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Identification of high zerumbone content in volatile oils of shampoo ginger (*Zingiber zerumbet*) from Odisha, India

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ABSTRACT

Zingiber zerumbet (L.) Roscoe ex Smith (Family Zingiberaceae) is a rhizomatous aromatic perennial species widely distributed throughout Southeast Asian countries, mainly found in India, Bangladesh, Malaysia, Vietnam and Sri Lanka. The rhizomes have wide applications for traditional as well as medicinal purposes and are extensively used in the treatment of inflammation, diarrhea, stomach cramps, bacterial infections, fever, flatulence, allergies and poisoning. The paper presents botany, traditional medicinal uses and bio-chemical characterization of essential oil of underground rhizomes of *Z. zerumbet* germplasm maintained at NBPGR Base Centre, Cuttack. Rhizomes showed a high essential oil content of 1.27 to 1.96% on DWB. Detail oil composition analyzed by GC/FID and GC/MS identified elite germplasm of *Z. zerumbet* (IC-647593) having high zerumbone content as the most predominate sesquiterpenoid compound (>77.42%) present in the essential oil, confirmed over the years. Other major constituents present in the essential oil were α-humulene (5.65-6.71%), camphene (3.47- 4.10%), humulene oxide I (4.33-5.22%), humulene oxide II (5.12-5.71%), cayophyllene oxide (2.52-2.94%) and camphor (1.90-2.05%). Zerumbone, a monocyclic sesquiterpene compound,is of tremendous importance due to its antitumor activity. Our studies suggested that *Z. zerumbet* assembled from Odisha, Eastern India having high oil and zerumbone content can be a potential natural source of zerumbone which has a high pharmaceutical demand.

Key words: Essential oil, medicinal properties, rhizome, shampoo ginger, zerumbone

INTRODUCTION

Zingiber zerumbet (L) Smith, commonly known as pinecone, shampoo or bitter ginger, is a perennial herbaceous plant belonging to the Zingiberaceae family. It is mainly found wild or sometimes cultivated in Southeast Asian countries, tropical and sub-tropical regions around the world in damp and partial shady areas (Anon., 1976). The leaves and inflorescences of the plant crop up from a thick knobby rhizome that grows under the soil surface. All the plant parts of *Z. zerumbet* have wide applications for traditional as well as medicinal purposes. It is used for the treatment of cough, cold, stomachache, ear inflammation, tonsillitis, colic pain, diarrhea, and skin diseases. The underground rhizome of the plant has been extensively used as an ethno-medicine for the treatment of diarrhea, bacterial infections, fever, sore throat, stomach cramps, inflammation, allergies, poisoning, and flatulence (Yob et al., 2011). The leaves are used for the treatment of joint pain and mature inflorescence is used as a natural shampoo being rich in surfactants (Rout et al., 2011). The plant parts including stems and rhizomes also serve as a seasoning agent in foods, while the floral buds are consumed as a vegetable. The leaves in the form of the decoction are used in therapies for joint pain. The creamy substance present in the mature inflorescence or cone of the herb is rich insaponins and thus is used as a natural shampoo and a key ingredient in shampoo products (Nag et al., 2013). The presence of zerumbone as the major chemical constituent, along with sesquiterpenoids likeother phytochemicals of the plant rhizomes may be attributed to their ethno-medicinal values (Jiwajinda et al., 2002).

The study on the essential oil of *Z. zerumbet* has been carried out by many researchers, wherein a major constituent was found to be zerumbone (Srivastava et al., 2000; Rana et al., 2012, 2017; Padalia et al., 2018). The rhizome essential oil as well its major constituent, zerumbone were shown to possess various properties including anti-oxidant, anti-inflammatory, anti-diabetic, anti-tumor, antimicrobial, antiviral, anti-malarial, anti-allergic, anti-secretory, antipyretic, analgesic, cyclooxygenase-2 suppressant, and anti-proliferative (Murakami et al., 2002, Sakinah et al., 2007, Abdul et al., 2008, Singh et al., 2019, Tian et al., 2020). The objective of the present study was to explore the composition of essential oil of rhizome of *Z. zerumbet* assembled from Odisha, India.

MATERIALS AND METHODS

Plant material

During exploration mission for germplasm collection in December 2018, occurrence of *Zingiber zerumbet,* a wild ginger, locally called as "*Gada ada*", was noticed in damp and shady hill slopes in Malkangiri district, Odisha (Fig. 1A). The germplasm samples of matured rhizome propagules of plant species free from insect damage and disease symptoms and without any mechanical injury were collected from the collection site by selective sampling method. Further, the rhizomes were vegetatively propagated, maintained and the live plants bearing accession no. IC-647593 (collection number RCM/PK/BR/39) was conserved in field gene bank of ICAR-NBPGR Base Centre, Cuttack (Fig. 1B). The morphological features of vegetative and floral parts (Fig. 1B-C) were examined and the detailed taxonomic characters were described. The ethno-botanical uses collected from local tribes were documented. Rhizomes were harvested at full maturity in the month of April and evaluated for rhizome and essential oil yielding traits during the four consecutive years from 2019 to 2023 for studying the chemical composition of rhizomes (Fig. 1D).

Fig. 1. *Zingiber zerumbet.* A. Natural occurrence in Malkangiri district, Odisha. B. Live plant maintained at ICAR-NBPGR, Cuttack. C. Heads bearing flowers. D. Rhizomes extracted for biochemical evalution

Isolation and GC/MS analysis of essential oil

For essential oil isolation, fresh rhizomes were collected, washed, cut into smaller pieces and dried in the shade. 500 g of dried rhizomes were hydro-distilled in a Clevenger apparatus for six hours to extract oil. The duration of hydrodistillation was optimized so the essential oil was fully extracted. The distilled essential oil was collected in a clean glass vial which was then treated with anhydrous sodium sulphate to remove traces of water, if any present. The essential oil samples were stored at 4ºC until further analysis. The GC/MS analysis of the essential oil was analyzed by Agilent technologies gas chromatograph equipped with HP-5MS column of silica capillary (60 m × 0.25 mm i.d.; film thickness 0.25 µm) fused with MSD 5975C mass selective detector. Helium was a carrier gas with a flow rate of onemilliliters per minute and an ionization voltage of 70 eV. The initial oven temperature was held at 40°C for 2 min followed by 250°C with a rate of 5°C per minute and the final temperature was held at 300°C at a rate of 30°C per minute for 10 minutes. The scanning value ranged from 45 to 650 amu. The values of mass spectra of components were matched to NIST11/Willey/ Adams library mass spectral for compound identification. Kovat's index on the HP-5MS column was used for further confirmation of the compounds (Adams, 2007).

RESULTS AND DISCUSSION

Habitat

The plant species grew in a wild state on damp and shady hill slopesin moist deciduous forest of Chitrakonda block, Malkangiri district, Odisha. The species distribution was rare, found in isolated patches in moist habitats and the germplasm statustends to decline rapidly due to forest degradation and habitat loss.

Morphological description

Perennial herbs; stem leafy, 1.0-1.5 m high; rhizome aromatic, horizontal, whitish outside, pale-yellow inside. Leaves elliptic-oblong or elliptic-lanceolate, 8-12 each side, 25-40 × 4.0-9.0 cm, acuminate, glabrous or very thinly silky when young, midrib sulcate; ligule 1.0-2.5 cm, rounded. Inflorescence on separate pseudo-stem, spike cylindric or ellipsoid-oblong, long-peduncled, stout, 6-11 × 3.5-4.5 cm, sheathed by bracts; bracts glabrous to glabrescent, green in flower, turning red afterwards; bracts at the base of the spike ovate or paraboloid, 3.7 cm, cuspidate; flowering bracts closely imbricate, truncately suborbicular, apiculate, with hirsute margin, green during flowering; bracteoles hyaline, with rounded tip; peduncle clothed with sheathing bracts. Flowers pale yellow, 4.5 cm long, tubular-ventricose, longer than bracts, lasts for few hours. Calyx 1.8 cm long, sheathing. Corolla-tube slender, 1.8 cm, upper segment broader than the two others, others lanceolate, acute 3-nerved. Labellum with lateral lobes suborbicular, 1.6-1.8 cm diameter. (when spread out), thin,wrinkled, nearly as long as corolla, many-nerved, mid-lobe deeply emarginate or lobed, lateral lobes broad and rounded, three-fourths as long, wrinkled. Stamen 1.5-2.0 cm, filament short, connective broad, beak with incurved edges; ovary 3-celled, style filiform, stigma small, sub-globose, ciliate. Capsule thin-walled, glabrous. Seeds arillate, black. Flowering: July - September.

Breeding method

The breeding method has been adopted mainly by vegetative propagation of rhizome by asexual reproduction.

Ethno-botanical use by tribal community

The *Paraja* tribes of Malkangiri district, Odisha, use the rhizome juice with honey against chronic cough and asthma. Paste of rhizome with black peppers is used against stomach pain, acidity and intestinal worms. Rhizome-paste is also applied on affected parts of snakebite to neutralise venom. Also used for scabies and skin diseases by women community. The spikes including floral buds are also consumed as vegetables. The cone-shaped spikes are long-lasting and sometimes employed in craft displays for ornamental purposes during festivals.

Phyto-chemical contents

Phyto-chemical composition of dry mature rhizomes showedtotal phenol content of 5.58 ± 0.10 mg GAE per g DW: total flavonoid content of 18.19 ± 0.96 mg QE per g DW, total sugars content of 3.05 ± 0.01, tannin content of 2.23 ± 0.11%, ash content of 10.51 ± 2.30 and extractive yield of 16.43% ± 0.30 (Table 1). The rhizome essential oil content was found to be 0.58% on fresh weight basis and 1.27 to 1.96% on dry weight basis. Essential oil was evaluated using GC-FID and GC-MS techniques for oil composition. Altogether, sixteen constituents, representing 97.47 to 98.88% of the total oil composition were identified. The essential oil was mainly composed of sesquiterpenoids, represented by oxygenated sesquiterpenes (80.14-81.19%) and sesquiterpene hydrocarbons (7.02-7.69%), and followed by monoterpene hydrocarbons (4.29-5.70%) and oxygenated monoterpenes (2.46-2.71%). Essential oil profile of *Z. zerumbet* (IC 647593) showed presence of zerumbone as the most predominate sesquiterpenoid compound in the range of 70.60 to 82% after validating it over the five consequent years from 2019 to 2023 (74.41%, 70.60%, 80.67%, 79.40%, 82.0%) which is higher than the earlier reports from the same region (Dash et al., 2019). Other major constituents present in the essential oil were α-humulene (5.65-6.71%), camphene (3.47-4.10%), humulene oxide I (4.33-5.22%), humulene oxide II (5.12-5.71%), cayophyllene oxide (2.52-2.94%) and camphor (1.90-2.05%) as reported by Raina and Misra (2022). The predominance of zerumbone, humulene, humulene oxides, and β-caryophyllene identified in present studies agreed with the previous reports by other workers from India (Baby et al., 2009; Rana et al., 2012, 2017; Padalia et al., 2018; Rawat et al., 2023) and other countries (Dung et al., 1993; Bhuiyan et al., 2009; Tian et al., 2020). Zerumbone, is a monocyclic sesquiterpene compound which has gained tremendous pharmacological importance due to antitumor activity and prevents the apoptosis in cancer cells. Therefore, this elite germplasm can be a natural source of this compound and hence needs to be multiplied and cultivated for its utilization.

Table 1. Phytochemical evaluation of dry rhizomes of *Zingiber zerumbet*

Biochemical trait	Range	Mean±SD
Moisture (%)	5.19–7.42	6.31±1.58
Extractive yield (%)	16.22–16.64	16.43±0.30
Total phenols (mg g^{-1})	5.51–5.65	5.58±0.10
Total flavonoids (mg g^{-1})	17.51–18.87	18.19±0.96
Tannins (%)	2.16–2.31	2.23±0.11
Total sugars (%)	3.05–3.08	3.05±0.01
Ash (%)	8.88–12.13	10.51±2.30
Essential oil (%)	1.27–1.96	1.66±0.26
Zerumbone (%)	74.15–82.0	77.42±4.77

CONCLUSION

This germplasm *Zingiber zerumbet* (IC647593) from eastern India with significant essential oil yield and zerumbone content could be exploited as potential source for this phyto-molecule having widespread use in the food-flavor and pharmaceutical products. The diverse biological activities of zerumbone make it useful to develop naturally derived therapeutics.

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