tocopherol	402.1
50	51.739	1.372	0	0.18	n-pentatriacontane	57
51	53.078	1.408	0	1.64	2-methyl octacosane	57
52	53.243	1.412	0	0.11	Cholesta-4,6-dien-3-ol	43.05
53	53.313	1.414	5.34	0.05	Stigmasterol acetate	43
54	53.537	1.42	0	0.15	Unidentified	396.2
55	53.889	1.429	0	2.27	Cholesterol	43
56	54.544	1.447	13.39	0	γ - sitosterol	43
57	54.749	1.452	0	0.2	6-methylcholest-5-en-3-ol	385.1
58	55.609	1.475	0	1.55	Ergost-5-en-3β-ol	43
59	56.124	1.489	11.01	15.71	Stigmasterol	55
60	57.01	1.512	0	0.03	Unidentified	206.9
61	57.182	1.517	10.55	12.21	β – sitosterol	42.95
62	57.611	1.528	0	0.12	Heptadecanal	413.25
63	57.842	1.534	0	0.1	Dimethylcholest-8(14)-en-3-ol	206.9
64	58.761	1.559	0	0.26	Cycloartenol	68.95
65	60.067	1.593	0	0.5	Methylene cycloartanol	55
Total identified %			97.87	98.55		

RT, Retention Time; **RTT**, Relative Retention Time to 37.698 (retention time phytol which is the major compound in the two species).

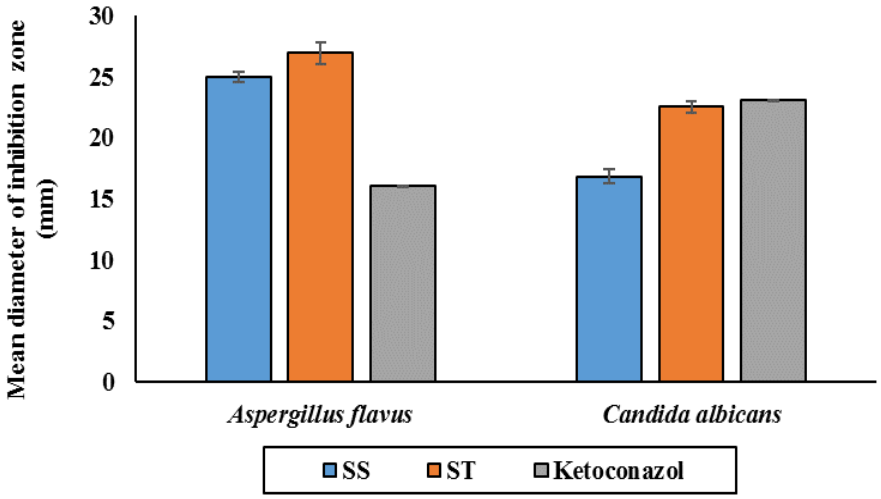

Figure 4. Antifungal activity of ethanolic extracts of the two studied *Sansevieria* species SS: *Sansevieria suffruticosa* N.E.Br.; ST: *Sansevieria trifasciata* Prain

Table 4: Results of GC/MS analysis of the saponifiable fraction of the aerial non-flowering parts of the two *Sansevieria* species

No	RT	RRT	Area %		Compound Name	Base m/z
			S. trifasciata Prain.	*S. suffruticosa* N.E.Br.		
1	23.856	0.592	3.15	1.63	Unidentified	205
2	24.195	0.600	0.61	0.44	Dodecanoic acid methyl ester	73.95
3	30.537	0.757	0.42	0.25	Tetradecanoic acid methyl ester	74
4	33.376	0.828	0.38	0.27	Pentadecanoic acid methyl ester	73.95
5	35.964	0.892	0.45	0	6- Octadecanoic acid methyl ester	41
6	36.086	0.895	23.38	27.08	Hexadecanoic acid methyl ester	74
7	37.792	0.937	0.43	0	Hexadecanoic acid ethyl ester	88
8	38.600	0.957	0.84	0.91	Heptadecanoic acid methyl ester	74
9	40.314	1	33.11	39.83	Linoleic acid methyl ester	67
10	40.444	1.003	0	22.27	8,11,14- Docosatrienoic acid methyl ester	55
11	40.479	1.004	27.61	0	9,12,15- Octadecatrienoic acid methyl ester	79
12	40.547	1.006	0	1.06	9-Octadecanoic acid methyl ester	55
13	41.023	1.018	5.02	3.49	n-Octadecanoic acid methyl ester	74
14	41.850	1.038	0.54	0.21	9,12- Octadecadienoic acid ethyl ester	67
15	42.014	1.042	0.33	0	9,12,15- Octadecatrienoic acid ethyl ester	79
16	44.660	1.108	0	0.16	9,11,13- Octadecatrienoic acid methyl ester	79
17	44.885	1.113	0.08	0	11,14- Eicosadienoic acid methyl ester	67
18	44.982	1.116	0	0.11	11- Eicosenoic acid methyl ester	55.05
19	45.540	1.129	1.20	0.30	18- Methylnonadecanoic acid methyl ester	74
20	46.167	1.145	0.58	1.64	Unidentified	99
21	50.353	1.249	0.88	0.35	Docosanoic acid methyl ester	73.95
22	53.611	1.329	0.19	0	Tricosanoic acid methyl ester	74
23	57.773	1.433	0.80	0	Tetracosanoic acid methyl ester	74
	Total identified %		96.27	96.73		

RT, Retention time; RTT, Relative Retention Time to 40.314 (retention time of 9, 12- Octadecadienoic acid methyl ester which is the major compound in the two species).

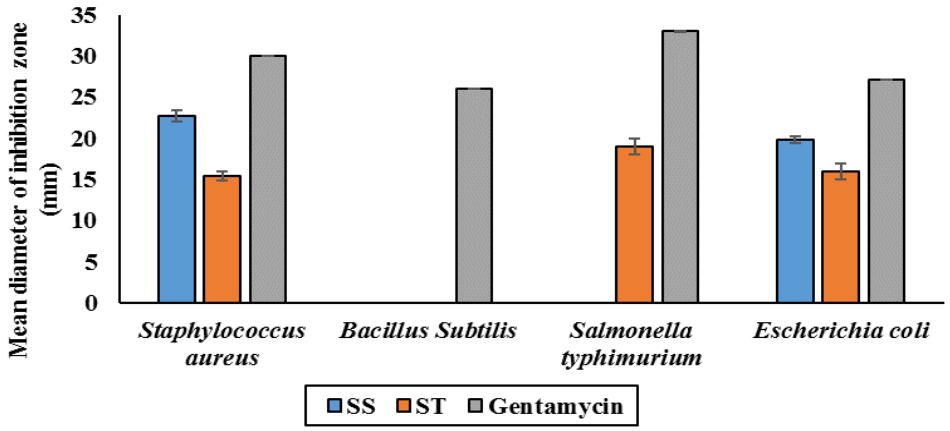

Figure 5; Antibacterial activity of ethanolic extracts of the two studied *Sansevieria* species
SS: *Sansevieria suffruticosa* N.E.Br.; ST: *Sansevieria trifasciata* Prain.

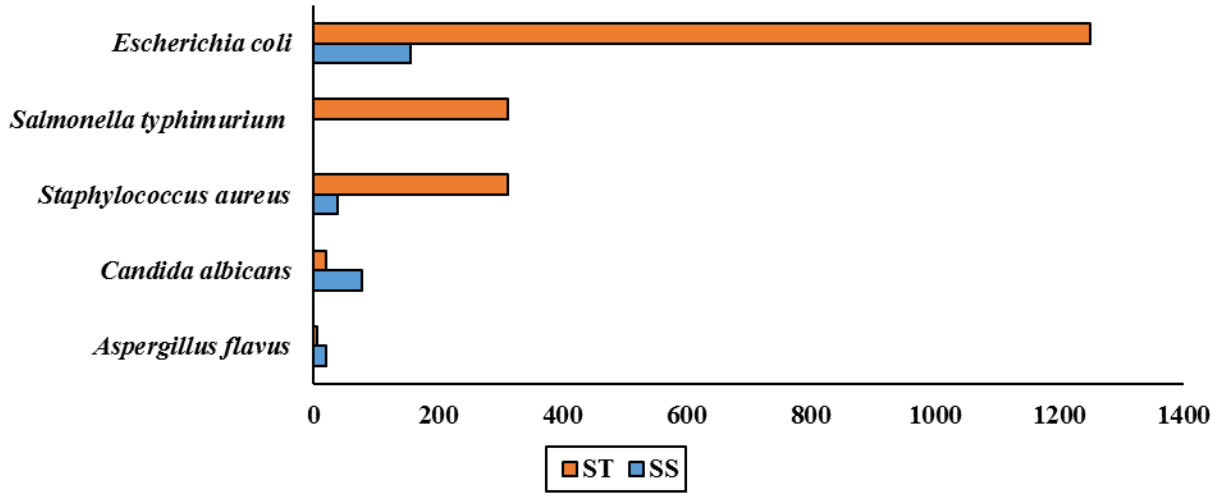

Figure 6: MIC of ethanolic extracts of *Sansevieria* species against bacterial and fungal strains
SS: *Sansevieria suffruticosa* N.E.Br.; ST: *Sansevieria trifasciata* Prain.

CONCLUSION

The plant identification is the first step to assurance efficacy, quality, and safety of a drug or an extract. Our present study clearly point out that RAPD markers as effective tool to authenticate our two *Sansevieria* species in the local herbal markets. Phytochemical identification and standardization of both plants were enabled by different parameters developed in this study. The lipoidal matter content of the two species showed high percentage of many compounds that have beneficial effect. The results of the preliminary phytochemical screening and antimicrobial evaluation indicate that *S. trifasciata* Prain. and *S. suffruticosa* N.E.Br. can be potential sources of phytomedicines with promising antifungal activities.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Noha Essam Fawaz: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Seham Salah El-Din El-Hawary, Mona El-Mahdy El-Tantawy, Mohamed Abdelatty Rabeh and Zeinab Yousef Ali: Conceived and designed the experiments, contributed reagents, materials, analysis tools or data; Wrote the paper.

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