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Terpene-Rich Medicinal Plant Spices for Flavoring of Processed Tropical Food

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Abstract

Tropical medicinal plant spices have remained underutilized in commercial food processing because of the inadequate chemical characterization of their terpene compositions. The activity of medicinal plants used in flavoring is due to their terpene contents. Terpene contents of *Aframomum danielli* seeds, *Xylopia aethiopica* fruits, *Syzygium aromaticum* leaves, *Piper guineense* seeds, and *Monodora myristica* seeds were determined using headspace solid-phase microextraction combined with gas chromatography-mass spectrometry. *P. guineense* is rich in terpinene and terpinolene (2.5 g per 100 g), *S. aromaticum* has caryophyllene (0.68 g per 100 g); *A. danielli* is rich in ocimene (1.65 g per 100 g); *X. aethiopica* is rich in ocimene (2.94 g per 100 g); *M. mystristica* is rich in delta carene (0.49 g per 100 g). Combinations of the five medicinal plant spices are being applied as flavorings in packaged tropical food.

Keywords

γ-Terpinene, *β*-Caryophyllene, Ocimene, Delta Carene, African Rain Forest, Microextraction GC-MS

1. Introduction

Terpenes comprise the largest class of natural products with more than 30,000 compounds [1]. But tropical medicinal spices have remained underutilized in commercial food processing because of the inadequate chemical characterization of their terpene compositions. Spices are aromatic flavoring added to food to enhance organoleptic properties, making it attractive and palatable [2]. The uses of terpenes as flavor, food additives, vitamins, sweeteners, pharmaceuticals, medicines, and folklore spices in aromatherapy products have benefitted human-

kind [3]. The wider functions of terpenes in plant biology include light-harvesting pigments in photosynthesis, attraction of insect pollinators, defense against feedants and pathogens, and as mediators in the interactions of plants with other organisms. Although many plant species contain the terpene biochemical pathways [4], the diversity of the chemical structures of terpenes implies that individual groups of related terpenes with the desired biological activities are found only in a few plant families. Furthermore, the concentrations of the terpenes and phytochemicals may be so low in the plant tissue that their potency may be variable and low accordingly [5] due to the dependence of the growth of medicinal plants on environmental conditions (soil type, mineral nutrients, climate, time of harvest). Only a few studies have applied reliable analytical tools to determine the terpene compositions of tropical medicinal plants [6] [7]. The terpene compositions of each medicinal spice are unique taxonomical properties [3] that have been applied since ancient times for characterizing the ethnomedicinal application of each plant species [8]-[12]. Therefore, the aim of this study was to determine the concentrations of terpenes in medicinal plants that are used as spices in traditional cultures in the tropical rain forests of Africa so as to encourage their utilization as flavoring, sweeteners, additives, and supplements in commercial food processing industries.

Some botanical background of African medicinal spices: *Aframomum danielli* (alligator pepper) is a perennial herbaceous plant indigenous to West Africa. This spice plant of the family *Zingiberaceae* is also known as "Olima" in Igbo [13]. The seeds are smooth and shining olives. It is traditionally used to preserve food due to its bacteriostatic properties [14]. *Xylopia aesthiopica* is an evergreen tree of the family *Annonaceae*, found in the coastal rain forests of tropical Africa. It is known as Uda in Igbo, Eru in Yoruba, and Illa in Ibibio. Dry fruit is used as spice; but the roots, leaves, and bark are used in ethnomedicine for treatment of hemorrhoids, and jaundice, as anthelmintics, and as lactating aid [15] [16]. *Syzygium aromaticum* is a tree of the *Myrtaceae* family. The leaves and flowers are spices used for traditional food preservative and for medicinal purposes [17]; but the volatile oil from the buds is used as antiseptic [18]. *Piper guineense* (Uziza; Ashanti pepper), *Piperaceae* is a vine in the West African rain forest. The flowers are dioecious. The male flower has 2 - 3 stamens; the female flower has 3 - 5 stigmas. For identification, the fruits are prolate and semi-spherical, and retain their curved stalks like tadpoles. *Piper guineense* has anti-sickling phytochemicals [19]. *Monodora myristica* is a deciduous ornamental evergreen tree in the rain forest, the trunk can be up to 2 meters in diameter; the leaves are orchid-like; its flowers are scented. The seeds are embedded in white sweet-smelling pulp of a long fruit. The seeds are aromatic spicy, added as stimulant to herbal medicines [16].

2. Materials and Methods

2.1. Plant Materials

Aframomum danielli (Alligator paper) dry seeds, *Xylopia aethiopica* (Udah: Ig-

bo) dry fruits, *Syzygium aromaticum* (Clove) fresh leaves and flowers, *Piper guineense* (Uziza: Igbo) dry seeds, and *Monodora mystritica* (Ehu: Igbo; Abo lakoshe: Yoruba) dry seeds were sourced from indigenous Igbo, Yoruba, and Ashanti markets in West African rain forests, and identified by plant taxonomists from Imo State Polytechnic, Owerri; Imo State University, Owerri; and by ethnobotanists at Green Healthcare Foundation Botanical Gardens in Owerri West, Nigeria. Seeds were cleaned, briskly washed with distilled water, air dried and ground to powder with motar and pistle. Flowers and leaves were air-dried between filter papers, followed by grinding to powder with motar and pistle. Powders of plant materials were stored in the fridge before chemical analyses.

2.2. Chemical Analysis

Terpene content was determined by Anresco Laboratories, San Francisco using their proprietary Headspace GC-MS method for comprehensive terpene profiling in Cannabis in which plant material is homogenized with dry ice, and 20 mg is loaded into the head space vial cap and incubated; followed by injection into Shimadzu QP 2010 SE GC-MS. The software used for acquiring/processing the spectra was Shimadzu GC-MS solution Version 4.42. Quality control for the terpene concentrations was assured by performing four points standard curve of all 27 terpenes including the method blank.

3. Results and Discussion

Possible terpene-rich medicinal plants and spices (**Table 1**) that are culturally utilized in food preparation were selected based on orally-communicated African ethno-botanic traditional history, and from the literature [16]. The tissues of the plants that are normally used in food preparations were analyzed. The results (**Table 1**) showed that each plant species has a characteristic set level of a mix of terpenes that determine the aroma and taste imprint of the plant. *Piper guineese* (Igbo: Uziza) seed is characterized by its high accumulation of *γ*-terpinene (1.23 g per 100 g) and terpinolene (1.1 g per 100 g) which are known for their turpentine, citrus-like orange and lemon flavors [20]. *Syzygium aromaticum* (Igbo: Osaragbogo-eze) leaves and flowers have the highest accumulation of caryophyllene (0.68 g per 100 g) and ocimene 2 (0.14 g per 100 g) known for their peppery, spicy, and woody taste. Ocimene 2 (1.65 g per 100 g) is also the colorless liquid with a pleasant odor and the dominant terpene in the seeds of *Aframomum danielli* (alligator pepper), and *Xylopia aethiopica* (2.9 g per 100 g) used as dietary supplements and spices in the rain forests of Africa [16] [21]. The abundant terpene in *Monodora mystristica* (Igbo: Ehu) seed is delta 3-carene (0.49 g per 100 g) known for its penetrating sweet, earthy, lemon flavor used as a condiment for soup flavoring in West Africa [16]. The medicinal plant spices are being deployed in the flavoring of processed tropical food thereby increasing the farm income of the indigenous (limited resources) farmers who cultivate the crops. In addition to the rich terpene composition of the five aromatic medicinal

Table 1. Terpene profiles of tropical African medicinal plants and spices (More details are available under supplementary materials).

Terpenes	*P. guineense*	*S. aromaticum*	*A. danielli*	*X. aethiopica*	*M. mystristica*
			(mg per 100 g)		
1) *α*-Pinene	117	-0-	32	81	215
2) *β*-Pinene	129	19	131	112	11
3) Camphene	25	-0-	-0-	15	-0-
4) *β*-Myrcene	67	-0-	131	55	147
5) Δ 3-Carene	260	-0-	-0-	20	493
6) Limonene	144	12	41	82	100
7) *α*-Terpinene	104	-0-	-0-	9	2
8) Ocimene 1	41	-0-	-0-	10	31
9) Ocimene 2	51	142	1655	2936	43
10) p-Cymene	21	-0-	-0-	-0-	288
11) Eucalyptol	14	36	248	104	-0-
12) *γ*-Terpinene	1229	53	53	307	2
13) Terpinolene	1105	38	11	51	-0-
14) Linalool	53	-0-	-0-	68	62
15) Isopulegol	-0-	-0-	5	-0-	2
16) Menthol	-0-	-0-	-0-	36	-0-
17) (-)-Borneol	-0-	-0-	-0-	6	-0-
18) Terpineol	15	-0-	126	32	10
19) Citronellol	-0-	-0-	-0-	-0-	37
20) Caryophyllene oxide	-0-	16	-0-	-0-	-0-
21) *β*-Caryophyllene	411	682	28	23	5
22) *α*-Humelene	130	125	-0-	6	3
23) Nerolidol 1	18	6	-0-	-0-	6
24) Nerolidol 2	22	-0-	-0-	-0-	-0-
25) Guaiol	23	-0-	-0-	-0-	-0-

plants studied, each plant species has its suite of pharmacologically important compounds that have been documented in great detail [16], a discussion of which is outside the scope of this research project.

The conventional approach for terpene analysis involves a solvent extraction followed by gas chromatography-flame ionization detection (GC-FID) analysis. The Anresco instrument suite involved headspace solid phase microextraction (HS-SPME) combined with GC-MS for the quantitation of terpenes. HS-SPME brings several advancements over solvent extraction method because it avoids destruction of the plant sample, requires micro-quantities of plant sample, and avoids interference from co-extracted materials which are extremely variable in solubility in solvent. The micro-quantity sample amount effectively forms a single-phase gas system in the headspace vial at equilibrium, thereby making the

terpene quantitation spectra extraordinarily accurate [22].

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Conflicts of Interest

The authors declare no financial conflict of interests in the MOU research projects.

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