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# Comparative Study on the Volatile Constituents of *Polyscias guilfoylei* and *Polyscias balfouriana* Leaves

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## Abstract

The essential oils of the fresh leaves of *Polyscias guilfoylei* and *Polyscias balfouriana* (Araliaceae) were separately prepared by hydro distillation and were characterized by gas chromatography-mass spectrometry (GC/MS). Forty-two compounds were identified representing 79.17% and 87.77% of the whole volatile constituents of *P. balfouriana* and *P. guilfoylei,* respectively. The oxygenated sesquiterpenes represents 10.56% and 20.08% of the whole volatile constituents of *P. balfouriana* and *P. guilfoylei*, respectively. *β*-chamigrene and *γ*-muurolene were the most abundant compounds in both oil samples. The antimicrobial effect of the samples was evaluated against two gram positive and two-gram negative bacteria in addition to two fungi using agar well diffusion method. *P. guilfoylei* showed moderate activity against *staphylococcus aureus* (MIC 313 µg/mL) and *Bacillus subtilis* (MIC 78.13 µg/mL). While *P. balfouriana* was active against *E. coli* (MIC 156.3 µg/mL) and *Candida albicans* (78.13 µg/mL). Cytotoxic activity was assessed for both plants' samples using MTT assay on Caco-2 cell lines. Results revealed that *P. guilfoylei* volatile constituents showed cytotoxicity with $IC_{50}$ value of $IC_{50}$ 70.62 µg/mL while *P. balfouriana* showed activity with $IC_{50}$ value of 232.17 µg/mL.

**Keywords:** Antimicrobial; Essential oils; Gas chromatography; Araliaceae; *Polyscias*

## Introduction

Since ancient times, people have resorted to nature, mainly to plants as medical and health sources to treat and prevent illness. Medicinal plants have been utilized to treat many diseases due to their multiple biological effects [1]. Natural plant products have few side effects, lower cost and they are easily available [2].

Volatile constituents derived from plants revealed a great importance for the production of many biologically active agents which are a vital source for drug industry. Essential oils have been reported to have antioxidant, anti-inflammatory, antimycotic, antibacterial, antiviral, anti-parasitic and cytotoxic activities. Also plant essential oils are widely used in the food, flavors, fragrances and pharmaceutical industries [3-5].

Family Araliaceae, includes 46 genera and 1415 species that are widely used in traditional and modern phytotherapy. Araliaceae is known to comprise wide spread classes of secondary metabolites such as triterpenes, triterpenoidal saponins, sterols, diterpenes and cerebrosides with wide range of biological activities [6]. Panax, the ginseng genus, is one of the most medicinally important genera in the family. Essential oil constituents from ginseng genus are rich in sesquiterpene hydrocarbons [7].

Genus *Polyscias* comprises 116 species they are widely used as ornamental plants also for many medicinal purposes mainly as anti-inflammatory, antitoxin, antibacterial and diuretic [8]. Among this genus, *Polyscias fruticosa* leaves was previously identified by GC/MS analysis essential oil and revealed high percentage of sesquiterpenes [9].

Since cancer is considered as one of the most predominant non-communicable diseases worldwide and is known as the second cause of death in most of the developed countries also considered as the fourth leading cause of death [10]. The lack of selectivity of chemotherapeutic agents is the most harmful problem leading to great damage to both cancer and normal cells. Thus, many studies seek alternative and complementary remedies to overcome the undesirable effects [11]. Another health threat facing human is the evolution of antibiotic resistant microorganisms. Infectious diseases are recently considered as a leading cause of morbidity and mortality globally and due to the reported adverse effects of current synthetic antimicrobial agents [12]. Searching for new sources of anticancer and antimicrobial agents that are effective, safe as well as selective have recently become the main target of drug discovery. Thus, we targeted the essential oils as a natural source to offer effective and quite safe cytotoxic and anti-infectious remedies.

*Polyscias guilfoylei* and *Polyscias balfouriana* present sources of important biologically active constituents. Pyrrolidine derivatives, flavonoid glycosides and tritepenoidal saponins have been investigated previously from the leaves of *P. balfouriana* [13]. In addition, tritepenoidal saponins from leaves of *P. guilfoylei* were reported [14] together with saponins of the aerial parts, which exhibited antiproliferative activity [15]. Nevertheless, the essential oils composition and the biological activities of both plant species have not yet been investigated. In this context, in our study we investigated the chemical composition of both species qualitatively and quantitatively using GC/MS analysis. Moreover, the antimicrobial and cytotoxic activities of essential oils obtained from the leaves of *P. guilfoylei* and *P. balfouriana* cultivated in Egypt were explored.

## Experimental

### Plant material

The fresh leaves of *Polyscias guilfoylei* and *Polyscias balfouriana* were collected from the Zohrya botanical garden, Giza, Egypt, in September 2014. The plants were identified morphologically by Mrs. Therese Labib, Consultant of Plant Taxonomy at the Ministry of Agriculture and El-Orman Botanical Garden, Giza, Egypt. Voucher specimens with codes PHG-P-PG-242 and PHG-P-PB-241 for *P. guilfoylei* and *P. balfouriana*, respectively, were deposited in the herbarium Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.

### Isolation of the volatile constituents

The volatile constituents of the fresh leaves of *P. guilfoylei* and *P. balfouriana*, (50 g) were separately obtained by hydro distillation for 4 h using a Clevenger-type apparatus. The extracted oils were dried over anhydrous sodium sulphate and kept in separate, sealed vials at 4°C for further analyses. The yield expressed in % w/w was determined in triplicate and calculated based on the initial plant weight.

### GC/MS analysis

Mass spectrum was recorded using Shimadzu GC-2010 plus gas chromatograph (Shimadzu Corporation, Kyoto, Japan) coupled to a quadrupole mass spectrometer Shimadzu QP-2010 equipped with Rtx-5MS fused bonded column (30 m × 0.25 mm i.d. × 0.25 µm film thickness) (Restek, USA) equipped with a split–spitless injector. The capillary column was directly coupled to a quadrupole mass spectrometer. The initial column temperature was kept at 45°C for 2 min (isothermal) and programmed to 300°C at a rate of 5°C/min and kept constant at 300°C for 5 min (isothermal). Detector and injector temperatures were 300 and 250°C, respectively. Helium carrier gas flow rate was 2 ml/min. Mass spectra were recorded applying the following condition: (equipment current) filament emission current, 60 mA; ionization voltage, 70 eV; ion source, 200°C. Diluted samples (0.5% v/v) were injected with split mode (split ratio 1:15). The sample (1 µL) was injected automatically to the chromatograph using AOC-20i auto sampler. GC solution® software ver. 2.4 (Shimadzu Corporation, Kyoto, Japan) was used for recording and integrating the chromatograms. Volatile components were identified by comparing their retention indices and mass spectra with those built in libraries (NIST Mass Spectral Library (December 2005), Wiley Registry of Mass Spectral Data 8th edition) and literature [16,17].

## Evaluation of Antimicrobial Activity

### Bacterial and fungal strains

The antimicrobial activity of the samples was determined using agar well diffusion method [18]. All samples were tested *in vitro* for their antibacterial activity against two Gram positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis*, and two Gram negative bacteria, *Salmonella typhi* and *Escherichia coli* using nutrient agar medium. Antifungal activity was carried out against *Aspergillus flavus* and *Candida albicans* using Sabouraud dextrose agar medium. Gentamycin was used as standard drug for Gram positive, Gram negative activities and Ketoconazole was used as standard drug for antifungal activity. DMSO was used as the negative control. The samples were tested at a concentration of 5 mg/mL against both bacterial and fungal strains.

### Determination of the mean zones of inhibition

The sterilized media were poured onto the sterilized petri dishes (20 mL, each petri dish) and allowed to solidify. Wells of 6 mm diameter were made in the solidified media with the help of sterile borer. A sterile swab was used to evenly distribute microbial suspension over the surface of solidified media and solutions of the tested samples were added to each well with the help of micropipette. The plates were incubated at 37°C for 24 h in case of the antibacterial activity and 48 h at 25°C for the antifungal activity. This experiment was carried out in triplicate and zones of inhibition were measured in mm scale.

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

The agar plate method was used to determine the MIC of the tested extracts. Nutrient agar (for bacteria) and Sabouraud dextrose agar (for fungi) were heated in the autoclave for 25 min at 121°C and allowed to cool to 45°C. Two-fold serial dilutions of each sample were added to the medium immediately before it was poured into the petri dishes. DMSO was used as the negative control. The culture of each organism in the nutrient broth (beef extract 5 g/L and peptone 10 g/L, pH=7.0) for bacteria and Sabouraud dextrose broth for fungi was diluted with sterile distilled water to $10^5$–$10^6$ CFU/mL. A loop of each suspension was inoculated in the appropriate medium with the sample or the control added. After inoculation, the plates were incubated at 37°C for 24 h for bacteria, and at 30°C for three to four days for fungi. The MIC was considered to be the lowest concentration that completely inhibited the visible growth of a microorganism compared with the control. Each test was performed in duplicate. Gentamycin and Ketoconazole were used as the positive controls [19].

## Results

The volatile constituents of both species *P. balfouriana* and *P. guilfoylei* were quantitatively and qualitatively analyzed using GC/MS technique (Figure 1). Volatile oils of both species were yellow and having a characteristic aromatic odor. The yields of *P. balfouriana* and *P. guilfoylei* leaves oil were 0.4 and 0.3% w/w, respectively. 42 components were totally identified, which representing 79.17% and 87.77% of the total volatile constituents of *P. balfouriana* and *P. guilfoylei* leaves, respectively. The identified compounds, their percentages and retention indices are listed in Table 1. From these results it is clearly obvious that sesquiterpene hydrocarbons represent the majority of the components existing in the essential oil of both plant species. Volatile oil analysis of *P. balfouriana* yielded 38 components, the most abundant components were five sesquiterpenes namely; *β*-chamigrene (14.84%), *γ*-muurolene (12.70%), caryophyllene oxide (5.55%), *β*-Elemene (4.77%), guaiazulene (3.94%) and one diterpene; phytol (3.92%). On the other hand, volatile oil constituents of *P. guilfoylei* yielded 36 components consisting of *β*-Chamigrene (13.90%), *γ*-muurolene (13.20%) and germacrene D-4-ol (10.24%), Globulol (9.1%), phytol (7.87%), guaiazulene (6.87%) were the major components. Literature review on the *Polyscias* genus revealed that *P. fruticosa* essential oil was rich in sesquiterpene hydrocarbons showing *β*-elemene, (*E*)-*γ*-bisabolene, germacrene-D, and *α*-bergamotene as major components of the oil [9].

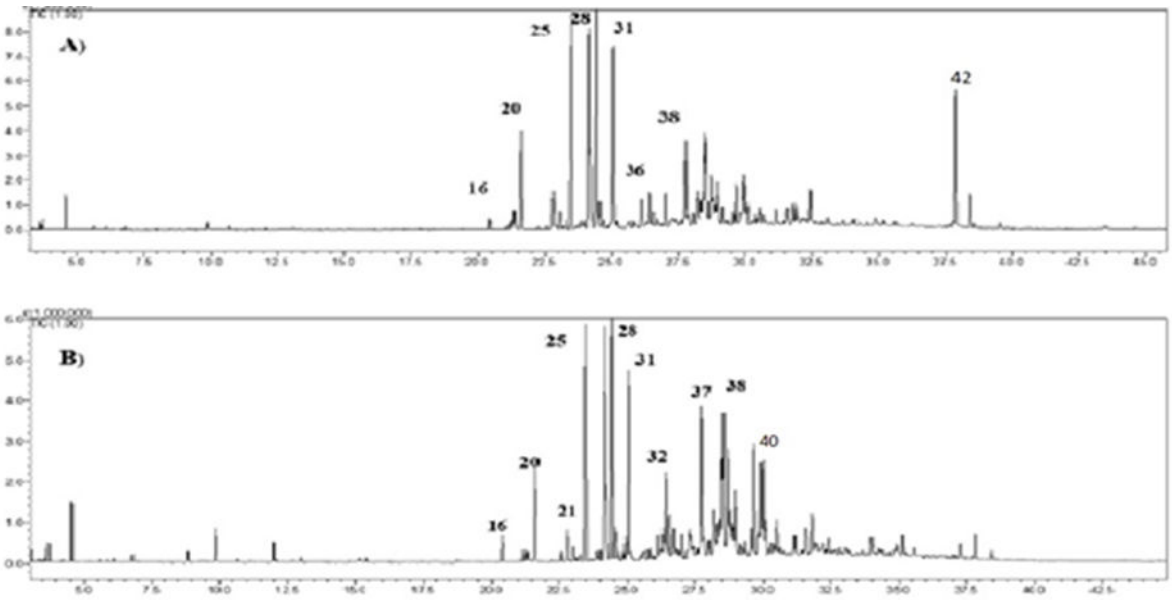


**Figure 1:** GLC-profile obtained with a Rtx-5MS column of the essential oils isolated by hydrodistillation for 4 h from A) *P. guilfoylei* and B) *P. balfouriana*. *Numbers on peaks describe compounds in Table 1.

| S.no | Compound name | KI | | Content% | | Identification method |
|---|---|---|---|---|---|---|
| | | Calculated | Reported | PBL | PGL | |
| 1 | Hexanal | 801 | 801 | Tr. | Tr. | MS, RI |
| 2 | Acetic acid, butyl ester | 813 | 813 | 1.8 | 1.02 | MS, RI |
| 3 | 1-Butanol, 3-methyl-, acetate | 869 | 872 | Tr. | Tr. | MS, RI |
| 4 | Heptanal | 894 | 890 | 0.29 | Tr. | MS, RI |
| 5 | Heptanal | 968 | 968 | 0.41 | - | MS, RI |
| 6 | Methyl-5-heptene-2-one | 987 | 988 | 0.12 | 0.17 | MS, RI |
| 7 | D-limonene | 1030 | 1030 | 0.23 | 0.12 | MS, RI |
| 8 | 2-Nonanone | 1097 | 1095 | Tr. | - | MS, RI |
| 9 | Octanal | 1103 | 1105 | 1.45 | 0.27 | MS, RI |
| 10 | Nonanal | 1109 | 1104 | Tr. | Tr. | MS, RI |
| 11 | Nonenol (*E*) | 1170 | 1167 | - | Tr. | MS, RI |
| 12 | Octanoic acid | 1177 | 1177 | Tr. | Tr. | MS, RI |
| 13 | 2-Decenal, (*E*) | 1269 | 1268 | 0.15 | 0.27 | MS, RI |
| 14 | *α*-Cubebene | 1357 | 1355 | 1.29 | 0.54 | MS, RI |
| 15 | Ylangene | 1378 | 1373 | - | Tr. | MS, RI |
| 16 | Copaene | 1382 | 1382 | 0.55 | 0.26 | MS, RI |
| 17 | *β*-Gurjunene | 1386 | 1388 | 1.2 | - | MS, RI |

| | | | | | | |
|---|---|---|---|---|---|---|
| 18 | *β*-Elemene | 1397 | 1393 | 4.77 | 4.96 | MS, RI |
| 19 | *α*-Santalen | 1426 | 1424 | Tr. | - | MS, RI |
| 20 | *α*-Ionone | 1434 | 1428 | 0.51 | Tr. | MS, RI |
| 21 | *α*-trans-Bergamotene | 1441 | 1432 | 0.3 | 0.16 | MS, RI |
| 22 | *α*-Guaiene | 1446 | 1442 | 1.4 | 0.65 | MS, RI |
| 23 | trans-Geranylacetone | 1458 | 1453 | 0.84 | - | MS, RI |
| 24 | *γ*-Muurolene | 1470 | 1477 | 12.7 | 13.2 | MS, RI |
| 25 | *γ*-Gurjunene | 1486 | 1475 | 0.37 | 0.41 | MS, RI |
| 26 | Eremophylene | 1486 | 1489 | 1.74 | - | MS, RI |
| 27 | *α*-Curcumene | 1490 | 1485 | 0.44 | 0.43 | MS, RI |
| 28 | *β*-Chamigrene | 1496 | 1499 | 14.84 | 13.9 | MS, RI |
| 29 | *β*-Selinene | 1499 | 1495 | 3.64 | 3.73 | MS, RI |
| 30 | *β*-Bisabolene | 1516 | 1514 | 2.9 | 2.17 | MS, RI |
| 31 | Germacrene D-4-ol | 1526 | 1526 | 1.47 | 10.24 | MS, RI |
| 32 | *δ*-Cadinene | 1533 | 1538 | 0.47 | 0.62 | MS, RI |
| 33 | *α*-Calacorene | 1554 | 1545 | - | 0.13 | MS, RI |
| 34 | Oleic acid | 1556 | 2097 | 0.5 | - | MS, RI |
| 35 | Carotol | 1608 | 1594 | 1.25 | 1.72 | MS, RI |
| 36 | Widdrol | 1653 | 1606 | - | 3.53 | MS, RI |
| 37 | Caryophyllene oxide | 1659 | 1666 | 5.55 | 0.32 | MS, RI |
| 38 | Globulol | 1670 | 1695 | 2.3 | 9.1 | MS, RI |
| 39 | Guaiazulene | 1690 | 1778 | 3.94 | 6.87 | MS, RI |
| 40 | Aristolene | 1761 | 1762 | 3.26 | 5.11 | MS, RI |
| 41 | 2-Pentadecanone, 6,10,14-trimethyl | 1847 | 1848 | 1.24 | Tr. | MS, RI |
| 42 | Phytol | 2123 | 2112 | 3.92 | 7.87 | MS, RI |
| 43 | Monoterpene hydrocarbons | | | 0.23 | 0.12 | |
| 44 | Sesquiterpene hydrocarbons | | | 49.43 | 51.4 | |
| 45 | Oxygen-containing Sesquiterpene | | | 1.056 | 20.08 | |
| 46 | Diterpenes | | | 3.92 | 7.87 | |
| 47 | Others | | | 15.03 | 8.3 | |
| 48 | Total identified components | | | 79.17 | 87.77 | |

**Table 1:** Essential oil composition of *Polyscias balfouriana* (*PB*L) and *Polyscias guilfoylei* leaves (*PG*L). KI: Kovats index; RI: Retention index.

In this study, the antimicrobial activity of the essential oil samples was evaluated against two gram positive (*Staphylococcus aureus*, *Bacillus subtilis*) and two-gram negative bacteria (*Salmonella typhi* and *Escherichia coli*) in addition to two fungi (*Aspergillus flavus* and *Candida albicans*) using the agar well diffusion technique. Final results of antimicrobial assay are summarized in Tables 2 and 3 showed the antimicrobial activity of the both oil samples.

| Microorganisms | Diameter of inhibition zone (mm) | | | |
|---|---|---|---|---|
| | PGL | PBL | Keto | Genta |
| **Gram Positive** | | | | |
| *Staphylococcus aureus* (RCMB 010010) | 19.37 ± 1.10 | 18.07 ± 1.10 | NT | 24.13 ± 1.21 |
| *Bacillus subtilis* (RCMB 015 (1) NNRL B-543) | 16.27 ± 1.42 | 17.10 ± 1.15 | NT | 25.97 ± 0.95 |
| **Gram Negative** | | | | |
| *Salmonella typhi* (RCMB 006 (1) ATCC 14028) | 15.17 ± 1.26 | 16.03 ± 0.75 | NT | 16.97 ± 0.95 |
| *Escherichia coli* (RCMB 010052 ATCC 25955) | 17.50 ± 1.32 | 17.20 ± 0.80 | NT | 30.03 ± 1.05 |
| **Fungi** | | | | |
| *Aspergillus flavus* (RCMB 002002) | 15.13 ± 0.81 | 14.17 ± 0.76 | 16.30 ± 0.81 | NT |
| *Candida albicans* (RCMB 05003 (1) 10231) | 24.67 ± 1.53 | 20.20 ± 0.72 | 20.23 ± 1.37 | NT |

**Table 2:** Mean inhibition zones of volatile constituents of *P. guilfoylei* leaves (*PG*L) and *P. balfouriana* leaves (*PB*L) against different ranges of environmental and clinically pathogenic microorganisms determined by the agar diffusion method. Data are measured in triplicates (n=3) Keto=Ketoconazole; Genta=Gentamycin; NT: Not tested; RCMB: Regional Center of Mycology and Biotechnology, Al-Azhar University.

| Microorganisms | Minimum Inhibitory Concentration (µg/mL) | | | |
|---|---|---|---|---|
| | PGL | PBL | Keto. | Genta. |
| **Gram Positive** | | | | |
| *Staphylococcus aureus* (RCMB 010010) | 313 | 19.53 | NT | 10 |
| *Bacillus subtilis* (RCMB 015 (1) NNRL B-543) | 78.13 | 156.3 | NT | 10 |
| **Gram Negative** | | | | |
| *Salmonella typhi* (RCMB 006 (1) ATCC 14028) | 625 | 313 | NT | 10 |
| *Escherichia coli* (RCMB 010052 ATCC 25955) | 156.3 | 313 | NT | 10 |
| **Fungi** | | | | |
| *Aspergillus flavus* (RCMB 002002) | 313 | 625 | 40 | NT |
| *Candida albicans* (RCMB 05003 (1) 10231) | 1125 | 78.13 | 40 | NT |

**Table 3:** Minimum Inhibitory Concentrations (MIC) volatile constituents of *P. guilfoylei* leaves (*PG*L) and *P. balfouriana* leaves (*PB*L) against different a range of environmental and clinically pathogenic microorganisms determined by the agar diffusion method. Data are measured in triplicates (n=3) Keto=Ketoconazole; Genta=Gentamycin; NT: Not tested; RCMB: Regional Center of Mycology and Biotechnology, Al-Azhar University.

Both *P. balfouriana* and *P. guilfoylei* essential oils exhibited antibacterial activity against the above-mentioned bacterial strains at the concentration of 5 mg/mL with mean inhibition zones diameter ranging between 19.37 and 15.17 mm for *P. guilfoylei*. The mean inhibition zones diameter for *P. balfouriana* ranged between 18.07 and 16.03 mm. In addition, a notable antifungal activity was exerted by *P. guilfoylei* and *P. balfouriana* against *Candida albicans* comparable to the standard drug with mean zones of inhibition diameter 24.67 mm for *P. guilfoylei* and 20.20 mm for *P. balfouriana*.

*P. balfouriana* exhibited a good antimicrobial activity against gram positive bacteria, where *Staphylococcus aureus* was the most susceptible bacteria to *P. balfouriana* with MIC value of (19.53 µg/mL), while *P. guilfoylei* revealed lower activity against *Staphylococcus aureus* with MIC value of 313 µg/mL but a better activity against *Bacillus subtilis* with MIC value of 78.13 µg/mL.

Both *P. balfouriana* and *P. guilfoylei* showed moderate activity against gram negative bacteria, *E. coli* was more susceptible to the volatile constituents of *P. balfouriana* (MIC 156.3 µg/mL) than the essential oil of *P. guilfoylei* (MIC 313 µg/mL). Concerning the antifungal activity, essential oil from the two species showed moderate activity except for *P. balfouriana*, which showed good activity against *Candida albicans* with the MIC value of 78.13 µg/mL.

Volatile constituents from both plants were tested for their cytotoxic activity on human colon carcinoma (Caco-2) cell line. The results revealed that *P. guilfoylei* volatile constituents showed cytotoxicity

against Caco-2 cell lines with $IC_{50}$ value of 70.62 µg/mL while *P. balfouriana's* essential oil constituents exhibited cytotoxic effect on Caco-2 cell lines with $IC_{50}$ value of 232.17 µg/mL.

## Discussion

Essential oils obtained were traditionally used for many infections and recently used in medicines for colds [13]. Sesquiterpenes from plant essential oil have been reported for their antimicrobial activities [14]. The mechanism(s) of the antibacterial action of most of the volatile constituents are still not totally understood. However, literature reports this it may be due to the lipophilic nature of terpenes which allow their penetration through the membranes of microorganisms [15], leading to severe changes within the microbial cell wall by both hydrocarbons and oxygenated components resulting in severe changes in cell wall structure. Also, the oil permeability causes the depletion of ions, reduction of membrane potential, and failure of the proton pump which finally lead to cell death [16]. It was also reported that the essential oil antibacterial activity is a result of the synergistic effect of the oil constituents together with the minor compounds [17,18]. Essential oils rich in sesquiterpenes were reported for cytotoxic activity, $\beta$-caryophyllene and $\alpha$-humulene are examples for potent cytotoxic sesquiterpenes ($IC_{50}$=40.9 and 24.8 µg/mL, respectively) tested against HT-29 cell lines [19]. These sesquiterpenes together with the other components in *Polyscias* leaves oil may be responsible for the cytotoxic effect of the volatile constituents.

## Conclusion

In this study, the volatile constituents of two *Polyscias* species; namely *P. guilfoylei* and *P. balfouriana* were chemically investigated using GC/MS chromatographic technique. A significant variation is noticed in the volatile constituents between both species. The results of their biological investigation present a solid base for recommending the use of volatile constituents of the leaves as antimicrobial agents. Additionally, the volatiles in the leaves of both *Polyscias* plants afforded a potential cytotoxic activity. In conclusion, these results provide evidence for the utilization of these species as alternative natural resources of medicinal interest.

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