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The floral essential oil composition and biological activity of *Solanum macranthum* Dunal

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Abstract
The essential oil from the floral part of *Solanum macranthum* Dunal was obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS). A total of seventeen compounds were identified representing 100% of the oil composition. Major components of the volatile oil were pentadecane (58.6%), (*E*)-nerolidol (15.4%), α-phellandrene (7.5%) and (*E*)-nerolidol acetate (5.6%). The essential oil exhibited strong antimicrobial activity (39-625 µg/ml) on pathogens employed in the assay. However, no appreciable cytotoxic effects on human breast carcinoma cells (Hs 578T) and human prostate carcinoma cells (PC-3) were observed. The floral volatile oil contents of *S. macranthum* and biological activity is being reported for the first time.

Keywords: *Solanum macranthum*, essential oil composition, pentadecane, nerolidol, antimicrobial activity.

1. Introduction
The genus *Solanum* (Solanaceae) comprises around 1700 species commonly found in the temperate and tropical regions of the world [1]. The genus is represented by some 25 species in Nigeria including five introductions: *S. wrightii* Benth, *S. melongena* L., *S. tuberosum* L., *S. mammorum* L. and *S. seaforthianum* Andr. (var. *disjunctum*) [2]. *Solanum macranthum* Dunal (syn. *S. wrightii* Benth) or 'Giant Star Potato Tree' is a shrub and an ornamental plant. The leaves are large, lobed and prickly. The fruits are small and oval shaped; flowers are very fragrant and change their color from white to pink to lavender, having all 3 colors at once. The very nice fragrant tree can be readily perceived from a distance when in full bloom [1, 3, 4]. Volatile constituents from the flowers of *S. stipulaceum* [5], *S. stuckertii* and *S. incisum* [6] and *Solanum tuberosum* [7] have been reported. Essential oil compositions of other parts of some *Solanum* species have also been analyzed by some researchers [8-13]. There is paucity of chemical and biological information on *S. macranthum*. However, in 1975, Hilal *et al*. [14] reported on a pharmacognostical study of *S. macranthum* grown in Egypt. In our previous work [9], the leaf and fruit essential oils of *S. macranthum* was shown to exhibit strong antimicrobial activity and the fruit oil demonstrated significant anti-proliferative activity against breast carcinoma cells (Hs 578T). Although no ethnomedicinal uses of *S. macranthum* have been documented, the plant is reputed to inhibit the growth of bacteria and fungi when applied to surfaces of other plants [1].
To the best of our knowledge, this is the first examination of the volatile components of *S. macranthum* floral essential oil, antimicrobial and cytotoxic activities. This work is an integral part of our chemical and biological studies on *S. macranthum* plant [9, 15].

2. Materials and methods
2.1 Plant material and oil extraction
Solanum macranthum flowers were collected in the month of July within the University of Ibadan, Nigeria. Plant samples were authenticated by Mr. F. Usang of the Herbarium Headquarters, Forest Research Institute of Nigeria (FRIN), Ibadan, Nigeria, where voucher specimen (FHI 106921) was deposited. The fresh flower sample of *S. macranthum* (500 g) was pulverized and hydrodistilled for 4 h using a Clevenger apparatus to give the light green colored essential oil in 0.3% yield.

2.2 Gas chromatography-mass spectrometry analysis (GC-MS)
The essential oil of *S. macranthum* was analyzed by GC-MS using an Agilent 6890 GC with Agilent 5973 mass selective detector [MSD, operated in the EI mode (electron energy = 70 eV), scan range = 45-400 amu, and scan rate = 3.99 scans/sec], and an Agilent Chem Station

data system. The GC column was an HP-5ms fused silica capillary with a (5% phenyl) polymethylsiloxane stationary phase, film thickness of 0.25 µm, a length of 30 m, and an internal diameter of 0.25 mm. The carrier gas was helium with a column head pressure of 48.7 kPa and a flow rate of 1.0 mL/min. Inlet temperature was 200 °C and interface temperature was 280 °C. The GC oven temperature program was used as follows: 40 °C initial temperature, hold for 10 min; increased at 3 °C/min to 200 °C; increased 2°/min to 220 °C. A 1% w/v solution of the sample in CH_2Cl_2 was prepared and 1 µL was injected using a splitless injection technique. Identification of oil 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 [16] and stored on the MS library [NIST database (G1036A, revision D.01.00)/ChemStation data system (G1701CA, version C.00.01.08)].

2.3 Antimicrobial screening

Essential oils were screened for antibacterial activity against the Gram-positive bacteria, *Bacillus cereus* (ATCC No. 14579) and *Staphylococcus aureus* (ATCC No 29213) and the Gram negative bacteria, *Pseudomonas aeruginosa* (ATCC No 27853) and *Escherichia coli* (ATCC No 254922). Minimum inhibitory concentration (MIC) was determined using microbroth dilution technique [17]. Dilutions of the oils were prepared in cation-adjusted Mueller Hinton Broth (CAMHB) beginning with 50 µL of 1% w/w solutions of essential oils in DMSO plus 50 µL CAMHB. The oil solutions were serially diluted (1:1) in CAMHB in 96-well plates. Organisms at a concentration of approximately 1.5×10^8 colony forming units (CFU)/mL were added to each well. Plates were incubated at 37 °C for 24 h; the final minimum inhibitory concentration (MIC) was determined as the lowest concentration without turbidity. Gentamicin was used as a positive control while DMSO was used as a negative control. Antifungal activity was determined as described above using *Candida albicans* (ATCC No. 10231) in yeast nitrogen base growth medium with approximately 7.5×10^7 CFU/mL. Antifungal activity against *Aspergillus niger* (ATCC No 16401) was determined as above using potato dextrose broth inoculated with *A. niger* hyphal culture diluted to a McFarland turbidity of 1.0. Amphotericin B was the positive control. Both C. *albicans* and *A. niger* were maintained at 32 °C and 25 °C respectively for 24 h.

2.4 Cell culture media

Human Hs 578T breast ductal carcinoma cells (ATCC. No. HTB-129) [27] were grown in 3% CO_2 environment at 37 °C in DMEM with 4500 mg glucose per litre of medium, supplemented with 10% fetal bovine serum, 10 µg bovine insulin, 100,000 units penicillin and 100 mg streptomycin per liter of medium, and buffered with 44 mM $NaHCO_3$, pH 7.35. Human PC-3 prostatic carcinoma cells (ATCC No. CRL-1435) [18] were grown in 3% CO_2 environment at 37 °C in RPMI-1640 medium with l-glutamine, supplemented with 10% fetal bovine serum, 100,000 units penicillin and 10.0 mg streptomycin per liter of medium, and buffered with 15 mM Hepes and 23.6 mM $NaHCO_3$.

2.5 Cytotoxicity screening

Hs 578T cells were plated into 96-well cell culture plates at 1.0×10^5 cells per well and PC-3 cells at 1.9×10^4 cells per well. The volume in each well was 100 µL for both cell types. After 48 h, supernatant fluid was removed by suction and replaced with 100 µL growth medium containing 2.5 or 1.0 µL of DMSO solution of oil (1% w/w in DMSO), giving a final concentration of 250 or 100 µg/mL, respectively, for each cell line. Hs 578T cells were tested at final concentration at 250 µg/mL and PC-3 cells at final concentration of 100 µg/mL. Solutions were added to wells in four replicates. Medium controls and DMSO controls (25 and 10 µL DMSO/mL) were used. Tingenone (250 and 100 µg/mL) was used as a positive control [19]. After the addition of compounds, plates were incubated for 48 h at 37 °C. The medium was then removed by suction and 100 µL of fresh medium was added to each well. In order to establish percent kill rates, the cell Titer 96® AQueous Non-Radioactive Cell Proliferation Assay was performed [20]. After colorimetric readings were recorded in quadruplicate (using a molecular Devices SpectraMAX Plus microplate reader, 490 nm), average absorbances, standard deviations, and percent kill ratios (% $kill_{cmpd}$/% $kill_{DMSO}$) were calculated.

3. Results & Discussion

The chemical composition of *S. macranthum* floral essential oil is summarized in Table 1. A total of seventeen compounds were identified representing 100% of the total oil composition. The oil was composed of pentadecane (58.6%), (*E*)-nerolidol (15.4%), α-phellandrene (7.5%) and (*E*)-nerolidol acetate (5.6%) in high amounts. Other constituents detected in appreciable quantities were 1,8-cineole (2.6%) and *p*-cymene (2.4%). Aliphatic hydrocarbons (62.9%), sesquiterpenoids (21.9%) represented by high amounts of the oxygenated derivatives (21.0%); and the monoterpenoids (14.9%) composed of low concentration of the oxygenated derivatives (4.7%) were the major classes of compounds identified in *S. macranthum* essential oil.

Higher amounts of sesquiterpene hydrocarbons (76.2%) were reported for *S. stipulaceum* floral essential oil [5] relative to *S. macranthum* (21.9%) in our study. Osorio *et al*. [5] indicated that the most abundant components in the oil were β-caryophyllene (25.8%), followed by γ-gurjunene (11.9%), β-gurjunene (8.2%), α-and selinene (5.3%). Floral essential oils of *S. stuckertii* and *S. incisum* [6] displayed a significant quantitative and qualitative chemical profiles. Major volatiles of *S. stuckertii* were myrcene (8.1%), aromadendrene (5.0%), β-pinene (4.7%), germacrene D (4.7%), *p*-cymene (4.5%), (*Z*)-β-ocimene (4.3%) and decane (4.1%), while floral components of *S. incisum* consisted of β-pinene (9.9%), limonene (6.0%), 1,8 cineole (4.8%) and myrcene (4.8%). In the same vein, considerable variations could be discerned from the leaf, fruit [9] and floral volatile oils composition of *S. macranthum*; presumably due to the fact that the essential oils are from different parts of the plant. 1,8-Cineole, *p*-cymene, nerolidol, and pentadecanal may be considered as common constituents of *S. macranthum* volatiles since these components are identified in the leaf, fruit and floral essential oils. β-Caryophyllene detected in high concentration (17.6%) in the fruit oil of *S. macranthum* [9] and floral oil of *S. stipulaceum* [5] was not present in *S. macranthum* floral oil. Comparison of the fruit and floral oils of *S. macranthum* revealed *p*-cymene, 1,8-cineole, and pentadecanal as common constituents of both oils. Principal constituents of the floral oil, pentadecane (58.6%) and nerolidol (15.4%) occurred in minor amounts (0.6% and 0.3% respectively) in the fruit oil sample. Nerolidol, occurring in significant amount in *S. macranthum* oil, is an important aroma compound in perfumery and food industry; and is reported as a component of some plant essential oils for example, *Thymus praecox* [21]. The presence of α-phellandrene

in the floral essential oil may furnish a light minty aroma exuded by the oil [22].

The floral volatile oil of *S. macranthum* demonstrated broad spectrum antimicrobial activity (Table 2). The essential oil exhibited stronger antibacterial effect on the Gram-positive bacteria: *B. cereus* and *S. aureus* (78 and 39 µg/mL, respectively) compared with the Gram-negative bacteria: *E. coli* and *P. aeruginosa* (625 µg/mL). *Aspergillus niger* was more susceptible to the effect of constituent(s) of the floral essential oil than *C. albicans*. The floral oil demonstrated comparable antimicrobial activity with the fruit oil of *S. macranthum* in our earlier study [9]. Crude methanol extracts of leaf, stem bark, roots and fruits of *S. macranthum* were also shown to exhibit strong broad spectrum antimicrobial activity [15]. It has been documented that Gram-positive bacteria are more sensitive to chemical compounds than Gram-negative bacteria due to the relative thickness of their cell walls [23]. This was observed for the bacteria strains in this study. It is well known that the biological activities of an essential oil may depend on the main constituents or a synergy between the major and some minor compounds. It should also be noted that prominent constituents of *S. macranthum* floral oil such as pentadecane, nerolidol, α-phellandrene and nerolidol acetate, along with other constituents have been shown to exhibit antimicrobial activity [24-26]. Furthermore, the floral oil exhibited no cytotoxic activity on Hs 578T cells and an insignificant effect on PC-3 carcinoma cell lines (14.8% kill, 100 µg/mL). However, the fruit essential oil of *S. macranthum* was found to demonstrate relatively significant cytotoxicity on both Hs 578T (79.39%, 250 µg/mL) and PC-3 (47.43%, 100 µg/mL) [9]. Major constituents in *S. macranthum* fruit oil – α-humulene (36.0%), methyl salicylate (8.1%), β-caryophyllene (7.8%), humulene epoxide 11 (5.5%) and caryophyllene oxide (5.0%) – were neither identified as components of the floral oil nor leaf oil. This distinction in chemical profiles may account for the significant cytotoxicity of the fruit essential oil compared to the floral oil in this study. The antitumor activities of caryophyllene oxide, β-caryophyllene and its potentiating effect on the anticancer activity of humulene have been reported [27-29].

3.1 Tables

Table 1: Chemical composition of *S. macranthum* floral essential oil.

RI[a]	RI[b]	Compound[c,d]	Area (%)	QI[e] (%)
940	939	α-Pinene	0.3	96
1003	1005	α-Phellandrene	7.5	94
1023	1026	*p*-Cymene	2.4	97
1031	1033	1,8-Cineole	2.6	98
1086	1088	α-Terpinolene	1.2	98
1100	1098	Linalool	0.9	90
1301	1300	Tridecane	1.9	95
1397	1398	Cyperene	0.1	94
1402	1400	Tetradecane	1.6	96
1501	1500	Pentadecane	58.6	96
1516	1508	(*E,E*)-α-Farnesene	0.8	99
1564	1564	(*E*)-Nerolidol	15.4	91
1697	1700	Heptadecane	0.4	96
1713	1714	Pentadecanal	0.3	91
1721	1714	(*E*)-Nerolidol acetate	5.6	91
2497	2500	Pentacosane	0.3	91
2697	2700	Heptacosane	0.1	95
		Total Identified	100	

[a]RI, calculated retention indices; [b]RI, retention index from literature; [c]Order of elution on HP-5ms capillary column; [d]Identification by comparison of the mass spectral and retention index data; [e]QI, 'quality index', reflects the fit comparison of experimental mass spectrum and NIST library mass spectrum.

Table 2: Antimicrobial activity of *S. macranthum* floral volatile oil (MIC, µg/mL)

Sample	B.c	S.a	E.c	P.a	C.a	A.n
S. macranthum	78	39	625	625	625	39
Positive control	1.22[a]	0.61[a]	2.44[a]	1.22[a]	0.61[b]	0.61[b]

B.c., *Bacillus cereus* (ATCC No. 14579); S.a, *Staphylococcus aureus* (ATCC No 29213); E.c, *Escherichia coli* (ATCC No. 25922); P.a, *Pseudomonas aeruginosa* (ATCC No. 27853); C.a, *Candida albicans* (ATCC No. 10231); A.n, *Aspergillus niger* (ATCC No. 16401). [a]Gentamicin sulphate; [b]Amphotericin B; Negative control, DMSO had zero effect in the assay.

4. Conclusions

The floral volatile constituents of *S. macranthum* have been analyzed and identified. The floral essential oil has been shown to exhibit significant antibacterial and antifungal activities against pathogens in the assay. The biological potential of this oil could be further explored and utilized.

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