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Communication

Volatile Chemical Constituents of *Piper aduncum* L and *Piper gibbilimbum* C. DC (Piperaceae) from Papua New Guinea

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Abstract: Exhaustive hydro-distillation of the leaves of *Piper aduncum* and fruits of *Piper gibbilimbum* (Piperaceae) afforded colorless and pale orange colored oils in 0.35 and 0.30 % yields, respectively. Detailed chemical analysis by GC/MS indicated the volatile constituents of *Piper aduncum* to be composed of dill apiole (43.3 %), β-caryophyllene (8.2 %), piperitione (6.7 %) and α-humulene (5.1 %), whilst the oil of *P. gibbilimbum* is dominated by the gibbilimbols A-D (74.2 %), with the remaining major constituents being the terpenes camphene (13.6 %) and α-pinene (6.5 %).

Keywords: *Piper aduncum*; *Piper gibbilimbum*; Piperaceae; dill apiole; gibbilimbol A-D.

Introduction

Piper aduncum is an exotic species in Papua New Guinea (PNG) that has been observed to invade many disturbed forest areas, reaching up to an altitude of about 2,000 meters above sea level [1]. It is a multistemmed evergreen shrub that can grow up to a height of about 6 m, has alternate leaves with short petioles and elliptic to lanceolate leaf blades that are about 12 - 20 cm long. All plant parts have a characteristic peppery odour. It has also been pointed out that *Piper aduncum* has the attributes of an

invasive species [1] and is thus now considered a major plant pest in almost all areas in PNG. The dried wood and branches are widely used for firewood, fencing and in temporary constructions, but easily rot away when subjected to long-term exposure to moisture.

Its usage has however been appreciated in many areas in medicinal applications. The people of Finschaffen in PNG use the extracts derived from this species as an antiseptic for wound healing [2], while other reports also indicate that the extracts are applied to treat diarrhoea in Peruvian ethnobotany [3] and as treatment for haemostasis and dysentery in Colombian ethnobotany [4]. 2',6'-Dihydroxy-4'-methoxychalcone, isolated from the inflorescence of *P. aduncum*, has been found to be selectively effective against *Leishmania amazonensis*, a disease described as having the potential to cause disfigurement and sometimes as fatal [5]. Furthermore, dill apiole, the major chemical constituent in *P. aduncum* was also found to impart 92 % mortality on mosquito larvae [6], suggesting the potential for its application as an agent for malarial vector control.

Piper gibbilimbum was recently described as being native to the island of New Guinea [7]. It is a widespread shrub, occasionally reaching up to a height of about 4 m. It is ecologically suited for growth in higher altitudes, usually between 1,000 m to 3,000 m above sea level. The leaves and fruits have an aromatic character. The medicinal use of the *P. gibbilimbum* in PNG as an antiseptic that heals abscess and skin ulceration as well as to treat fever has been reported [8]. The juices extracted from the heated barks are used on internal sores [9]. The leaves are also used as wrappers for cooked food and animal proteins in the Highlands societies of PNG.

Phytochemical studies of the compositions of these two species of *Piper* from PNG have been reported [10,13-17]. Four new cytotoxic alkenylphenol compounds, designated gibbilimbols A-D were obtained from *P. gibbilimbum* and their chemical structures established from spectroscopic evidence [10]. These compounds were found to be toxic to brine shrimps (*Artemia salina*) and cytotoxic towards nasopharyngeal carcinoma cells and strategies for their synthesis have been recently described [11, 12].

Phytochemical investigation of *P. aduncum* revealed a new amide, designated as aduncamide [13], three new dihydrochalcones, designated as piperaduncins A – C [14], five new prenylated *p*-hydroxy-benzoic acid derivatives [15], five new unusual monoterpene substituted dihydrochalcones, designated adunctins A – E [16], three new natural products derivatives of chromene and benzoic acids [17] as well as other known chemical compounds.

The volatile oil constituents of *P. gibbilimbum* and *P. aduncum* from PNG have not been documented in the literature, although those of *P. aduncum* from other geographical localities have been reported. Vila and co-workers [18] reported high sesquiterpene contents in Panama samples, with β-caryophyllene and aromadendrene being the major components, whilst Bolivian samples were composed predominantly of monoterpenes, with 1,8-cineole being the major component. In contrast, Maia and co-workers [19] reported high dill apiole contents (31.5 – 97.3 %) in samples obtained from the Amazon region. Malaysian and Fijian [20] samples were reported to contain 64.5 % and 58.0 % dill apiole, respectively, while samples from Cuba recorded 82.2 % composition [21]. By far the highest reported dill apiole contents in *Piper aduncum* were those from the Amazonian region, as reported by Maia and co-workers [19], with levels of 97.3 %. This observation further confirms an earlier postulation that chemical compositions can differ widely within the same species across different geographical locations [22].

Interestingly, comparative studies conducted on the feeding habits of caterpillars (Lepidoptera) on two exotic *Piper* species (*P. aduncum* and *P. umbellatum*) with the native host species of PNG indicated a higher preference for the two introduced species [23]. The higher assemblage and feeding preference as well as species diversity on *P. aduncum* is particularly interesting. Such assemblage, feeding preference and specificity of the caterpillars on specific host species could be correlated to the adaptability of the caterpillars to accumulate such high concentration of the toxic phytochemicals in their body organs. The accumulation of these toxic phytochemical substances may serve as a mechanism for chemical defence in these caterpillar species.

In an ongoing research program to study the essential oil composition of the endemic aromatic plant species of PNG, we report herein, a complete analysis of the essential oil distillates from the leaves of *P. aduncum* and fruits of *P. gibbilimbum* found in PNG.

Results and Discussion

The foliar oil distillates obtained from *P. aduncum* L and *P. gibbilimbum* C.DC (Piperaceae) afforded colorless and pale orange coloured oils in 0.35 and 0.30 % yields, respectively. The results of the GC/MS analysis of the volatile oils of the two *Piper* species are presented in Table 1. It is apparent from the data shown that the components of *P. aduncum* are mainly mono- and sesquiterpenes, dominated by dill apiole, whilst those of *P. gibbilimbum* are predominantly sesquiterpenes with the gibbilimbols being the dominant components.

In the analysis of the essential oil of *P. aduncum*, a total of 46 components were identified. The major component was identified as dill apiole or 4,5-dimethoxy-6-(2-propenyl)-1,3-benzo-dioxole (43.3 %), as confirmed by NMR, together with other minor components such as β-caryophyllene (8.3 %), piperitione (6.7 %) and α-humulene (5.1 %). These components accounted for 63.4 percent of the total oil composition while the other minor components made up the balance. The results obtained in this study for *P. aduncum* correlate with those of the published data for the chemical composition in the Amazon region, Malaysia, Fiji and Cuba [19-21]. The dill apiole content in *P. aduncum* from these localities are in agreement with each other while the chemical compositions for the Panama and Bolivia species have β-caryophyllene and 1,8-cineole as the main constituents respectively.

The essential oil derived from *P. gibbilimbum* is dominated by the gibbilimbols A-D (74.2 %) with the remaining constituents being the terpenes camphene (13.6 %) and α-pinene (6.5 %). The gibbilimbols were identified on the basis of their published mass spectral data [10].

Our analysis of the chemical constituents in the volatile oil constituents in the two species of *Piper* showed no correlation in their chemical composition, however, toxicity analyses of the chemical constituents indicate high lethality towards brine shrimps (*Artemia salina*) and molluscs. In spite of the toxicity of the chemical constituents found in the leaf extracts of the two species of *Piper* studied, it is interesting to note that *P. aduncum* is the highly preferred host for a number of caterpillar species [23]. Such selectivity in the assemblages and feeding preferences of the caterpillar may suggest that the accumulation of the phytochemicals, in particular dill apiole, derived from this species could serve as a chemical defence for the caterpillars to deter excessive predation.

Table 1. The essential oil components (% area) of *P. aduncum* and *P. gibbilimbum* (Piperaceae) and their corresponding retention indices (RI).

***P. aduncum* L (Piperaceae)**			***P. aduncum* L (Piperaceae)**		
Components	**RI**	**%**	**Components**	**RI**	**%**
α-thujene	940	0.2	δ-cadinene	1545	0.3
α-pinene	953	0.6	α-selinene	1546	0.5
myrcene	995	0.2	δ-cadinene	1556	1.2
β-pinene	1000	0.3	γ-cadinene	1558	0.6
α-phellandrene	1026	0.2	myristicin	1563	0.6
α-terpinene	1036	0.2	*cis*-calamene	1566	0.4
cis-β-ocimene	1043	0.8	nerolidol	1578	1.0
ρ-cymene	1046	1.3	spathulenol	1636	0.8
limonene	1050	0.8	caryophyllene oxide	1647	1.9
trans-β-ocimene	1056	2.5	viridiflorol	1658	1.5
γ-terpinene	1068	0.8	dill apiole	1662	43.3
α-terpinolene	1105	0.3	germacrenene-D-1,10-epoxide	1700	0.6
terpinen-4-ol	1212	1.8	τ-cadinol	1710	1.1
piperitione	1292	6.7			
α-copaene	1412	1.7	***P. gibbilimbum* C. DC (Piperaceae)**		
β-elemene	1420	0.4	α-pinene	952	6.5
β-caryophyllene	1468	8.2	camphene	972	13.6
calarene	1475	0.2	myrcene	995	0.3
aromadendrene	1486	0.2	ρ-cymene	1045	0.6
β-santalene	1492	0.3	limonene	1049	3.2
α-humulene	1505	5.1	terpinolene	1111	0.7
E,E-α-farnescene	1516	3.0	AR-curcumene	1505	0.5
germacrene-D	1529	2.5	gibbilimbol D	1789	46.0
α-amorphene	1535	0.4	gibbilimbol C	1805	19.2
α-muurolene	1537	0.6	gibbilimbol B	1997	7.7
β-selinene	1542	1.0	gibbilimbol A	2016	1.3

Conclusions

Analyses of the volatile oil constituents from the leaves and fruits of *Piper aduncum* and *Piper gibbilimbum* indicate dill apiole to be the main component of *P. aduncum,* while the main components found in *P. gibbilimbum* were prenylated phenolic compounds identified as gibbilimbols A-D. The fruit oil of *P. gibbilimbum* is the main natural source of the gibbilimbols A-D. This work provides the first report of the analysis of these two plant species from Papua New Guinea.

Experimental

Samples of *P. aduncum* were obtained from Isan village, Teptep in the Finnisterre mountain Ranges in the Morobe Province while the samples of *P. gibbilimbum* were collected from Mt. Giluwe, Ialibu, in the Southern Highlands Province. Voucher specimens of both species have been deposited at the

University of PNG herbarium in Port Moresby. The fresh plant leaves were subjected to exhaustive hydro-distillation for eight hours using a standard all glass distillation setup. The pure oils obtained were dried over anhydrous magnesium sulphate and the oil analyzed by gas chromatography-mass spectrometry (GC-MS) on an Agilent 6890 Gas Chromatograph, equipped with a split/splitless injector, a 7963 Mass Selective Detector (MSD). Chromatography was performed on a BPX-5 capillary column (50m x 0.22mm ID and 1μM film thickness – SGE, Melbourne) terminated at the MSD operating at: transfer temperature: 310^{o}C; ionization 70 eV, source temperature: 230^{o}C; quadrupole temperature 150^{o}C and scanning a mass range 35-550 m/z. The injector temperature was 250^{o}C and the carrier gas was helium at 23.10 psi and an average velocity of 28cm/sec to the MSD. The column oven was programmed as follows: initial temperature: 50 ^{o}C; initial time 1.0 min; program rate 4^{o}C/min; final temperature 300^{o}C; final time 10 min. The individual chemical constituents in the oil distillates were determined by comparing the retention indices, established libraries of the mass spectral data [25] or published literature citing the mass spectral information. NMR data for the confirmation of dill apiole in the essential oil from *P. aduncum* were recorded on a Bruker-DRX-500 (500 MHz) NMR spectrometer using $CDCl_3$ as solvent and operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C. 2D NMR was measured with standard pulse programs and acquisition parameters.

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