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journal homepage: www.elsevier.com/locate/jepPsychoneuropharmacological activities and chemical composition of essential oil of fresh fruits of *Piper guineense* (Piperaceae) in mice

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ABSTRACT

Ethnopharmacological relevance: *Piper guineense* Schum & Thonn (Piperaceae) is a medicinal plant used in the Southern States of Nigeria to treat fever, mental disorders and febrile convulsions.

Aims of the study: This study aims at determining the chemical composition and the central nervous system (CNS) activities of the essential oil obtained from the plant's fresh fruits in order to rationalize its folkloric use.

Materials and methods: Essential oil of *P. guineense* (EOPG) obtained by hydrodistillation was analysed by GC/MS. EOPG (50–200 mg/kg, i.p.) was evaluated for behavioural, hypothermic, sedative, muscle relaxant, anti-psychotic and anticonvulsant activities using standard procedures.

Results and discussion: Analysis of the oil reveals 44 compounds of which 30 compounds constituting 84.7% were identified. The oil was characterized by sesquiterpenoids (64.4%) while only four monoterpenoids (21.3%) were found present in the oil. Major compounds identified were β -sesquiphellandrene (20.9%), linalool (6.1%), limonene (5.8%), Z- β -bisabolene (5.4%) and α -pinene (5.3%). The EOPG (50–200 mg/kg, i.p.) caused significant ($p < 0.01$) inhibition on rearing [$F_{(4,20)}=43$], locomotor [$F_{(4,20)}=22$] activity and decreased head dips in hole board [$F_{(4,20)}=7$] indicating CNS depressant effect; decreased rectal temperature [$F_{(4,20)}=7-16$], signifying hypothermic activity; decreased ketamine-induced sleep latency [$F_{(4,20)}=7.8$] and prolonged total sleeping time [$F_{(4,20)}=8.8$], indicating sedative effect; reduced muscular tone on the hind-limb grip test [$F_{(4,20)}=22$], inclined board [$F_{(4,20)}=4-49$] and rota rod [$F_{(4,20)}=13-106$], implying muscle relaxant activity; induced catalepsy [$F_{(4,20)}=47-136$], inhibited apomorphine-induced climbing behaviour [$F_{(4,20)}=9$] and inhibited apomorphine-induced locomotor [$F_{(4,20)}=16$], suggesting anti-psychotic effect; and protected mice against pentylenetetrazole-induced convulsions, indicating anticonvulsant potential.

Conclusion: The most abundant component of the fresh fruits essential oil of *P. guineense* was β -sesquiphellandrene (20.9%); and the oil possesses CNS depressant, hypothermic, sedative, muscle relaxant, antipsychotic and anticonvulsant activities, thus providing scientific basis for its ethnomedicinal applications.

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1. Introduction

Screening of natural products with the aim of identifying new compounds and activities is on the increase lately. Medicinal plants provide a veritable source for discovery of new biological molecules

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which also provide plausible rationale and justification for traditional uses in several regions of the world (Da Silva et al., 2014).

Piper guineense Schum & Thonn (Piperaceae), commonly called West African Black Pepper, is an herbaceous climber commonly found in the African tropical forest zones, with hundreds species distributed in tropical regions of the world (Olonisakin et al., 2006). Locally in Nigeria, it is commonly known as *Uziza* and *Iyere* among the Igbos and Yorubas respectively. *P. guineense* has culinary, medicinal, cosmetic and insecticidal applications (Okwute, 1992; Arong et al., 2011).

In folkmedicine, it is used in the treatment of rheumatism and bronchitis (Sofowora, 2008), cough, stomach disorder, intestinal diseases

and gonorrhoea (Mensah et al., 2008; Sumathykutty et al., 1999), obstetrics and fertility enhancement in women (Mbongue et al., 2005; Udoh et al., 1999; Noumi et al., 1998), control of weight/obesity (Mba, 1994), the seeds as an aphrodisiac (Mbongue et al., 2005) and in the treatment of mental illness (Odugbemi, 2008).

P. guineense found within the same species and geographical area has been reported to vary in their chemical composition, for example, Ekundayo et al. (1988) showed myristicin, safrole, sarisan and elemicin as main compounds; Oyedeji et al. (2005) reported β -pinene, α -pinene and germacrene-B as major components; Olonisakin et al. (2006) indicated β -pinene, *D*-Limonene, caryophyllene and car-3-ene as most abundant constituents, while Oboh et al. (2013) reported β -pinene, α -pinene, 1,8-cineole and γ -terpinene as the major components of the plant fruit essential oil from this Nigerian spp. The variations in chemical constituents of this plant species could contribute to its divergent biological activities and this therefore necessitate evaluating its chemical composition along with pharmacological activities.

Biological studies attributed to the plant include antioxidant and anti-diabetic (Oboh et al., 2013; Etim et al., 2013), hypolipidemic and hypokalaemic (Nwaichi and Igbino, 2012), insecticidal (Adewoyin et al., 2006), anti-microbial (Oyedeji et al., 2005), sedative activity (Tankam and Ito, 2013) and anticonvulsant activity of aqueous extract of its seed (Abila et al., 1993). The acute toxicity study of the oil conducted in our laboratory indicates the LD₅₀ value of the oil to be 693 mg/kg, i.p. in mice (Unpublished data).

Some of the traditional uses of *P. guineense* are closely associated with the central nervous system; hence it becomes imperative to further evaluate the essential oil of its fresh fruits for possible central activities. In the present work, the essential oil of *P. guineense* extracted by hydrodistillation was evaluated in terms of CNS activities in experimental animals using standard procedures to possibly validate some of its ethnomedicinal uses. Furthermore, the chemical composition of this particular essential oil species was carried out to determine its chemotype.

2. Materials and methods

2.1. Plant identification and authentication

The fruits of *P. guineense* were authenticated by Mr. G. Ibhanebor, of the herbarium unit, Department of Botany, Obafemi Awolowo University (OAU) Ile-Ife, Nigeria. Voucher specimen comprising the leaves and fruits was deposited as IFE 16772.

2.2. Plant collection and extraction of the essential oil

Fresh fruits of *P. guineense* were purchased from the Central Market, Ondo, Ondo State in 2012. The fruits were commuted into smaller particles and subjected to hydrodistillation using a Clevenger-type apparatus (BP, 1988). The essential oil (EOPG) was dried over magnesium sulphate crystal and stored in an air tight bottle, and kept refrigerated until use. The oil was emulsified with Tween 80 prior to administration in all the tests with the final concentration of Tween being $\leq 5\%$ v/v. Tween 80 (5% v/v) was used as the negative group for all tests.

2.3. GC–MS analysis of the essential oil

Essential oil of *P. guineense* (EOPG) was analysed by GC/MS. The GC/MS analysis was carried out on the Hewlett–Packard Model 5971, GC/MS using a helium as gas carrier, 1 mL flow rate, 30 psi inlet pressure, and split ratio 1:30. Temperatures of the column were programmed from 35 to 180 °C at a rate of 4 °C/min, then heated at a rate of 10 °C/min from 180 to 250 °C/min. Mass spectra were

recorded from 30 to 450 m/z. Individual components were identified by matching their 70 eV mass spectra with those of the spectrometer database using Wiley L-built library as well as by comparison of the fragmentation pattern with those reported in the literature.

2.4. Pharmacological experiments

2.4.1. Materials and equipment

Metler Toledo balance (Switzerland), Plexiglas observation cage (25 × 25 × 30 cm³), digital thermometer, suspended iron rod (30 cm long, 0.3 cm diameter and 36 cm above the table), adjustable rectangular wooden inclined board (30 × 60 cm², 30°, 65°), and Rota-Rod machine (Ugo Basile Rota-Rod, Model 7650).

2.4.2. Drugs

Ketamine HCL (Alpha Pharm. Nig.), diazepam (Valium[®] Roche, Switzerland), pentylenetetrazole (Sigma, USA), strychnine (Sigma, Switzerland, MSDS), fluoxetine (FLUTEX[®] Medbios, India), normal saline (Unique Pharm. Nig. Ltd.), haloperidol HCL, Apomorphine HCL, normal saline (Unique Pharm. Nig. Ltd.), and other reagents were of analytical grade.

2.4.3. Laboratory animals

Adult male and female albino mice (VOM strain) 18–25 g were obtained from the Animal house, Department of Pharmacology, Faculty of Pharmacy, OAU, Ile-Ife. The animals were maintained on standard animal pellets and water *ad libitum*. The ethical clearance for this research was obtained through the Faculty Postgraduate Committee and all animal experiment was carried out in strict compliance with the National Institute of Health (NIH, 1985) as being implemented by the OAU Research Committee.

2.4.4. General experimental design

Animals were randomly selected into 5 groups ($n=5$). Group I serves as the negative control which received the vehicle (5% Tween 80, 10 ml/kg) only. Test groups II–IV were treated with the EOPG at doses of 50, 100 and 200 mg/kg respectively, while the positive control group received the appropriate standard drug. All treatments were by intraperitoneal (i.p.) route.

2.4.5. Effect of the EOPG on novelty-induced behaviours (NIB) in mice

The novelty induced behavioural effects scores of rearing and locomotion were performed according to Onigbogi et al. (2000) with minor modification. Each mouse was placed inside Plexiglas's cage and observed for rearing (20 min) and locomotive activity (20 min) after 30 min of pre-treatment. The floor of the cage was divided into 16 equal squares and the number of squares crossed with all the fore and hind limbs was counted as locomotion, while rearing was the number of times the animal places its fore paws against the wall of the cage or in the air. The positive control group V received diazepam (1 mg/kg, i.p.). Head-dips performed by the mouse were also counted for a period of 5 min (Takeda et al., 1998). Diazepam (1 mg/kg, i.p.) also serves as the standard treatment.

2.4.6. Effect of the EOPG on rectal temperature

The effect of the EOPG on body temperature of mice was determined using a thermo probe digital thermometer. The rectal temperature of each mouse was taken by inserting the probe 2 cm deep into the anus at time 0, 30, 60 and 120 min posttreatment. Mean \pm SEM were calculated for each treated group (Al-Naggar et al., 2003).

2.4.7. Effect of the EOPG on ketamine induced-hypnosis

The effect of the EOPG on ketamine-induced sleeping time was measured as described by Mimura et al. (1990). The animals were

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