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Volatile oil composition of *Curcuma oligantha* Trimen from Sri Lanka

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Abstract
Genus *Curcuma* is medicinally important. Among five *Curcuma* species grown in Sri Lanka, *C. oligantha* is unexplored. Therefore, current study was conducted to identify plant and establish chemical profile of the rhizome of *C. oligantha* essential oil. Mature whole plants of *C. oligantha* were collected in the flowering season in 2016 in Badulla district. Voucher specimens of the plants were authenticated & deposited at the National Herbarium, Peradeniya, Sri Lanka. Dried powdered samples were extracted by hydro-distillation with Clevenger's apparatus (6 h). GC-MS analysis was carried out on a RTX WAX capillary column. Morphologically, leaf surface and red-brown short stem, pubescent corolla tube, labellum yellow centrally were observed. Microscopically, single layer palisade cells, diamond shaped prismatic crystals, anomocytic stomata, annular and reticulate xylem vessels, dumbbell shape starch grains were observed. Major chemical constituents found in rhizome were β-caryophyllene (15.07%), phytol (13.38%), and α-humulene (8.24%).

Keywords: *Curcuma oligantha*, GC-MS, microscopy

1. Introduction
Plants of genus *Curcuma* are known to have anti-inflammatory, and antioxidant properties, and which is given from acute to chronic situations [1-3]. Among more than ninety species are accounted for *Curcuma*, five species (*C. albiflora*, *C. zedoaria*, *C. longa*, *C. aromatica*, and *C. oligantha*) are reported in Sri Lanka [4, 5]. *C. oligantha* might be endemic to Sri Lanka and its detailed study has not being conducted. Therefore, current study was conducted to identify plant and establish chemical profile of the whole plant of *C. oligantha* on essential oil.

2. Materials and methods
Matured whole plants of *C. oligantha* were collected in the flowering season from 2016 to early 2017 in Badulla district (Mahiyanganaya: N 7º 18' 31", E 81º 6' 16" and Badulla: N 6º 59' 19", E 81º 30' 42"). Voucher specimens of the plants (herbariums) were authenticated & deposited at the National Herbarium, Peradeniya, Sri Lanka (My ref 6/01/H/03). Collected samples were cleaned by tap water and cut into small pieces. All procedures were carried according to WHO guidelines and other published data [6, 7]. Fresh samples were obtained for microscopic study. Cut samples were dried under shade to obtain 8-10% moisture content. Then they were grinded and sieved from 40 mesh size sieve. Powdered samples were extracted by hydro-distillation with Clevenger's apparatus (6 h). GC-MS analysis was carried out on a Thermoscientific Trace 1300 detector and with RTX WAX capillary column. Mode of operating conditions was split (1:50), and the oven temperature program was 60 ºC (after 10.00 min) to 240 ºC at 5 ºC/min with Helium as carrier gas. Identification of constituents was done by matching 70 eV mass spectra, 250 ºC quad temperature, 250 ºC source temperature, 50 - 450 (amu) scan parameters, and direct matching and reverse matching with NIST library. Determination of compounds in GC-MS requires matching the retention time of same compounds with standard on two chromatographic columns.

2.2 Equations
Kovat (1958) introduced a retention index scheme. The retention index (I) of particular compound was calculated according to the formulae [8, 9].

$I = 100 [n + (N-n) \frac{log\ t_u - log\ t_s}{log\ t_L - log\ t_s}]$ where; n: number of carbon atoms in the smaller alkane, N: number of carbon atoms in larger alkane, t_u: retention time of unknown compound, t_s: retention time of smaller carbon atom, and t_L: retention time of larger carbon atom.

Standard alkane series was used number of carbons from 10 to 27 (e.g. 10, 11, 12 …etc.). To calculate retention index of unknown compounds for current study, above formulae modified as follows;

$$I = 100 [n + \frac{t_u - t_s}{t_L - t_s}]$$

where n is the number of carbon atoms in the smaller alkane, t_u: retention time of unknown compound, t_s: retention time of smaller carbon atom, and t_L: retention time of larger carbon atom.

3. Results & Discussion

3.1 Morphology of *C. oligantha*

The plant of *C. oligantha* was 12-20 cm in height and the rhizome was ovoid, oblong and elongated. 5-7 leaves were observed and they were pale green and mottled with dark green lamina; ovate, acute, rounded or cordate at the base, surface glabrous, red-brown short stem base. Corolla tube was pubescent and white; apiculate apex, lateral lobes smaller, rounded at the apex, lateral staminods white, ovate, sub-acute, labellum yellow at least centrally, slowly emarginated, margin undulating, anther crestless, basal spurs, pubescent, converging at the tips. Coma was absent; few flowered, one flower opening at a time, fertile bracts few, lanceolate, lightly pubescent and acute apex, joined lower quarter, more or less triangular, elongated and narrowed at the apex, and inner bracteoles smaller (Plate 1). Organoleptic characters of rhizome of *C. oligantha* were pale white inside, camphor smell, and pungent taste.

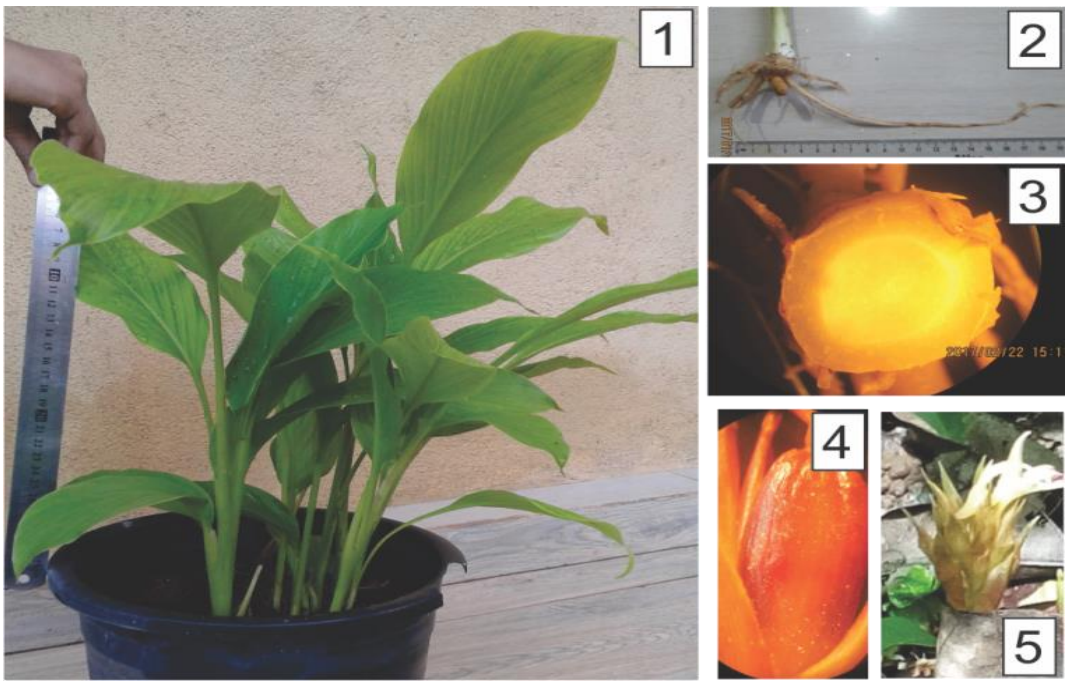

Plate 1: Morphology of *C. oligantha*; 1: whole plant, 2: root, 3: rhizome inside, 4: fruit, 5: flower bract.

3.2 Microscopy of *C. oligantha*

Single layer palisade cells were observed in adaxial side continuously in leaf lamina. Main vascular bundles were attached to both side of the lamina. Daughter vascular bundles were between both sides of the lamina (i.e. center). Parenchyma with cuboidal, cubic, and diamond shaped prismatic crystals of calcium oxalate and three sizes of prismatic crystal were found 3-4 crystals were found in single cell in midrib and petiole area parenchyma near vascular bundles. Phloem strands undivided. Air canals were single arc pectinating with main veins and embedded in a distinct abaxial band of collenchyma. Vascular bundles were collateral of the leaf. Stomata were anomocytic. Surface layer was cutinized in rhizome. Parenchyma cells of wide cortex loaded with many starch grains per cell, and they were loaded with oil. Collateral poly arc vascular bundles and annular and reticulate xylem vessels were found. Starch grains (SGs) were simple and hilum of starch grain was not distinct. They were triangular, circular, kidney shaped, elongated, dumbbell shaped. However, dumbbell shaped and triangular shaped SGs were prominent. Three sizes of starch grains were found (small: 5-7 µm, medium: 12-16 µm, and large: 20-27 µm). Oil loaded cells were found in rhizomes. Oil filled cells adjacent to coloring matter filled cells were observe (Plate 2).

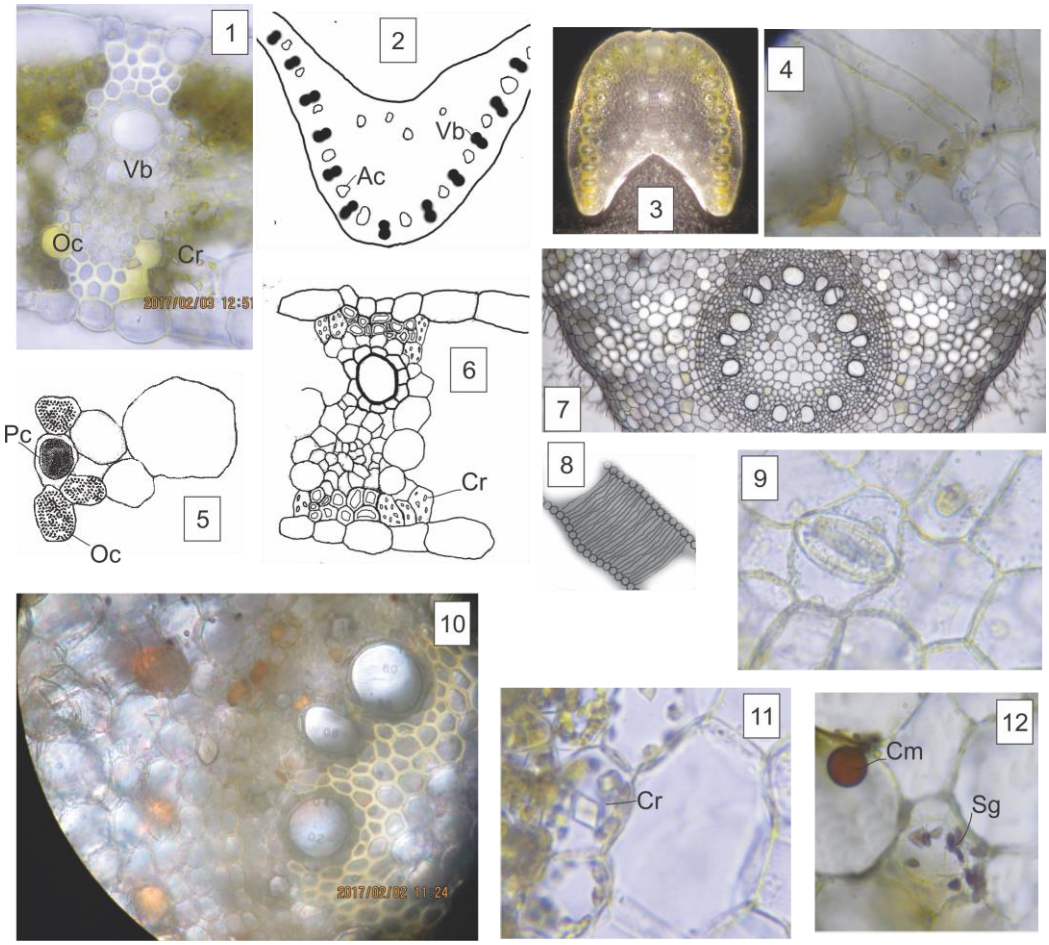

Plate 2: Microscopic study; 1: leaf lamina T.S., 2: petiole T.S., 3: midrib T.S., 4: rhizome hair, 5: rhizome oil filled cells, 6: scematic diagram of lamina T.S., 7: rhizome T.S., 8: annular vessels, 9: anomocytic stomata, 10: rhizome T.S. 11: prismatic crystals, 12: starch grains. Vb: vascular bundle, Oc: oil filled cells, Cr: crystals, Cm: colouring matter, Sg: starch grains.

3.3 GC-MS analysis of *C. oligantha*

The rhizome of *C. oligantha* possesses aliphatic constituents, β-sitosterol, curcumin etc [10]. *C. oligantha* is a poorly explored plant, which is grown in Sri Lanka. Therefore, GC-MS profile of *C. oligantha* was obtained and reported in the table 1. Thirty-nine chemical constituents were found. However, fourteen compounds were identified. Experiment was conducted based on RTX-wax column. RI referernce values were obtained from polyethylene glycol (PEG) and DB-Wax column based values to show RI (calculated) similarity [8, 9].

Table 1: GC-MS profile of *C. oligantha* rhizome essential oil.

Compound	RI cal	RI ref	Area%
α-Copaene	1409	1491	0.87
α-Bourbonene	1436	1500	0.06
β-Caryophyllene	1512	1598	15.07
Terpinen-4-ol	1520	1601	1.82
β-Elemene	1554	1590	0.19
Aromadendrene	1560	1620	0.19
α-Humulene	1585	1667	8.24
α-Terpineol	1616	1694	0.14
γ-Elemene	1649	1639	1.86
α-Ionone	1774	1843	0.19
Caryophyllene oxide	1906	1986	5.82
Spathulenol	2045	2126	1.97
Falcarinol	2284	2035	0.13
Phytol	2533	2613	13.38

RI: retention index, RI cal: RI calculated, RI ref: RI reference.

Awasthi *et al.* found that the major constituents of the *C. longa* rhizome oil are ar-turmerone (31.7%), α-turmerone (12.9%), β-turmerone (12.0%) and (Z)-β-ocimene (5.5%). Moreover, the major constituents in the *C. longa* leaf oil are α-phellandrene (9.1%), terpinolene (8.8%), 1, 8-cineole (7.3%) and undecanol (7.1%) and p-cymene (5.5%) [11]. But, major chemical constituents found in rhizome of *C. oligantha* were β-caryophyllene (15.07%), phytol (13.38%), and α-humulene (8.24%).

β-caryophyllene selectively binds to the CB_2 receptor (responsible for the psychomodulatory effects) and CB_2 receptor (inhibits inflammation, pain, atherosclerosis, and osteoporosis). Therefore, β-caryophyllene inhibits adenylate cylcase and activates the mitogen-activated kinases in primary human monocytes. Moreover, β-caryophyllene inhibits induced pro-inflammatory cytokine (e.g. lipopolysaccharide) in peripheral blood and phosphorylation in monocytes [12]. Phytol and α-humulene inhibit inflammatory responses by reducing cytokine production [13, 14]. α-humulene inhibits the lipopolysaccharide-induced NF-kB activation, neutrophil migration, pro-inflammatory cytokines (e.g. TNF-α and IL-1b) and signalling pathways (e.g. MAP kinases) leads to upregulation of B_1 receptor [15, 16]. Phytol presents in vitamins (e.g. K and E) and other tocopherols [17]. Further, phytol is shown antinociceptive, antioxidant, anti-allergic, as well as immunostimulant properties (long-term memory induction). Although, phytol activates both innate and acquired immunity, it has shown antimicrobial activity against *Mycobacterium tuberculosis* and *Staphylococcus aureus* [17].

4. Conclusions

Since *Curcuma* species possess similar morphological characters, adulteration might be occurred. By leaf and flower characters, *C. oligantha* can be identified from other *Curcuma* species available in Sri Lanka; leaves were pale green and mottled with dark green lamina, surface glabrous, and red-brown short stem base. Coma was absent and lightly pubescent joined lower quarter. Microscopically, prismatic crystals (cuboidal, cubic, and diamond shaped and starch grains (triangular, circular, kidney shaped, elongated, dumbbell shaped) were distinct characters. By GC-MS analysis, it was found that medicinally important phytochemicals; major chemical constituents found in rhizome of *C. oligantha* were β-caryophyllene (15.07%), phytol (13.38%), and α-humulene (8.24%).

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