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Short Paper

Comparative GC-MS analysis of all *Curcuma* species grown in Sri Lanka by multivariate test

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Abstract *Curcuma* is clinically valuable genus in Traditional Medicine. People use various plants under the same vernacular name may lead to adulteration or substitution. Whole plants of *Curcuma* species were collected in 2016 in the flowering season. Voucher specimens of the plants were authenticated from the National Herbarium, Peradeniya. Essential oils were extracted from Clevenger's apparatus and analyzed separately by GC-MS. The analyses were carried out with RTX WAX capillary column. Sampling and experiments were done according to WHO guidelines. One hundred sixty four phytochemicals were analyzed by simple correspondence and by cluster variable method. By cluster varibale as per phytochemicals present, mainly two groups were identified. *C. albiflora* and *C. oligantha* were identified as one group and the rest of the three plants were kept in the other group. A total of 64 constituents of essential oil obtained from whole plant of *C. albiflora* were identified by GC-MS, where α-pinene (10.87 %), caryophyllene oxide (8.85 %), alcanfor (5.12 %), aromadendrene oxide-(1) (4.81 %), n-hexadecanoic acid (4.74 %), α-famesene (3.93 %), camphene (3.52 %), and isoborneol (3.4 %) were detected as major compounds. The essential oil of *C. aromatica* possesses 7–methanoazulene (13.75 %) and curcumene (25.71%). Caryophyllene (15.07%), phytol (13.38%), humulene (8.24%), elemene (6.11%), caryophyllene oxide (5.82%) were found in *C. oligantha*. This preliminary study has identified chemical markers present in all *Curcuma* species grown in Sri Lanka.

Keywords: *Curcuma* species, essential oils, GC-MS, multivariate.

1 Introduction

Curcuma is an important genus in Traditional Medicine of Sri Lanka. Commonly known as turmeric, five species are reported in Sri Lanka (e.g. *C. albiflora* Thw., *C. aromatica* Salisb., *C. Longa* L., *C. oligantha* Trimen., *C.*

zedoaria Roscoe). These plant materials are known to be used for centuries to manage various health conditions. People use turmeric in culinary as a flavor or color. In addition, *Curcuma* species show antioxidant, anti-inflammatory, hypocholestraemic, choleratic, antimicrobial, antirheumatic, antifibrotic, antivenomous, antidiabetic, antihepatotoxic, anticancerous, larvicidal, pheromone, insecticidal, anti-plasmodium, hyperprotective, platelet aggregation inhibitory, antiarthritic, COX-1 inhibitory, antiviral and antiproliferative activity etc (Afzal *et al*. 2013; Tholkappiyavathi *et al*. 2013; Abbasi and Shah 2015; Krup *et al*. 2013; Sikha, *et al*. 2015;).

However, many plants are reported under the same *Sinhalese* vernacular name. For example, the people in Mahiyanganaya area use both *C. aromatica*, and *C. zedoaria* as '*Harankaha'*, while the people in Erathna area (Kegalle district) name *C. aromatica* as '*Beheth-harankaha'*. In literature, *Zingiber zerumbet*, *C. albiflora*, and *C. zedoaria* are reported as '*Harankaha' (*Dassanayake 1983). Regionally people have used various plants under the same vernacular name, even those plants have different chemical compositions and medicinal uses. Furthermore, *C. albiflora* and *C. oligantha* are unexplored plants. This is the first report of the chemical constituents of these different species of *Curcuma*, and the study was conducted to understand the similarities in terms of phytochemical constituents by analyzing data using multivariate test on five species available in Sri Lanka, *C. albiflora, C. aromatica, C. longa, C. oligantha*, and *C. zedoaria.* Therefore, the present study will provide important information on similarities and dissimilarities of *Curcuma* plants grown in Sri Lanka by chemical analysis.

2 Material and Methods

2.1 Samples and chemical analysis

Whole plants of *Curcuma* species were collected in the year 2016 from wet and dry zones of Sri Lanka in the flowering season; *C. albiflora* (Kitulgala, Kegalle and Erathna, Ratnapura district), *C. aromatica* (Erathna, Rathnapura), *C. longa* (Kahathuduwa, Colombo district), *C. oligantha* (Hebarawa, Badulla district), *C. zedoaria* (Gonapola, Colombo district). Voucher specimens of the plants were authenticated and deposited in the National Herbarium, Peradeniya, Sri Lanka, and Herbal Technology Section, Industrial Technology Institute for future reference. Phytochemicals in essential oil extracted from Clevenger's apparatus of five *Curcuma* species were analyzed by GC-MS. 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 ^{o}C (after 10.00 min) to 240 ^{o}C at 5 ^{o}C/min with helium as carrier gas. Identification of constituents was done by matching 1700 eV mass spectra, 250 ^{o}C quad temperature, 250 ^{o}C source temperature, 50 - 450 (amu) scan parameters, and matching with NIST library. The fragmentation pattern and the retention time were compared with the standards to confirm the presence of a particular phytochemical. Sampling and analysis were done according to WHO guidelines (WHO 2012).

2.2 Statistical Analysis

Statistical tests were performed using Minitab 17. Multivariate test was used to determine the complex relationship among variables (Amel 2015). 164 phytochemicals were analyzed by simple correspondence analysis and by cluster variable method. But trace elements were discarded. Statistical analysis examined the relationships between the 5 species and the associations between variables in two dimensions, and similar phytochemical contents were identified from their positions as described in Greenacre (1983).

3 Results and Discussion

Phytochemicals detected in the extracts prepared from *Curcuma albiflora*, *C. aromatica*, *C. longa*, *C. oligantha*, and *C. zedoaria* are summarized in Table 1.

Table 1: Chemical profiles of five *Curcuma* species grown in Sri Lanka (AF: *Curcuma albiflora,* AR: *C. aromatica*, CL: *C. longa*, CO: *C. oligantha*, CZ: *C. zedoaria).*

Compound	**Area%**				
	AF	AR	CL	CO	CZ
alpha ylangene	0.47	0	0	0	0
Guaia-1(10),11-diene	1.07	0	0	0	0
myrtenal	0	0	0.1	0	0
o-Cymene	0.2	0	0	0	0
p-cymene	0	0	13.3	0	0
thymol	0	0	0.2	0	0
(-)-Alcanfor	5.12	0	0	0	0
(-)-Myrtenol	0.81	0	0	0	0
(-)-Spathulenol	0	0	0	1.97	0

Table 1. Continued.

Compound	AF	AR	CL	CO	CZ
(1R)-(+)-Nopinon	0.2	0	0	0	0
(E)-bocimene	0	0	0.3	0	0
(E)-nerolidol	0	0	0.5	0	0
(E)-p-famesene	0	0	0.2	0	0
(Z)-p-ocimene	0	0	0.1	0	0
1- bisabolone	0	1.5	0	0	0
1(10),4-furanodien-6-one	0	0	0	0	0
2(10)-Pinen-3-ol	0.75	0	0	0	0
2-heptanol	0	1	0	0	0
2-octanol	0	0	0	0	0
2-Tridecanone	0.15	0	0	0	0
3-carene	0	0	0.9	0	0
6 - Camphenol	0.12	0	0	0	0
8-tunnerone	0	0	0	0	0
á-copaene	0.85	0	0	0	0
a-curcumene	0	0	0.2	0	0
a-humulene	0	0	0.2	0	0
á-Linalool	0.69	0	0	0	0
alpha- Bourbonene	0	0	0	0.06	0
alpha Copaene	0	0	0	1.89	0
alpha Terpineol	0	0	0.14	0	0
alpha-Bisabolene epoxide	0	0	0	0	0
alpha-Famesene	4.01	0	0	0	0
á-Myrcene	0	0	0	0	0
Andrographolide	0	0	0	0.07	0
a-phellandrene	0	0	18.2	0	0
a-pinene	14.5	0.3	2.6	0	0.4
ar-curcurnene	0	3.1	0	0	0
Aromadendrene oxide-(1)	4.81	0	0	0.38	0
ar-turmerol	0	0.4	0.2	0	0
ar-turnerone	0	6.3	0.1	0	0
Asarone	0	0	0	0.98	0
a-terpinene	0	0	0.4	0	0
a-terpineol	0.64	0.6	0.9	0	0
a-thujene	0	0	0.1	0	0
a-turmerone	0	6.7	0.3	0	0
Azulene	0	0	0	0.37	0
bisabolene	0	0	0.2	0	0
B-elernene	0	1.4	0	0	0
B-sesquiphellandrene	0	1.7	0	0	0
carnphene	0	0.7	0	0	0
Caryophyllene	2.57	0	0	15.07	0
Caryophyllene oxide	9.35	0	0.4	5.82	0

Table 1. Continued.

Compound	AF	AR	CL	CO	CZ
cawacrol	0	0	0.1	0	0
ç-Elemene	0	0	0	6.11	0
cineole	0	0	14.6	0	0
Cinnamal	0	0	0.4	0	0
cis-&elernenone	0	0.2	0	0	0
cis-sabinol	0	0	1	0	0
cis-Verbenol	0.28	0	0	0	0
cubedol	0	0	0	0	0
curcuphenol	0	0.2	0	0	0
curdione	2.83	0	0.5	0	0
curzerene	0	0.2	0	0	0
curzerenone	0	11	0	0	0
de hydro-p-cymene	0	0	0.1	0	0
Denderalasin	0.6	0	0	0	0
d-Mannose	0	0	0	0.08	0
Doconexent	0.5	0	0	0	0
Elemene	0	0	0	1.86	0
elemicin	0	0	0.2	0	0
endo-Borneol	1.44	0	0	0	0
epicurzerenone	0	0	0	0	0
Eremophila-1(10),11-diene	0.91	0	0	0	0
Eucalyptol	2.21	0	0	0	0
Falcarinol	0.13	0	0	0.13	0
Gamolenic Acid	0	0	0	0.1	0
gerrnacrone	0	0.5	0	0	0
Humulene	0	0	0	8.24	0
Ionone	0	0	0	0.19	0
Isoaromadendrene epoxide	0.69	0	0	0	0
Ledene oxide-(II)	2.11	0	0	0.52	0
limonene	0	1	3.3	0	0
linalool	0	0.2	1.2	0	0
myrcene	0	0	1.8	0	0
Myrtanal	0.11	0	0	0	0
myrtenol	0	0	0	0	0
Naphthalene	0	0	0	0.78	0
Neocurdione	0.87	0	0	0	0
n-Hexadecanoic acid	4.74	0	0	0	0
Patchoulane	0.16	0	0	0	0
p-bisabolene	0	1.8	0	0	0
p-caryophyllene	0	0.3	0.5	0	0
p-cymen-8-01	0	0	2.4	0	0
Phytol	0.99	0	0	13.38	0
Pinocarvone	0.46	0	0	0	0

Table 1. Continued.

Compound	AF	AR	CL	CO	CZ
ppinene	0	0	7.2	0	0
p-pinene	0	0.4	0	0	0
p-sesquiphellandrene	0	0	0.2	0	0
p-turrnerone(curlone)	0	0.9	0	0	0
myrcene	0	0.2	0	0	0
terpinen-4-01	0.25	0.5	0.8	1.82	0
terpinolene	0	0	11.6	0	0
trans-á-Ionone	0	0	0	0.2	0
trans-Carvone oxide	0.11	0	0	0	0
trans-Geranylacetone	0.15	0	0	0	0
T-rnuurolol	0	0.1	0	0	0
y-terpinene	0	0	1	0	0
zingiberene	0	0.8	0.5	0	0
2-Undecanone	0	0	0	0	0.02
Verbenone	0	0	0	0	0.03
1-Carvon	0	0	0	0	0.04
Carvon	0	0	0	0	0.05
Pichtosine	0	0	0	0	0.05
1 Phelendrine	0	0	0	0	0.07
Cyclene	0	0	0	0	0.08
beta Myrcene	0	0	0	0	0.15
Carbinol	0	0	0	0	0.15
2-Nonanone	0	0	0	0	0.27
2-Nonanol	0	0.5	0	0	0.29
Phenyldihydropyran	0	0	0	0	0.31
Linderazulene	0	0	0	0	0.32
Terpineol	0	0	0	0	0.33
beta Pinene	0	0	0	0	0.41
1-Limonene	0	0	0	0	0.54
dl-Limonene	0	0	0	0	0.61
Sabinene	0	0	0.4	0	0.61
1 ,8-cineole	0	5.5	0	0	1.01
delta Elemene	0	0	0	0	1.05
Borneol	0	1.1	0.3	0	1.69
Camphene	3.64	0	0	0	1.87
beta Elemene	0	0	0	0	1.98
Germacrene B	0	0.3	0	0	3.98
Isoborneol	3.46	3.4	0	0	4.04
beta Eudesmene	0	0	0	0	4.97
Germacrone	0	0	0.2	0	5.17
Benzofuran	0	0	0	0	8.84
Aromadendrene	0	0	0	0.19	11.75
Camphor	0	32.3	0	0	11.82
Debromofiliforminol	0	0	0	0	31.46

Among chemicals in *C. albiflora*, alpha-pinene (14.5%), caryophyllene oxide (9.35%), and alconfor (5.12%) have been found as major compounds. Caryophyllene (15.07%), humulene (8.24%), and phytol (13.38) were found as major compounds in *C. oligantha.* Camphor (32.3%), debromofiliforminol (31.46%), and alpha-phellendrene (18.2%) were found as major compounds in *C. longa, C. zedoaria*, and *C. aromatica* essential oil.

By cluster varibale analysis of phytochemicals present in five speces, mainly two subgroups were clustered; *C. albiflora* and *C. oligantha* into one and *C. aromatica* and *C. zedoaria* into another. These two subgroups are separated from *C. longa* (Figure 1).

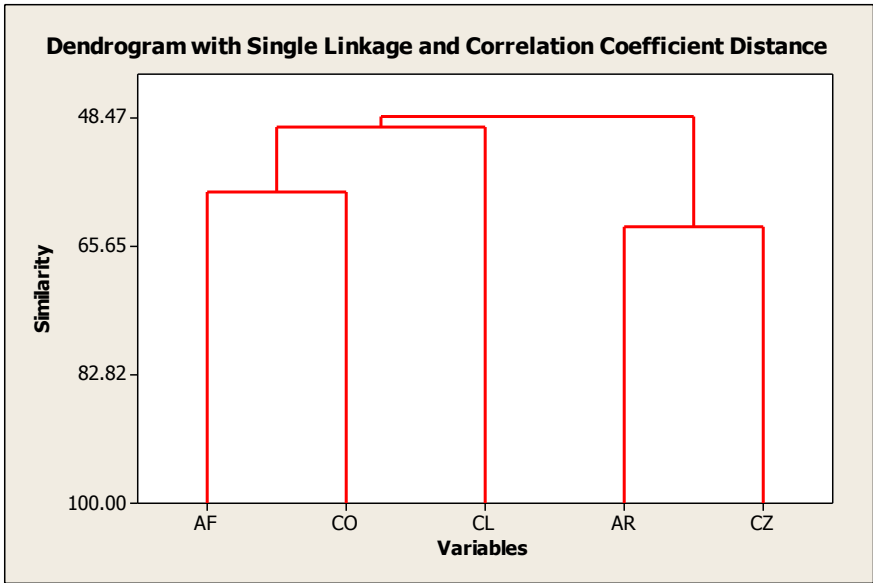

Fig. 1: **Cluster variable analysis of dendrogram obtained from phytochemicals present in five *Curcuma* species in Sri Lanka** (AF: *C. albiflora*, AR: *C. aromatica*, CL: *C. longa*, CO: *C. oligantha*, CZ: *C. zedoaria).*

This is in agreement with the seperation of *Curcuma* species according to morphological characters explained by Dassanayake (1983). Symmetric row and column plot and column plot from Simple Correspondence Analysis showed comparable results (Figure 2).

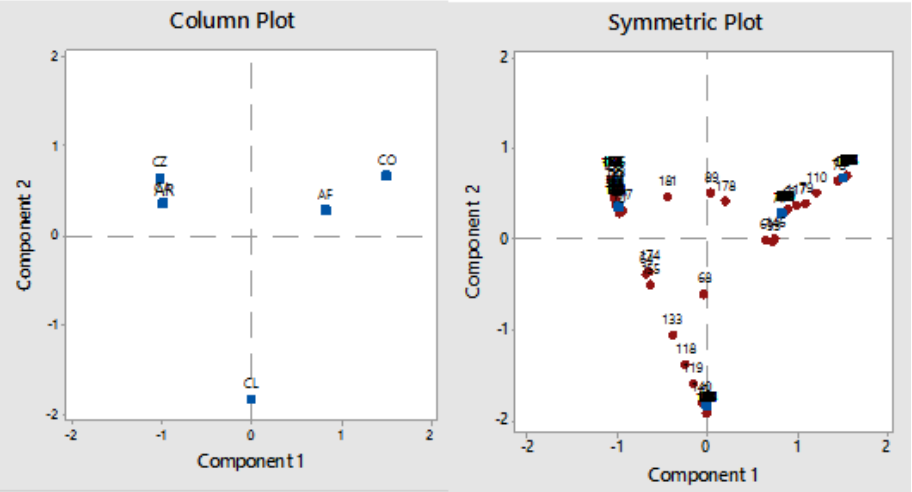

Fig. 2: **Column and symmetric plot obtained from phytochemicals present in five *Curcuma* species in Sri Lanka** (AF: *C. albiflora*, AR: *C. aromatica*, CL: *C. longa*, CO: *C. oligantha*, CZ: *C. zedoaria).*

The present study has identified chemical markers present in all *Curcuma* species grown in Sri Lanka. However, collection of more samples of same species from different locations is needed to be analyzed for the establishment of chemo-taxonomical identity and distribution pattern by multivariate test.

4 Conclusion

Cluster variable method and simple correspondence analysis enabled the clustering of *Curcuma* species grown in Sri Lanka into two major groups based on their phytochemical composition. The chemo-taxonomy of *Curcuma* plants grown in Sri Lanka could be established with the extension of number samples.

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