Original Research Article

Karyotype and Phytochemical Analysis of Four Species of the *Zingiberaceae* Family and Their Taxonomic Relevance In Systematic Botany

Abstract

Zingiberaceae consists of herbaceous plants that grow in humid tropical and subtropical regions. Economically, they are used as spices and flavoring agents, dyes and ornamental plants. This study assessed the kryotype and phytochemical compositions of four species of *Zingiberaceae* family to establish their taxonomic relationships for systematic consideration. Mitotic studies were carried out by cutting actively tender root tips developing from the plant's rhizomes. The karyotype analysis of the investigated *Zingiberaceae* specieswere based on chromosome pair, type and length, while the phytochemicals of the samples methanolic extracts were identified using gas chromatography mass spectrometry (GCMS). The results of the karyotype analysis showed that different chromosome pairs and total length of the plant species showed different types of chromosomes like *Z.officinale* indicated (3 submetacentric, 1 metacentric and 3 subtelocentric); *E. cadamomum* indicated (3 submetacentric, 2 metacentric and 2 subtelocentric); *C. longa* indicated 4 subtelocentric and 3 submetacentric) while *A. meleguata* showed 2 submetacentric, 4 subtelocentric and 1 metacentric). Phytochemicals identified from the methanolic extraction of the four testsamplesusing GC-MS analysis showed the presence of twelve (12) compounds on *Zingiber officinale*, twentyone (21) compounds on *Elettaria cardamomum*, seventeen (17) compounds on *Cucuma longa* and thirteen (13) compounds on *Aframomum melegueta*. The identified phytochemicals include Zingeron, Oleic acids, Eucalypto, aR-Tumerol, gama-Sistosterol among others which are used in different pharmaceutical applications. The four selected *zingibereceae* plants exhibited similarities in their phytochemical class by having terpenes and fatty acids. These findings suggest that phytochemical compositions as well as karyotype specifications of plant species should be a considering factor in taxonomy classification of plants families. However, there is the need for more taxonomic studies using other lines of taxonomic studies.

Keywords: kryotype; Phytochemical; *Zingiberaceae*; Taxonomic; chromosomes; Botany.

1. Introduction

Diversity in the plant kingdom has made it imperative to classify plants so as to logically organize and communicate information which helps identify unknown species by comparing them with known species as well as classify species of the same family. This is important because plants are used in diverse ways ranging from ornamental, therapeutic, dietary as well as medicinal purposes depending on their bioactive composition, structure, etc. Plant taxonomy allows the grouping of plants based on certain characteristics such as structure, leaf type and retention, habit, climatic adaptation, chromosome numbers, morphology and composition etc. The chromosomes of different types of plants or even plants of same species vary. Karyotype analysis which provides information on chromosome number, size, shape and arrangement is a basic cytogenetic tool that helps to define taxonomic relationship between plant species, genera or family (Soliman, 2002). Karyotype analysis has been considered as a reliable guide in studies of taxonomic and evolutionary relationships (Bennet, 1987). It is a useful tool in plant taxonomy that provides insights in plant species.

Plant species produce different types of natural products of which the biosynthetic pathways of producing these compounds differ from one taxon to another. A phytochemical analysis of plants which provides chemical markers that help classify these plants becomes very relevant in taxonomic systems. Information from phytochemical analysis that include phenolic constituents, alkaloids, terpenoids, free amino acids, fats and oils, cyanogenic compounds, proteins, DNA, and RNA most often helps in plant species classification.Use of various cytological and molecular markers are becoming more popular in the past two decades due to their accuracy and the fact that unlike morphological markers, they are not prone to environmental influences (Bennet & Smith, 2011). molecular markers are broadly applied for identification, population studies, phylogenetic evaluation and genetic linkage mapping in many plant species (Williams, et al., 1990)

Zingiberaceae represents moderately sized family of monocotyledon which comprises of approximately 1,400 species in more than 50 genera (Rachkeree., et al 2018). *Zingiberaceae* consists of herbaceous perennial plants that grow well in humid tropical and subtropical areas (Ama., et al 2019). Almost all parts of *Zingiberaceae* plants are used by mankind as a source of food (spices and flavoring agents), in traditional medicine and to produce natural dyes (Burkill., et al 1966). Due to the fact that discrepancies have been observed in the number of genera and

species in *Zingiberaceae*, coupled with lack of phylogenetic relationships among them, there is continuous addition of new species among them. The evidence is clear that the family is in active state of evolution and relationship between several newly described genera and species are yet to be established. Therefore, the confusion and controversies over the phylogenetic relationship among some species in the family *Zingiberaceae* needs an immediate attention hence, the need for taxonomic studies and re-characterization of the four species of the family *Zingiberaceae* namely; *Z. officinale*, C. longa, A. *melegueta, and E. cardamomum,* investigated in this study.

2. MATERIALS AND METHODS

2.1. Study Locations

The laboratory studies were carried out at International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria. Ibadan is located between latitude 7^{o} 23' 16" N and longitude 3^{o} 53' 47" E as stated by Nigerian Meteorological Agency (NiMet, 2014). Herbarium preparations were carried out in the Laboratory of Plant Science and Biotechnology, Imo State University, Owerri. According to NiMet, (2014) report, Owerri is located between latitude 5^{o} 10' N and 6^{o} 0' N and longitude 6^{o} 35' N and 7^{o} 0' E South East Nigeria.

2.2. Specimen Collection

The herbarium specimens and living plants were collected from various parts of Imo State. More than ten (10) samples per species were collected for analysis. Preparation of herbarium specimens was followedusing standard herbarium collection method (Larsen & Jenjittikul, 2001). Aseptic polythene bags were used during the specimen collection to avoid damages on specimens during field collections. The flowers were preserved in specimen bottles with 70% alcohol concentration. Voucher specimens was prepared, authenticated, numbered and deposited at Imo state university Owerri with voucher numbers IMSUH 1025 to 1028 for references.

2.3. Specimen Identification

The identification of specimens was done by Prof. F. N. Mbagwu, a Plant taxonomist in the Department of Plant Science and Biotechnology, Imo State University, Owerri and authenticated at Forest Herbarium Institute Ibadan, using identification keys and matching herbarium samples.

2.4. Karotype Analysis

Karyotype formulae were based on at least nine high-quality mitotic metaphase spreads. The degree of karyotype asymmetry was estimated with Stebbins's method. Chromosome classifications were made by a standardized nomenclature. KaryoType software version 2.0, was used to account for the karyological parameters automatically Statistical analysis was conducted using JMP Pro.14.1 software (SAS Institute Inc., Cary, NC, United States). The lengths of the chromosomes in the ideograms were based on the calculated mean values (Altinordu., et al 2016).

2,5, GC-MS Analysis

The GC-MS analysis was done at Zaria, kaduna state Nigeria. The compounds in the sample were identified using agilent GC-MS (Agilent 19091-433HP, USA) coupled to a mass spectrophotometer. The initial column temperature was 35°C with a hold time of 3 minutes. The temperature was programmed to rise by 8°C/min with a final temperature of 280°C. In the process, 1µl of the sample was injected into the port and immediately vaporized and moved down the column with helium as the carrier gas with flow rate of 1 ml/min. The MS Spectrum was taken at 70 eV. The identification of the compounds was done by comparing the spectrum of unknown compounds with the spectrum of known compounds in NIST structural library (Ikpa & Tochukwu, 2024).

3. Results and discussion

3.1. Karyotype Result

The result of the karyotype analysis from the selected four species of *zingiberaceae* indicated that the chromosomes have subtelocentric, submetacentric and metacentric at different total length and pairs of chromosomes (Table 1). The result revealed chromosome types as indicated;*Aframomummelegueta*showed 2 metacentric (2.42±0.10 & 3.47±0.08), 1 metacentric (3.51±0.10), 4 subtelocentric (3.34±0.09; 4.71±0.09; 3.69±0.09 & 3.42±0.10), *Z. Officinale*showed 3 submetacentric (3.42±0.07; 6.05±0.05 & 4.66±0.07), 1 metacentric (5.80±0.05) and 3 subtelocentric (4.41±0.08, 2.42±0.08 &2.24±0.05), *ElettariaCardamomum*showed 3 submetacentric (2.53±0.04; 5.57±0.01 & 3.73±0.08), 2 metacentric (5,61±0.04 & 4.51±0.05) and 2 subtelocentric (2.53±0.07 &2.70±0.10) while C*ucuma longa L* showed 3 submetacentric 4

subtelocentric (4.22±0.60, 3.22±0.50, 4.11±0.70 & 3.51±0.80) and 3 submetacentric at (4.96±0.91; 6.20±0.10 & 3.51±0.80).The karyotype result suggests that the visual analysis of the chromosome of the selected species are closely related by having similar types of chromosomes with related length at different pairs of chromosomes of the test samples.

Table 1: Result of the karyotype analysis of the four selected species of zingibreaceae family

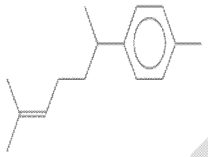
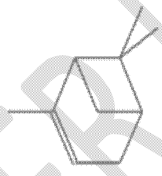
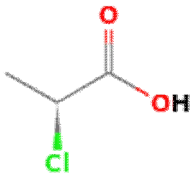
	Aframomum melegueta		*Cucuma Longa L*		*Elettaria Cardamomum*		*Zingiber Officinale*	
Chromosome pair	TL	TYPE	TL	TYPE	TL	TYPE	TL	TYPE
1	3.45 ± 0.07	Submet	5.57 ±0.01	Submet	4.22±0.60	Subtle	3.34±0.09	Subtle
2	4.41 ±0.08	subtel	4.46 ±0.01	Metal	3.22±0.50	Subtle	2.47±0.10	Submet
3	5.80 ±0.05	Metal	3.73 ±0.08	Submet	4.11±0.70	Subtle	3.67±0.08	Submet
4	6.05 ±0.05	submet	5.61 ±0.04	Metal	3.51±0.80	subtel	4.71±0.09	Subtle
5	4.55 ±0.07	Submet	4.51 ±0.05	Metal	4.98 ±0.91	Submet	3.69±0.09	Subtle
6	2.42 ±0.08	Subtle	2.53 ±0.04	Submet	6.20 ±0.10	Submet	3.51±0.10	Metal
7	2.24 ±0.05	subtel	2.70 ±0.10	Subtle	6.73 ±0.10	submet	3.42±0.10	subtel

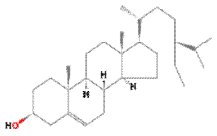
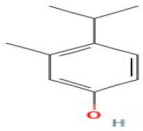
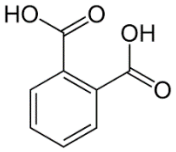
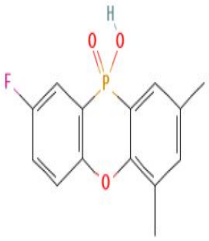
TL=Total length: Submet=Submetacentric: Subtle=Subtelocentric: metal=metalocentric

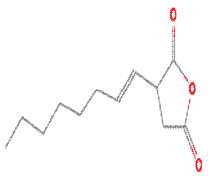
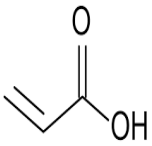
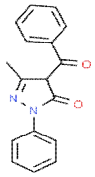
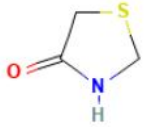
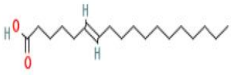
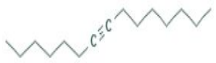
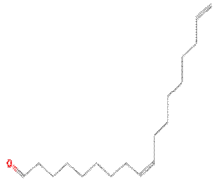
3.2.1. Phytochemical Result

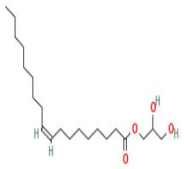
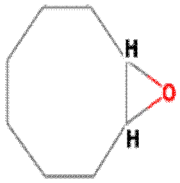
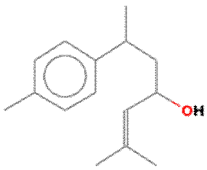
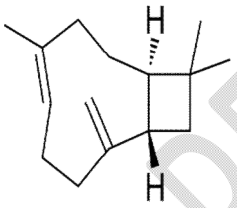
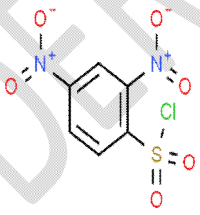
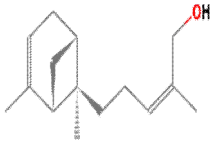
Phytochemicals identified from the methanolic extraction of the four test samples using GC-MS analysis showed the presence of twelve (12) compounds on *Z. officinale*, twenty one (21) compounds on *E. cadamomum*, seventeen (17) compounds on *C.longa* and thirteen (13) compounds on *A. meleguata*. The identified phytochemicals include Zingeron, Oleic acids, Eucalypto, aR-Tumerol, gama-Sistosterol among others which are used in different pharmaceutical applications.

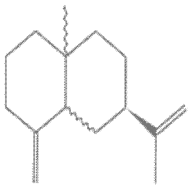
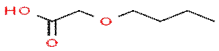
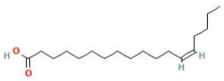
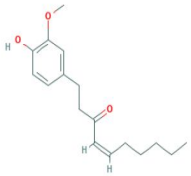
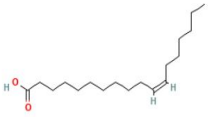
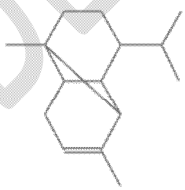
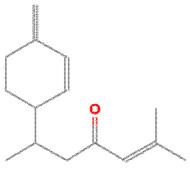
Table 2. The result of the merged GC-MS of the crude extract of the four *Zingiberaceae* species studied

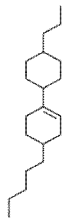
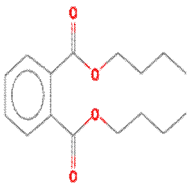
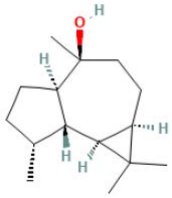
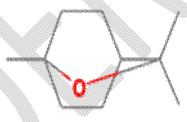
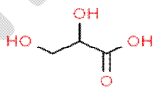
S/N	NAME OF COMPOUNDS	STRUCTURE	*A. melegueta*		*C. longa*		*E. cardamomum*		*Z. officinale*	
			RT	%	RT	%	RT	%	RT	%
1	α-Curcumene		-	-	7.089	1.08	-	-	-	-
2	α-pinene		-	-	-	-	17.621	12.12	-	-
3	(R*,R*)-5-Hydroxy-4-methyl-3-heptanone		-	-	-	-	11.877	22.87	-	-
4	(S)-(-)-2-Chloropropionic acid		-	-	18.592	0.61	-	-	-	-

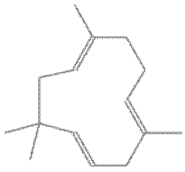
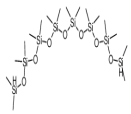
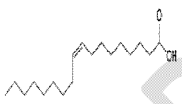
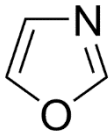
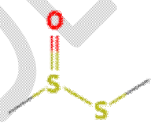
5	.beta.-Pinene		6.301	1.59	-	-	-	-	-	-
6	.gamma.-Sitosterol		-	-	-	-	-		31.623	0.05
7	O-Cymen		-	-	9.221	3.60	-	-	-	-
8	1,2-Benzenedicarboxylic acid		-	-	-	-	17.486	1.17	-	-
9	10H-Phenoxaphosphine, 8-fluoro-10-hydroxy-2,4-dimethyl-, 10-oxide		23.191	2.89	-	-	-	-	-	-
10	1H-Cycloprop[e]azulene decahydro-1,1,4,7-tetramethyl-, [1aR-(1a. alpha., 4beta., 4a. beta., 7. beta., b7a. beta., 7b.alpha.)]-		-	-	-	-	19.102	6.18	-	-

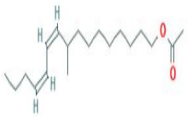
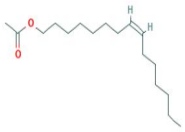
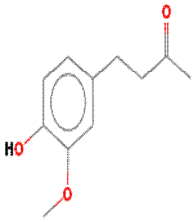
11	1-Octenylsuccinic anhydride		-	-	-	-	18.208	0.66	-	-
12	2-Propenoic acid		-	-	-	-	20.564	2.33	-	-
13	3H-Pyrazol-3-one, 4-benzoyl-2,4-dihydro-5-methyl-2-phenyl-		23.395	1.17	23.193	6.12	-	-	-	-
14	4-Thiazolidinone		-	-	-	-	23.91	1.54	-	-
15	5-Eicosene, (E)-		-	-	-	-	-	-	31.190	8.92
16	6-Octadecenoic acid		-	=	=	=	23.602	2.60	-	-
17	7-Pentadecyne		-	-	-	-	20.268	2.33		
18	9,12-Octadecadienoic acid (Z,Z)-		-	-	19.737	9.87	-	-	25.762	3.69
19	9,17-Octadecadienal, (Z)-		-	-	-	-	-	-	27.420	2.62

20	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester		-	-	-	-	-	-	29.961	1.90
21	9-Oxabicyclo[6.1.0] nonane, cis-		18.744	0.48	18.745	3.07	-	-	-	-
22	aR-Turmerol		-	-	19.308	28.37	-	-	-	-
23	b-Caryophyllene		17.530	6.80	-	-	-	-	-	-
24	Benzenesulfonyl chloride, 2,4-dinitro-		-	-	16.954	0.75	-	-	-	-
25	Bergamotol		-	-	-	-	14.435	6.31	-	-

26	B-selinine		15.405	1.10	-	-	-	-	-	-
27	Butoxyacetic acid		-	-	-	-	12.699	0.96	-	-
28	Carbonic acid, butyl dodecyl ester		-	-	15.404	1.47	18.800	5.41	-	-
29	cis-13-Octadecenoic acid		-	-	-	-	18.800	5.41	-	-
30	Cis-6-Shogaol		-	-	-	-	-	-	10.798	6.50
31	cis-Vaccenic acid		-	-	-	-	19.425	12.69	-	-
32	Copaene		19.307	30.89	17.403	2.20	-	-	-	-
33	Curlone		-	-	-	-	14.708	2.49	-	-

34	Cyclohexene, 4-pentyl-1-(4-propylcyclohexyl)-		20.120	12.19	-	-	-	-	-	-
35	Cyperene		-	-	-	-	-	-	28.006	7.53
36	Dibutyl phthalate		16.422	1.34	-	-	-	-	-	-
37	Dioctyl ether		-	-	-	-	14.527	1.70	-	-
38	Epiglobulol		19.742	17.44	5.777	3.82	-	-	-	-
39	Eucalyptol		-	-	9.849	1.92	-	-	-	-
40	Glyceric acid		-	-	-	-	23.110	1.24	-	-
41	Hentriacontane		-	-	-	-	-	-	29.734	6.22
42	Heptadecanoic acid, 10-methyl-,		-	-	18.983	0.58	-	-	-	

	methyl ester									
43	Hexadecanoic acid		-	-	-	-	16.982	2.94	-	-
44	Humulene		19.742	14.81	-	-	-	-	-	-
45	n-Hexadecanoic acid		17.400	1.93	17.533	9.01	-	-	-	-
46	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-		-	-	-	-	29.714	0.59	-	-
47	Oleic Acid		-	-	19.496	10.26	19.604	5.64	21.382	2.48
48	Oxazole, 5-hexyl-2,4-dimethyl-		-	-	-	-	15.474	2.39	-	-
49	S-Methyl methanethiosulfinate		-	-	18.412	0.61	-	-	-	-
50	Terpinolene		-	-	12.896	0.63	-	-	-	-
51	Tricosane		-	-	-	-	-	-	25.980	1.05

52	Z,Z-10,12-Hexadecadien-1-ol acetat		19.984	5.22	-	-	-	-	-	-
53	Z-8-Pentadecen-1-ol acetate		-	-	-	-	-	-	33.871	8.74
54	Zingerone		-	-	-	-	-	-	10.133	39.42

S/N=Serial number; RT=Retention time; %=Percentage area

3.2.2. Phytochemicals Classification

The test samples, *Aframomum melegueta, Cucuma longa, Elettaria cardamomum, and Zingiber officinale* can all be classified under the same zingibereceae family based on their phytochemical similarities that made them have similar therapeutic properties, which include anticancer, antibacterial, antifungal, antiviral, antihyperglycemic, analgesic, anti-inflammatory, antiparasitic and antihypertensive properties (Hartati,, et al 2014). Some of the identified phytochemicals such asn-Hexadecanoic acid, Epiglobulol, Copaene and 3H-Pyrazol-3-one, 4-benzoyl-2,4-dihydro-5-methyl-2-phenyl- revealed that there are closer relationship between *A. melegueta and C. longa*. These compounds probably are contributing factors to their medicinal properties. Moreso, the presence of oleic acid in *C. longa, E. cadamomum and Z. officinale* suggests a close relationship in the species which might be responsible to their close relationship in their medicinal properties.

Table 3: Phytochemical classification of the identified compounds from the four test samples (four Zingibereceae species studied)

S/N	Phytochemical Class	*Aframomum melegueta*	*Cucuma Longa L*	*Elettaria Cardamomum*	*Zingiber Officinale*
1	Terpenes	+	+	+	+
2	Fatty acids	+	+	+	+
3	Alkaloids	+	+	+	-
4	Heterocyclics	+	+	+	-
5	Hormones	-	+	-	+
6	Sulfurnates	-	+	-	-
7	Carboxylic acids	-	+	+	-
8	Phenolic compounds	-	-	-	+
9	Ethers	-	-	+	-
10	Ogano metal	-	-	+	-
11	Phthalates	+	-	-	-

+ = Present; - = Absent

The four selected *zingibereceae* plants exhibited similarities in their phytochemical class compositions by having terpenes and fatty acids.

Terpenes have many functions in plants such as a thermo-protectant, signaling functions, and not limited to, pigments, flavoring, and solvents but also have various medicinal uses (Yang et al., 2012).

Terpene is a natural compound with various medical properties and found in both plants and animals. Among natural products that mediate antagonistic and

beneficial interactions within the organism, terpene play a variety of roles. Terpene protects many living organisms like microorganisms, animals and plants from abiotic and biotic stresses (Gershenzon, 2007)related to the use of the rhizohmes of *Zingiberaceae* families like *C. longa and Z officinale* for the treatment wounds (Julung et al., 2023), and management of cancer (Julung et al., 2024). Terpene can ward off pathogens, predators, and competitors. Living organisms use terpene for multiple reasons like medicinal purposes and communications about food, mates, or enemies (Gershenzon, 2007). It is impressive how different organisms like different plant species of *Zingiberaceae*families use terpene for common purposes even though terpene contain many forms and varieties (Gershenzon, 2007). Fatty acids have been recorded as an important compound for cardiac functioning (Calder, 2015). The selected plants have been recorded to be good herbal agents for cardiovascular related health issues management, for instance; *A. melegueta* for treatment of young and elderly hypertensive patients (Lawal et al., 2018). *E cardamomum* for lowering gut modulation and hypertension (Anwarul et al., 2008), while *Z. officinale and C. longa* has modulatory effects on hypertension and hyperglycemia (Madkor et al., 2011).

Table 4: Similarities of phytochemical compounds in the four species of *Zingiberaceiae* investigated

S/N	Phytochemical	A. melegueta	C longa	E. cardomommum	Z. officinale
1.	Terpenes	+	+	+	+
2.	Fatty Acids	+	+	+	+

+ = Present; - = Absent

Table 5: Differences in phytochemical compounds of the four species of *Zingiberaceiae*investigated

S/N	Phytochemicals	*A. melegueta*	*C. longa*	*E. cardomomum*	*Z. officinale*
1.	Alkaloids	+	+	+	-

2.	Heterocydis	+	+	+	-
3.	Hormones	-	+	-	+
4.	Sulfurnates	-	+	-	+
5.	Carboxylic acids	-	+	+	-
6.	Phenolic compounds	-	-	-	+
7.	Esters	-	-	+	-
8.	Ogano metal	-	-	+	-
9.	Phthalates	+	-	-	-

+ = Present; - = Absent

Aframomum melegueta, Cucuma longa, Elettaria cardamomum, and Zingiber officinale can all be classified under the same zingibereceae family based on their phytochemical similarities like terpenes that made them similar therapeutic properties, which include anticancer, antibacterial, antifungal, antiviral, antihyperglycemic, analgesic, anti-inflammatory antiparasitic and antihypertensive effects (Cox-Georgian et al., 2019)Compared to other species, Zingiber species are unique phytoceuticals with strong therapeutic properties which probably were considered for placing them in one family of plant kingdom (Ghosh, 2014; Mustapha et al., 2017). These properties like protecting DNA damage and skin diseases (Khaki et al., 2009) probably may be due their similarities of having hormones in their phytochemical composition which is unique for only *C.longa and Z. officinale* among the four test samples.

The presence of organosilicon compound in E. *cardamomum* may be contributory to its aphrodisiac properties while the presence of phlatelets in *A. melegueta* can be attributed to the unique uses of *A. melegueta* to manage hypotension. Considering the higher concentration of terpenes and fatty acid in the phytochemical classification of the four test samples the bioactivities of these samples can be a considering factor in plants taxonomy classification.

Conclusion

Taxonomically, some phytochemicals identified among the four species of *zingiberaceae* studied revealed that they belong to a common ancestor. The presence of oleic acid in *C. long*a, *E. cardamomum* and *Z. officinale* indicating closer relationship than *E. cardamom* while the presence of terpenes and fatty acid placed the four samples in the same family.

Based on this, the species investigated showed taxonomic relationship with each other using their phytochemical position. The karyotype result which indicates similar type of chromosomes with related length at different pairs of chromosomes also suggests a taxonomic relationship of the selected species. Based on the findings in this work, the following recommendations are made:

1. Comprehensive cytogenetic of the plant species should be conducted.
2. The molecular characteristics of the plant species used in this study should be carried out in order to strengthen the taxonomic placement of these species.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

1. Authors have declared that no competing interests exist.

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