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Preliminary GC-MS analysis of two tropical bamboos (*Bambusa vulgaris* Schrad. ex J. C. Wendl. var. *vulgaris* Hort. and *Bambusa vulgaris* Schrad. ex J. C. Wendl. var. *vittata* A. et C. Rivière)

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Abstract: Tropical bamboo shoot solvent extract component identification by gas chromatography–mass spectrometry (GC/MS) is non-existing. To investigate and exploit their nutritional and biomedical potentials, extracts from air-dried young shoots from *Bambusa vulgaris* var. *vulgaris* (green bamboo) and *B. vulgaris* var. *vittata* (yellow variety) from Ghana were pre-treated by Soxhlet and Supersonic techniques using solvents with different polarities. Their chemical components were analyzed by GC-MS and compared with fractions from an extensively edible Asian variety (*Phyllostachys pubescens*). Octane, 2-methyl-Naphthalene and 1,1Bicyclohexyl were the main *n*-hexane Supersonic technique fractions identified from *B. vulgaris* var. *vulgaris* crown internode. Their retention times are 5.01, 19.86 and 20.75 mins., and 90 per cent, 90 per cent and 91 per cent match qualities respectively. The more polar methanol identified two chemicals, which are: p-xylene and 1,2-Benzenedicarboxylic acid-butyl 2-methylpropyl ester. Their retention times are 5.89 and 30.16 mins., but have greater match qualities (97% and 94% respectively) than the n-hexane fractions. *B. vulgaris* var. *vittata* is rich in 6,10,14-trimethylpentadecan-2-one from Soxhlet extraction with ether. Its retention time is 30.10 mins., and matching quality of 98 per cent. It has anti-microbial properties, while properties from *B. vulgaris* var. *vulgaris* fractions indicate they are generally unsafe for consumption. The major component identified in Supersonic ether extractions from *P. pubescens* is 3,7,11,15-tetramethyl-2-hexadecen-1-ol (phytol) at retention time of 28.62 mins., and match quality of 93 per cent. At present the tropical bamboos are not exploited as raw materials of biomedicine or vegetable. However, their local and worldwide edibility could be tapped out with further intensive researches.

Keywords: Bamboo extract, GC/MS, match quality, retention time, soxhlet extraction, supersonic technique.

INTRODUCTION

Bamboo shoots are delicious and are ideally utilized as vegetable worldwide, as they

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are toxic-free, low in fat, high in edible fibre and rich in mineral elements. According to the Indian Council of Forestry Research and Education, bamboo shoots, which are popular in Asian cuisine, are made up of 88.8 percent moisture, 3.9 percent protein, 0.5 percent fat, 5.7 percent carbohydrates and 1.1 minerals. The amino acid content of bamboo is higher than cabbage, carrot, onion and pumpkin. They also contain 17 different types of enzymes and more than 10 kinds of mineral elements, such as chromium, zinc, manganese, iron, magnesium, nickel and cobalt (Clarke-Brown, 2010). Moso bamboo is regarded as the most important bamboo species in China. In its native country, China, moso forests cover about 7.4 million acres and it is primarily grown for the edible shoots and its timber. Medically, it is a traditional Chinese herb in clearing fever and for detoxification. Recent research indicates the leaf extracts have additional activities, which include antioxidant, antiseptic, anti-bacterial effects, anti-cancer and anti-aging properties as well as other biological activities and physiological functions (Jiang Zehui, 2007).

However, for over the 1200 global bamboo species, several of them cannot be directly eaten. Bamboo utilization for food and other resources is linked with its chemical composition. The tropical African bamboos are hardly used for foodstuffs, pulp and paper production or as suitable construction materials since information regarding their properties are scant unlike those extensively investigated for their Asian counterparts. Thus, the popular moso bamboos (*Phyllostachys* spp.) are well known for their edible shoots in Indonesia and other Asian countries (Jian Zehui, 2007). Bamboo contains cellulose, hemi-cellulose and lignin (especially in the cell wall) as major constituents (Jiang, 2004). Other organic components include starch (2-6%), deoxidized saccharide (2%), fat (2-4%), and protein (0.8-6%); minor components include resins, tannins, waxes and inorganic salts, which make up ash and extractives (Li, 2004). The present work was carried out to analyze the essential chemicals in two widely known and abundant Ghanaian varieties (*Bambusa vulgaris* Schrad. ex J. C. Wendl. var. *vulgaris* Hort. [green bamboo] and *Bambusa vulgaris* Schrad. ex J. C. Wendl. var. *vittata* A. et C. Rivière [yellow bamboo]) using Gas Chromatography/ Mass Spectrometry (GC-MS). The results were compared with data from *P. pubescens*.

MATERIALS AND METHODS

GC-MS analysis of chemical compounds in two tropical bamboos and *P. pubescens*

Middle sections (1.5cm above and below) of the 4th and 12th internodes from the bases and crowns respectively of two representative culms of 8 month-old young shoots of green bamboo (*B. vulgaris* var. *vulgaris*) and the yellow variety (*B. vulgaris* var. *vittata*) (Fig. 1) were harvested in January, 2008 from strands in the Kumasi Metropolis, which is in the semi-deciduous forest of Ghana. The sections were dried to 10 per cent mc and individually ground by the Wiley Mill into powdered particles that passed through a No. 40 mesh sieve (425μm) but retained on a No. 60 mesh sieve (250μm). Leaves of Asian moso bamboo (*P. pubescens*) were similarly milled. Fine samples

Figure 1a *B. vulgaris vulgaris*

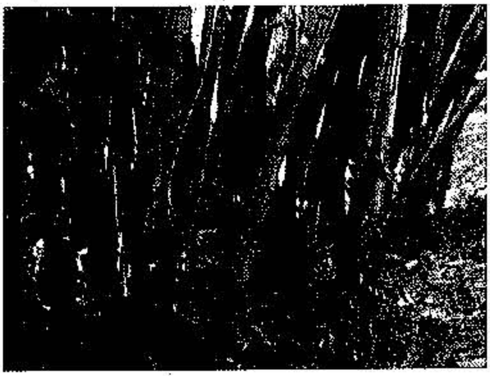
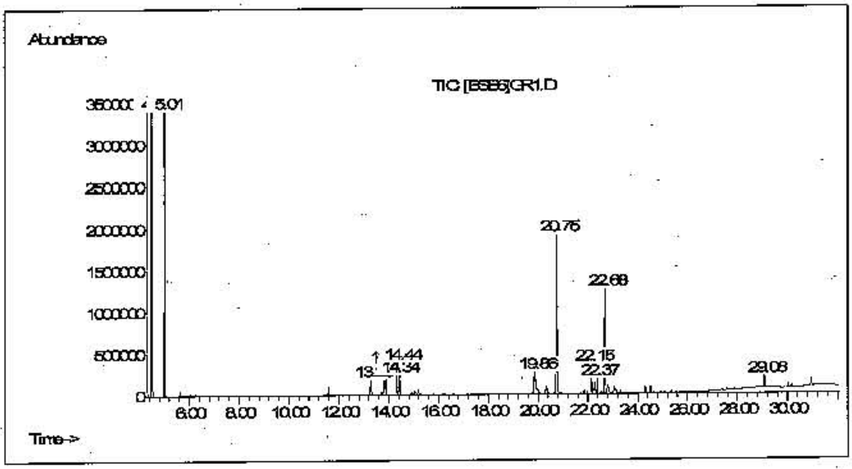
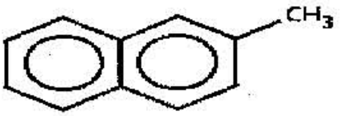


Figure 1b. *B. vulgaris "vittata"*

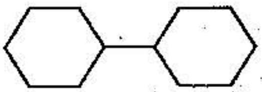


RT:5.01 Octane, match quality 90%
RT:19.86 2-methyl-Naphthalene, match quality 90%
RT:20.75 1,1'-Bicyclohexyl, match quality 91%
Non-polar Hexane: Green bamboo crown internode

Figure 2a. Spectrum of octane, 2-methyl-Naphthalene and 1,1'-Bicyclohexyl identified from the internode of green bamboo crown using GC-MS.



B. Chemical structure of 1,1'-Bicyclohexyl (Mol. formula: $C_{12}H_{22}$; Mol. wt: 166.3031).



A. Chemical structure 2-methyl-Naphthalene (Molecular formula: $C_{11}H_{10}$; Mol. wt: 142. 2).

Figure 2b. The chemical structures and molecular weights of 2-methyl-Naphthalene (A) and 1,1'-Bicyclohexyl (B)

(2g) of material granularity of 60-mesh from each bamboo were employed for the identification of the profiles of their chemicals using GC-MS. Two methods of extraction were conducted to pre-treat the samples: (a) Soxhlet extraction using ether as solvent at 6 cycles per hour for 6 h, (b) Supersonic extraction, which lasted for 60 min for each sample using three solvents of increasing polarity – n-Hexane, ether and methanol. Samples were kept in their respective solvents for 24 h before extraction. After extraction, contents from each sample were centrifuged (at 1200 rpm for 15mins.) and filtered. The extracts in the various solvents were prepared for the chemicals to be separated individually. Each sample (1μl) was injected into an Agilent 1100 Series GC-MS and the compound peaks identified by Chromatographs especially for chemicals with higher matching qualities in the bamboos. Their retention times and molecular weights were compared with compounds from valid standards. The structures of the components were determined on the basis of the spectroscopic techniques.

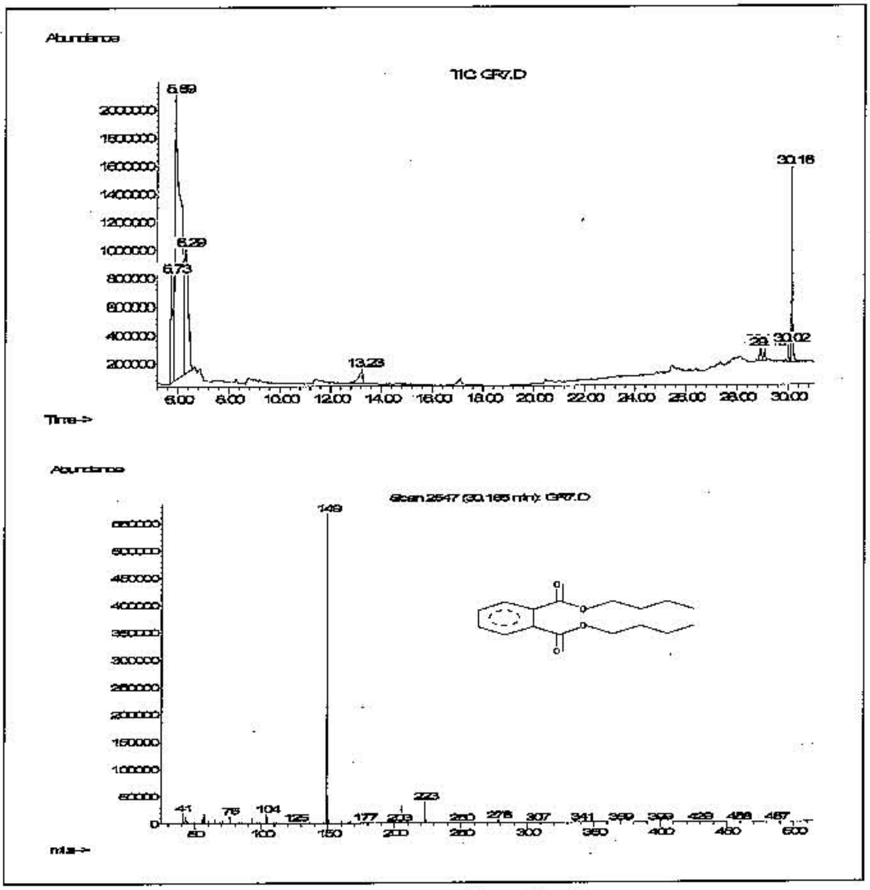
RESULTS

The main chemical components of tropical bamboos compared with those of moso bamboo and analyzed by GC-MS are summarized in Table 1. The detailed analyses of the bamboo components, their identified peaks, retention times and trends between

Table 1. Major chemicals identified from different parts of tropical (*B. vulgaris vulgaris* and *B. vulgaris vitata*) and Asian (*P. pubescens*) bamboos using GC-MS and solvents of different polarities.

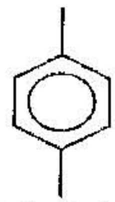
Species	Bamboo position	Chemical component present	Solvent; method used	Retention time (mins.)	Match quality (%)
B. vulgaris vulgaris (green bamboo)	Crown Internode	Octane	n-Hexane; Supersonic	5.01	90
		2-methyl-Naphthalene		19.86	90
		1,1Bicyclohexyl		20.75	91
		p-xylene		5.89	97
		1,2-Benzenedicar-boxylic acid-butyl 2-methylpropyl ester	Methanol; Supersonic	30.16	94
B. vulgaris 'vittata' (yellow bamboo)	Basal Internode	6,10,14-Trimethylpen-tadecan-2-one (phytone)	Ether; Soxhlet	30.10	98
P. pubescens (moso bamboo)	Leaves	3,7,11,15-tetramethyl-2-hexadecen-1-ol (phytol)	Ether; Supersonic	28.62	93

the species are described in chromatograms and spectra (Figs. 2-5). Octane, 2-methyl-Naphthalene and 1,1 Bicyclohexyl were the main *n*-hexane fractions identified from *B. vulgaris* var *vulgaris* crown internode using the Supersonic technique. Their respective retention times are 5.01, 19.86 and 20.75 mins., with matching qualities of 90 per cent, 90 per cent and 91 per cent (Fig. 2). However, from the more polar methanol two chemicals were identified: p-xylene and 1,2-Benzenedicarboxylic acid-butyl 2-methylpropyl ester. Their retention times are 5.89 and 30.16 mins., and matching qualities 97 per cent and 94 per cent respectively (Fig. 3). For *B. vulgaris* var. *vittata* the only chemical identified was 6,10,14-trimethylpentadecan-2-one from Soxhlet extraction with ether. Its retention time is 30.10 mins., and matching quality



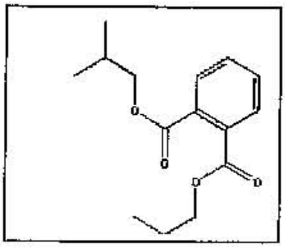
RT:5.89 p-Xylene, match quality 97
RT:30.16 1,2-Benzenedicarboxylic acid-butyl 2-methylpropyl ester, match quality 94.
POLAR METHANOL: *B. vulgaris vulgaris* crown internode.

Figure 3a. Chromatogram and spectrum of p-Xylene and 1,2-Benzenedicarboxylic acid-butyl 2-methylpropyl ester (TOP) and scan of the latter (BELOW) identified from the internode of *B. vulgaris vulgaris* crown using GC-MS.



The chemical structure of para-xylene
Molecular Formula: C_8H_{10}
Molecular Weight: 106.165
IUPAC Name: 1,4-Dimethylbenzene

1,4-dimethylbenzene



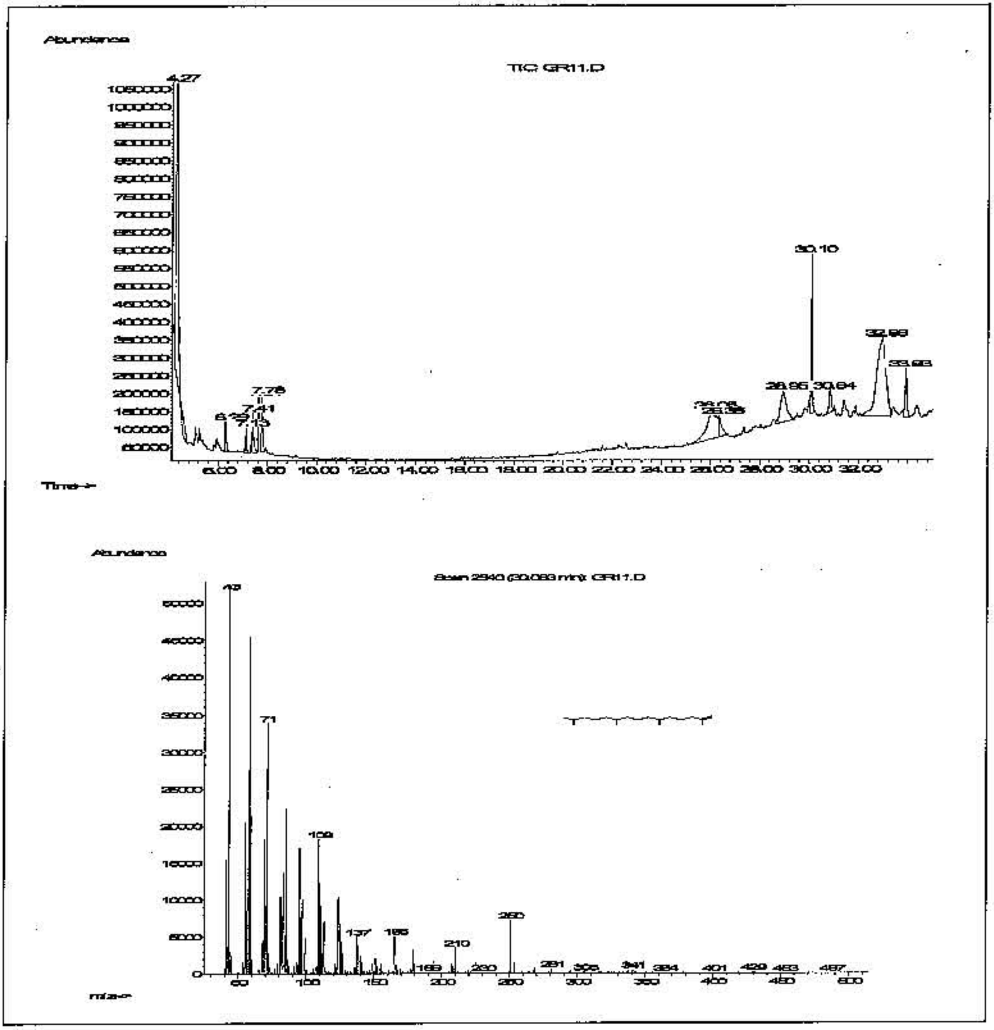
The chemical structure of 1,2-Benzenedicarboxylic acid-butyl 2-methylpropyl ester.
Molecular Formula: $C_{16}H_{22}O_4$
Molecular Wt: 278.34348

Figure 3b. The molecular structures of p-Xylene and 1,2-Benzenedicarboxylic acid-butyl 2-methylpropyl ester respectively.

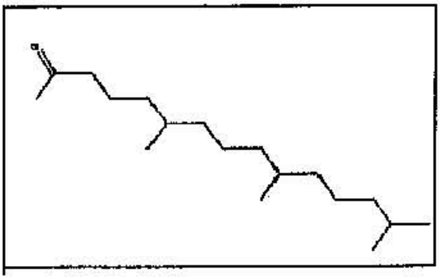
98 per cent (Fig. 4). From the moso bamboo (*P. pubescens*) leaves, 3,7,11,15-tetramethyl-2-hexadecen-1-ol (phytol) was detected when Supersonic extraction was employed using ether. Its retention time is 28.62 mins., and matching quality 93 per cent (Fig. 5). Generally, more chemicals were identified when the Supersonic technique and ether were employed than from Soxhlet extraction and methanol or n-hexane solvents.

DISCUSSION

Bamboo shoots are a popular cuisine in many countries (China, Japan, India, Indonesia, etc.) with a long history (Jiang Zehui, 2007). However, the scant information regarding the chemical analysis of tropical bamboos has created the assumption that they probably contain some lethal chemicals, which make them unsafe for human consumption. On the contrary, Asian bamboo utilization includes pulp and paper, flooring tiles, veneer production and is most importantly employed as vegetable or food crop based on the knowledge of their safe chemical compounds. The major components currently detected and identified by GC-MS for the tropical varieties were variable. Features including their peak series with different retention times, match qualities and comparable molecular weights suggest that, for the tropical species under investigation, five main components were identified from *B. vulgaris* var. *vulgaris*, namely, octane, 2-methyl-Naphthalene, 1,1 Bicyclohexyl extracted in n-Hexane, p-xylene and 1,2-Benzenedicarboxylic acid-butyl2-methylpropyl ester detected in methanol. Their match qualities ranged between 90 and 97. The last two compounds had the best match quality of 97 and 94 respectively. In all cases, these components were removed by the Supersonic extraction technique. A greater number of the chemicals identified from the tropical varieties are obnoxious and would likely cause health problems. The

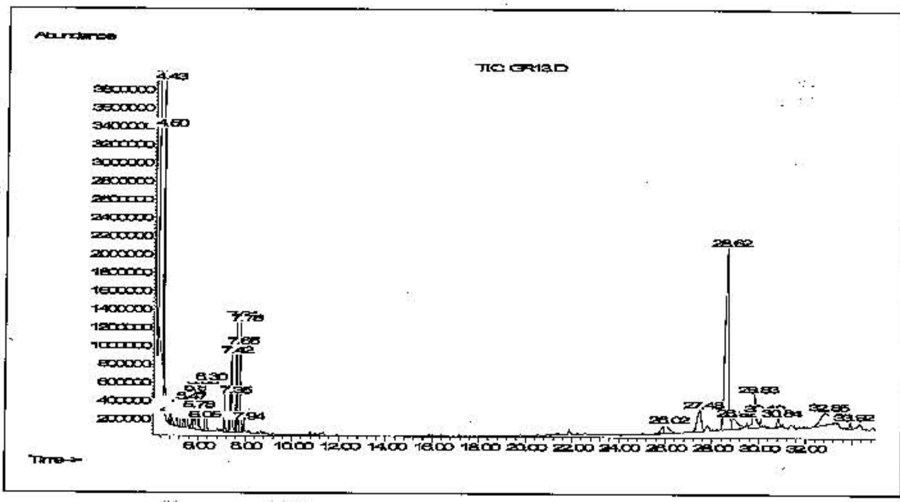


B. vulgaris var. *vittata*. Solvent: Ether RT:30.106,10,14-trimethylpentadecan-2-one,match quality 98

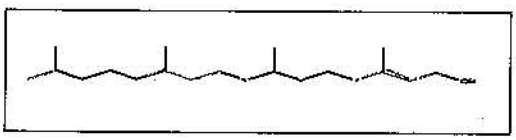


Chemical structure of 6,10,14-trimethylpentadecan-2-one
Mol. Formula: $C_{18}H_{36}O$
Mol. Wt.: 268.4778

Figure 4. Chromatogram [TOP] spectrum or scan [MIDDLE] and chemical structure [BOTTOM] of 6,10,14-Trimethylpentadecan-2-one (Phytone) identified from internode of *B. vulgaris* var. *vittata* using GC-MS.



Name Phytol
RT:28.62 Phytol match quality 93
Molecular Formula $C_{20}H_{40}O$
Ether extractive supersonic, *P. pubescens* leaves



Chemical structure of 3,7,11,15-tetramethyl-2-hexadecen-1-ol (phytol);
Mol. Formula: $C_{20}H_{40}O$
Mol. Wt.: 296.53

Figure 5. Chromatogram [TOP] spectrum or scan [MIDDLE] and chemical structure [BOTTOM] of 3,7,11,15-tetramethyl-2-hexadecen-1-ol (phytol) identified from *P. pubescens* leaves using GC-MS.

octane identified is fuel as in gasoline. Khan and Pandya (2006) noted that it causes hepatotoxicity in albino rats. Anon. (2000) also reported of its major hazards as respiratory tract, skin and eye irritations as well as central nervous system depression. When ingested, it causes nausea, vomiting, stomach pain, headache, lung congestion, drunkenness symptoms and kidney problems. 2-Methyl-Naphthalene causes organ and system toxicity as well as death in rats (Anon., 2003). It induces pulmonary alveolar proteinosis but does not possess unequivocal carcinogenic potential in B6C3F 1 mice (Murata *et al.*, 1997). Anon. (2008) reported that it is harmful if swallowed and causes skin, eye and respiratory irritations. 1,1 Bicyclohexyl has anti-muscuranic properties. Health exposure effects may be delayed. However, ingestion may cause gastrointestinal irritation with nausea, vomiting and diarrhoea. It may also cause CNS depression, while inhalation may cause respiratory tract irritation and may lead to pulmonary oedema. Inhalation at high concentrations may cause CNS despair and asphyxiation. It may cause skin irritations, dermatitis and cyanosis of the extremities, eye irritation, chemical conjunctivitis and corneal damage although its toxicological properties have not been fully investigated (Anon., 2009). A petroleum-derived compound, p-xylene is for modeling plastics, polyethylene terephthalate (PET), etc. However, Faust (1994) reported that Hipolito (1980) studied xylene poisoning in laboratory personnel who had been exposed 1.5-18 years to the chemical and described symptoms such as chronic headache, chest pain, electrocardiographic abnormalities, dyspnea, cyanosis of hands, fever, leukopenia, malaise, impaired lung function, and confusion. 1,2-Benzenedicarboxylic acid-butyl 2-methylpropyl ester is toxic. When over-exposed, it causes damage to these target organs: lungs, the reproductive system, liver, upper respiratory tract, CNS, eye, lens or cornea, skin redness and irritations (Anon., 2007). Their presence in the green bamboo could make the bamboo unsafe for consumption. From *B. vulgaris* var. *vittata* basal internode extract, only one main component was identified, namely 6,10,14-Trimethylpentadecan-2-one (phytone). In the composition of oils of *Philomis* spp., this compound is antimicrobial in function. Extracted in ether by Soxhlet extraction, its matching quality is the greatest (i.e. 98).

The present methods employed indicate that the main chemical identified in *P. pubescens* leaf extract is 3,7,11,15-tetramethyl-2-hexadecen-1-ol (*i.e.* phytol). For it to be extracted in ether by the Supersonic method but with a retention time of 28.62 and matching quality of 93 indicates its uniqueness from those of the tropical bamboos. The compound is important for the synthesis of vitamins E and K. Wu Yi-qiang *et al.* (2009) explored the potential of *P. pubescens* as resource utilization in biomedicine and determined the following main chemical components by means of Py-GC/MS from 69 peaks. These are pectic acid (12.22%), pectone (11.36%), propanoic acid, 3-amino-2-methyl-(7.57%), 2-propanone, 1-hydroxy- (6.07%), 2,3-butanedione (3.78%), propanoic acid, 2-oxo-, methyl-(3.68%), propanamide, N-acetyl-(3.04%), propanoyl chloride (2.78%), 3-hexanone(1.62%), 2-butenal, (E)- (1.52%), propanal(1.49%), 1,3-dioxol-2-one (1.44%), *etc.* They concluded that the pyrolysis of by-products of *P. pubescens* may be used as raw materials of biomedicine. Moso-bamboo (*P. pubescens*) is well known as an edible shoot in Asia, while its stems are used as tableware due to its characteristic odour (Toshiyuki, 2010). Phytol may cause irritation of the digestive tract. However, Lim (2006) reported of relative safety and efficacy of phytol and stated that on-going studies on antibacterial immunity demonstrate that phytol is a novel and an effective or superior adjuvant with little toxicity and also elicits anti-bacterial immune responses. Chlorophylls in green vegetables constitute an important source of an isoprenoid component, phytol (3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol, $C_{20}H_{40}O$), a branched aliphatic alcohol, also present as the fatty acid side chain in tocopherols. Since phytols are hydrophobic, they are capable of interacting with the cell membrane. However, there is as yet no definitive report on the adjuvanticity of phytol or any synthetic derivatives such as hydrogenated phytol or phytanol, named PHIS-01 (Patent pending) which has been studied. It is present in *Calotropis procera* (Ait.) R.Br (Okiei *et al.*, 2009).

Of the total compounds identified from the extracts of all the three bamboos, none of them was common to each of the varieties or the two pre-treatment methods (*i.e.*

Soxhlet and Supersonic techniques) employed for the extraction of the chemical compounds. However, for now the Supersonic procedure with ether coupled with GC-MS screening has been established as a valuable tool in the extraction and assessment of phyto-chemicals from tropical bamboos. From this preliminary investigation it is evident that variation in the profiles of compounds in the tropical bamboos exists. The study has revealed that components from the young shoot extracts are not healthy for human consumption, unlike the moso bamboos, which possess anti-mutagenic, anti-microbial and antioxidant properties. It is only the young shoot from *B. vulgaris* var. *vittata* whose main extract component (6,10,14-Trimethylpentadecan-2-one) is antimicrobial in function. Since this study is the first attempt in identifying chemical components of tropical bamboo resources regarding their suitability as safe vegetables, compounds in their young shoots need further and comprehensive analyses to establish their bio-health properties or safe utilization function as food (or otherwise). Further studies should involve their toxicological safety evaluation to establish their non-toxic classification standard.

CONCLUSION

Octane, 2-methyl- Naphthalene and 1,1Bicyclohexyl were the major *n*-hexane fractions identified from *B. vulgaris* var. *vulgaris* crown internode. Methanol fractions included p-xylene and 1,2-Benzenedicarboxylic acid-butyl 2-methylpropyl ester with greater match qualities (97 and 94 per cent respectively) than the n-hexane fractions (90, 90 and 91 per cent respectively). Their properties reveal they are unsafe for human consumption. *B. vulgaris* var *vittata* is rich in 6,10,14-trimethylpentadecan-2-one from Soxhlet extraction with ether with match quality of 98 per cent, and has anti-microbial properties. The major component identified in Supersonic ether extraction is 3,7,11,15-tetramethyl-2-hexadecen-1-ol (phytol) from *P. pubescens* with match quality of 93 per cent. Generally, retention times for all the components varied and followed no trend. Supersonic technique with ether identified more components than Soxhlet extraction with methanol or n-hexane. For the tropical bamboos to be used as raw materials of biomedicine or vegetable, they need further intensive investigations.

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