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Contributed Paper

Betel-like-scented *Piper* Plants as Diverse Sources of Industrial and Medicinal Aromatic Chemicals

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ABSTRACT

Piper betle (Piperaceae) or betel leaf, known locally as "*Phlu*" has been used by people in Thailand for chewing for a long time. Additionally, the leaves are used for traditional remedies and folk customs, such as for weddings and housewarming ceremonies. More recently, the aromatic oil industry has used the leaves for oil distillation. Moreover, the oils are used in several household products. Over the past 12 years of our research on *Piper* species, we found that among the more than 43 species recorded, there are some plants other than *P. betle* that possess a betel-like scent, viz. *P. betloides*, *P. crocatum*, *P. maculaphyllum*, *P. rubroglandulosum*, *P. semiimmersum*, *P. submultinerve*, *P. tricolor*, and *P. yinkiangense*. As it was expected that these plants would contain similar useful chemicals, their extracts were screened for the chemical contents by GC-MS. The extracts contain some important chemical substances that are similar to the betel extract, namely, eugenol, isoeugenol, chavicol, caryophyllene, sabinene, phellandrene, germacrene A and germacrene D, and sesquiterpenes. The results indicate that the eight plant species would have as high a potential as *P. betle* for industrial purposes. Moreover, as the plants are wild species they have a greater vigor, thus growing well and with more branching than betel. The diverse *Piper* species studied and documented are important for sustainable uses and can enable conservation management for posterity.

Keywords: betel plant, betel-like-scented plants, GC-MS, *Piper* species, Thailand

1. INTRODUCTION

The plants in the genus *Piper* are of great interest because they are useful in many functional aspects, for instance, as spices, medicines, and insecticides [1-3]. The betel plant, *P. betle*, is a well-known and important species that contains important chemical

substances, including essential oils and substances, such as chavicol, cineol, and eugenol which can be used for medicinal and insecticidal purposes [4,5]. Indeed, it has been reported that eugenol has anti-oxidant and anti-inflammatory properties [4]. It is also known to be stimulating, anesthetic, and psychoactive [6,7]. Even though the betel plant is of great economic importance, there are inherent problems with its cultivation, with one common cause being that the roots and leaves often rot because of infection by Phytophthora parasitica Dast [8,9]. Banka and Teo [9] have reported that leaf spots caused by bacteria are another problem.

Two *Piper* species from Cameroon, *P. nigrum* and *P. guineenense*, were studied [10] to identify the aromatic target components responsible for the characteristic odor of these valuable spices and food-flavoring products. The main compounds detected were as follows: *P. nigrum* contains germacrene D (11.01%), limonene (10.26%), β-pinene (10.02%), α-phellandrene (8.56%), β-caryophyllene (7.29%), α-pinene (6.40%) and cis-beta-ocimene (3.19%); *P. guineense* (black) contains β-caryophyllene (57.59%), β-elemene (5.10%), bicyclogermacrene (5.05%) and α-humulene (4.86%); and *P. guineense* (white) contains β-caryophyllene (51.75%), cis-β-ocimene (6.61%), limonene (5.88%), β-pinene (4.56%), linalool (3.97%) and α-humulene (3.29%).

Investigations of the genus *Piper* in Thailand [11,12] reveals that of the 43 known *Piper* species, there are numerous species that possess a betel-like scent. All of these species are blossoming, wild species that produce abundant branches and leaves. Furthermore, these species are hardy and resistant to diseases, and some of them have a stronger scent than the betel plants. Therefore, these species are predicted to have an economic potential greater than that of the betel plants.

Betel oil contains several substances, such as chavicol, terpene, and sesquiterpene [13], hydroxychavicol, hydroxychavicol acetate, allylpyrocatechol, chavibetol, piperbetol, methylpiperbetol, piperol A, and piperol B [14], chavibetol, chavibetol acetate and caryophyllene [15], allopyrocatecholdiacetate, safrole and B-phellandrene [16], and eugenol and hydroxychavicol [17]. Both the crude extracts of *P. betle* leaves and the purified compounds have been found to possess important antiseptic (e.g., oral hygiene), anti-diabetic, cardiovascular, anti-inflammatory/immunemodulatory, antiulcer, hepato protective, and anti-infective properties [14] and are also active against many species of bacteria and fungi [16,18]. The chemical composition of natural products was successfully accessed by gas chromatography-mass spectrometry (GC-MS) analysis as reported by Phutdhawong et al. [19], Quang et al. [20], and Norkaew et al. [21]. The properties of the major compounds are as follows. Isoeugenol, an isomer of eugenol, is a phenylpropene that is synthesized from eugenol and is a constituent of the essential oils of plants. Chavibetol, another isomer of eugenol, is an aromatic compound with a spicy odor [22]. Caryophyllene is a spicy, clove-like aroma [23]. Phellandrenes are used in fragrances because of their pleasant aromas; the odor of β-phellandrene has been described as peppery-minty and slightly citrusy [24]. Sabinene is a natural bicyclic monoterpene, which is one of the chemical compounds that contributes to the spiciness of black pepper (*P. nigrum*) [25]. Sesquiterpenes, a class of volatile compounds, are typically produced for their antimicrobial and insecticidal properties [26,27].

In Thailand, betel oil has been used in modern medicine as an antiseptic component in gels, balms, and cosmetics for hands, feet, and body, as an anti-inflammatory, and as a

treatment for many diseases. Examples of applications of betel oil are as follows: "Plumix", a product by Information Service Center, Institute of Science and Technology Research, Thailand for inhibition of gastrointestinal pathogens in chickens [28]; "betel oil", by Agricultural and Agro-Industrial Product Improvement Institute, Kasetsart University, Thailand, claimed to have an anti-microorganism effect [29]; "Plugenol gel", a product by Thai Herbal Products Co., Ltd, for treatment of microbial infection and inflammatory [30]. The research in this study aims to investigate various betel-like-scented species for a preliminary chemical information analysis using hexane extracts.

2. MATERIALS AND METHODS

2.1 Plant Materials

The 43 *Piper* species in Thailand were explored in the areas as described by Chaveerach et al. [11,12] and screened for their betel scent. Species identifications were done following the literature and compared with the specimens kept at Khon Kaen University. Leaf samples from several individuals of the betel-like-scented species were collected for chemical extraction. Voucher specimens were kept at the Department of Biology, Faculty of Science, Khon Kaen University. No specific permits were required for the described field studies because the locations are not privately owned nor are they protected in any way. Moreover the field studies did not involve endangered or protected species.

2.2 Preparation of Chemical Extracts

The extracts were prepared, and the chemical contents were analyzed by gas chromatography-mass spectrometry (GC-MS). The leaf samples were rinsed with water and air-dried to get rid of water. The 25 g of leaf samples were ground into a powder, mixed with a 120 mL hexane solvent (analytical grade), and filtered at room temperature. A 90 mL filtrate was obtained and stored at -20°C until used for the GC-MS analysis.

2.3 GC-MS Analysis and Identification of Components

The GC-MS analysis of the crude extracts was performed using an Agilent Technologies GC 6890 N/5973 inert MS fused with a capillary column (30.0 m × 250 mm × 0.25 mm). Helium gas was used as the carrier gas at a constant flow rate of 1 mL/min. The injection and mass-transferred line temperature were set at 280°C. The oven temperature was programmed for 70°C to 120°C at 3°C/min, then held isothermally for 2 min, and finally raised to 270°C at 5°C/min. A 1 μL aliquot of the crude extract was injected in the split mode. The relative percentage of the crude constituents was expressed as the percentage using peak area normalization. Identification of the components of the crude extracts was assigned by comparison of the mass spectra obtained with those of the reference compounds stored in the Wiley 7N.1 library.

3. RESULTS AND DISCUSSION

3.1 The Betel-Like-Scented Piper Species

There were nine species found to be aromatic betel-like plants, viz. *P. betle* L., *P. betloides* Chaveer. & Tanomtong, *P. crocatum* Ruiz et Pavon, *P. maculaphyllum* Chaveer. & Sudmoon, *P. rubroglandulosum* Chaveer. & Mokkamul, *P. semiimmersum* C.DC., *P. submultinerve* C.DC., *P. tricolor* Y.C.Tseng, and *P. yinkiangense* Y.C.Tseng. The voucher specimen numbers and sites of specimen collection are shown in Table 1. *Piper betle* is found as a cultivated plant in all regions of Thailand. Except for *P. betle*, most of the species are wild, however *P. maculaphyllum*

and *P. crocatum* are often collected from the wild and grown as decorative plants. The images of all of the betel-like-scented *Piper* species are shown in Figure 1.

Table 1. Details of specimen collection.

Species	Voucher specimen number	Site of specimen collection
P. betle L.	A. Chaveerach 16	Cultivated, Muang District, Khon Kaen Province, Northeastern Thailand
P. betloides Chaveer. & Tanomtong	A. Chaveerach 171	Doi Suthep-Pui National Park, Chiang Mai Province, Northern Thailand
P. crocatum Ruiz et Pavon	A. Chaveerach 12	Garden and Development Department, Queen Sirikit Botanic Garden, Chiang Mai Province, Northern Thailand
P. maculaphyllum Chaveer. & Sudmoon	A. Chaveerach 126	Punyaban Waterfall, Lum Nam Kraburi National Park, Ranong Province, Southern Thailand
P. rubroglandulosum Chaveer. & Mokkamul	A. Chaveerach 319	Khao Phra Thaeo Wildlife Conservation Development and Extension Center, Phuket Province, Southern Thailand
P. semiimmersum C.DC.	A. Chaveerach 115	Sri Pungnga National Park, Pungnga Province, Southern Thailand
P. submultinerve C.DC.	A. Chaveerach 223	Doi Suthep-Pui National Park, Chiang Mai Province, Northern Thailand
P. tricolor Y.C.Tseng	A. Chaveerach 64	Sri Pungnga National Park, Pungnga Province, Southern Thailand
P. yinkiangense Y.C.Tseng	A. Chaveerach 133	Khao Sok National Park, Surat Thani Province, Southern Thailand

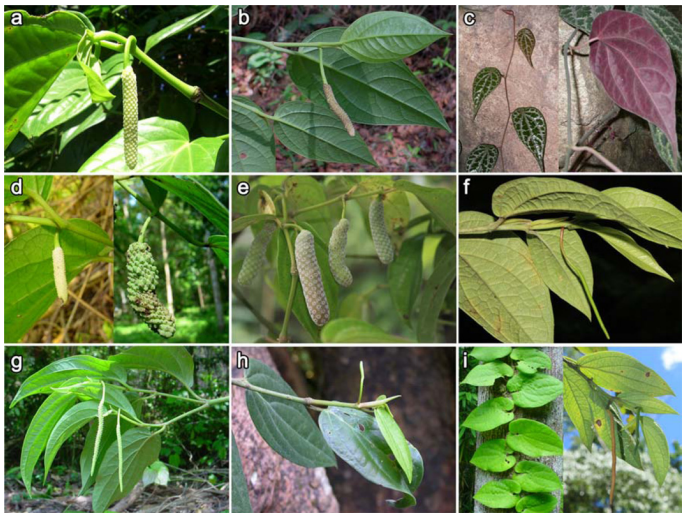

Figure 1. *Piper betle* (a) and the eight betel-like-scented species: *P. betloides* (b), *P. crocatum* (c), *P. maculaphyllum* (d), *P. rubroglandulosum* (e), *P. semiimmersum* (f), *P. submultinerve* (g), *P. tricolor* (h), and *P. yinkiangense* (i).

3.2 Chemical Identification of the Betel-Like-Scented Species Using GC-MS

The preliminary phytochemical screening of the hexane crude extract of nine *Piper* species shows the presence of different aromatic compounds. The total ionic chromatographs (TIC), showing the peak identities of the compounds from the nine individual species are given in Figure 2. The identified chemical compounds and their relative contents in each species are provided in Table 2.

Piper betle contains major chemicals, such as 80.52% eugenol (or isoeugenol, 4-cyclopropyl-2-methoxyphenol or chavibetol), which are identical to the constituents in earlier studies [15-17]. It also contains caryophyllene and some other minor chemicals, as shown in Table 2.

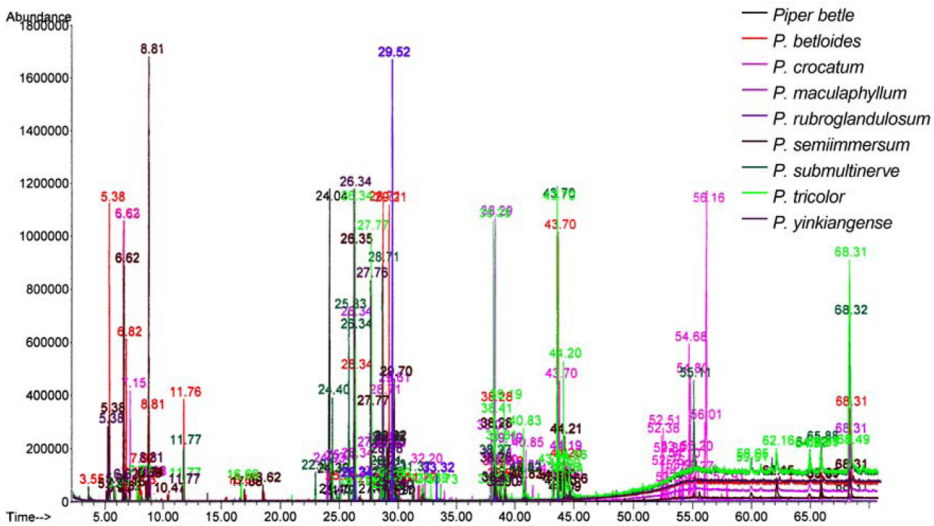

Figure 2. GC-MS chromatograms of hexane crude extracts from the leaves of the nine examined *Piper* species.

Table 2. Preliminary phytochemicals identified in the hexane crude extracts from the leaves of the nine examined *Piper* species.

Compound	Formula	Relative content**								
		P. betle	*P. betloides*	*P. crocatum*	*P. maculaphyllum*	*P. rubroglandulosum*	*P. semiimmersum*	*P. submultinerve*	*P. tricolor*	*P. yinkiangense*
Eugenol or Isoeugenol or Chavibetol or 4-cyclopropyl-2-methoxyphenol	$C_{10}H_{12}O_2$	80.52	-	-	3.24	-	-	-	-	-
Germacrene-D or β-cubebene or α-cubebene or α-ylangene or Naphthalene, 1,2,3,4,4a,5,6, 8a-octahydro or Bicyclo [4.4.0]dec-1-ene, 2-isopropyl-5-m	$C_{15}H_{24}$	5.21	13.29	4.59	3.78	-	-	12.13	-	-
Germacrene-A	$C_{15}H_{24}$	-	-	-	24.26	-	-	-	-	-
Trans-caryophyllene	$C_{15}H_{24}$	3.83	5.89	2.06	13.44	5.33	15.30	8.56	14.59	39.44
α-amorphene	$C_{15}H_{24}$	3.32	-	-	-	-	-	-	-	-
α-humulene or α-caryophyllene or cis,cis,cis-1,1,4,8-tetramethyl-4, 7, 10-cycloundecatriene	$C_{15}H_{24}$	-	-	1.12	3.61	-	5.21	-	9.43	35.03
Phytol or Phytol isomer	$C_{20}H_{40}O$	1.67	8.93	4.37	-	-	-	24.75	-	-
Bicyclogermacrene or 1H-cycloprop[e]azulene or Naphthalene,1,2,3,4,4a,5,6, 8a-octahydro	$C_{15}H_{24}$	1.24	12.55	-	10.98	-	-	-	-	-
α-pinene or Tricyclene or γ-terpinene or 4-carene or Tricycle[2.2.1.02,6] heptane,1,7,7-trimethyl-3-carene	$C_{10}H_{16}$	-	9.42	-	-	-	3.40	-	-	6.03
Sabinene or β-phellandrene or β-terpinene or β-thujene or β-pinene or Cyclohexane or 4-methylene-1-(1-methylethyl)	$C_{10}H_{16}$	-	18.43	10.17	-	-	32.91	-	-	3.65
1,3-dimethyl-4-azaphenanthrene or 4'methy-2 phenylindole	$C_{15}H_{13}N$	-	9.16	-	-	-	-	-	-	-
L-linalool	$C_{10}H_{18}O$	-	4.38	-	-	-	-	2.38	-	-
1,8-cineole	$C_{10}H_{18}O$	-	-	-	1.39	-	-	-	-	-
4-allyloxy-6-methoxy-N, N-dimethyl-1,3,5-	$C_{14}H_{19}NO_2$	-	-	6.54	-	-	-	-	-	-
β-myrcene	$C_{10}H_{16}$	-	-	4.01	-	-	-	-	-	-
β-elemene	$C_{15}H_{24}$	-	-	-	3.01	-	-	-	-	-
β-bisabo	$C_{15}H_{24}$	-	-	-	-	-	5.98	-	-	-
Methyl (25R)-5-oxo-A-nor-3, 5-secospirost	$C_{27}H_{42}O_5$	-	-	3.65	-	-	-	-	-	-
Palmitic acid	$C_{16}H_{32}O_2$	-	-	2.26	-	-	-	-	2.71	-
24(Z)-methyl-25-homocholesterol	$C_{29}H_{50}O$	-	-	-	9.66	-	-	19.15	-	-
or 1,5-dimethyl-6-(1,5-dimethylhexyl) -15,16	$C_{15}H_{22}$	-	-	-	3.39	-	-	-	-	-
1-(1,5-dimethyl-4-hexenyl) -4-methylbenzene	$C_{15}H_{24}O$	-	-	-	-	89.83	-	-	-	-
2,6-bis(1,1-dimethylethyl) -4-methylphenol (butylated hydroxytoluene)	$C_{18}H_{34}O_2$	-	-	-	-	-	5.00	-	-	-
9-octadecenoic acid or Heptadecene-(8)-carbonic acid-(1)	$C_{20}H_{38}$	-	4.13	-	-	-	4.06	-	13.85	-
or 9,12,15-octadecatrien-1-ol,(z,z,z)- Neophytadiene										

Table 2. Continued.

Compound	Formula	Relative content**								
		P. betle	*P. betloides*	*P. crocatum*	*P. maculaphyllum*	*P. rubroglandulosum*	*P. semiimmersum*	*P. submultinerve*	*P. tricolor*	*P. yinkiangense*
Vitamin e	$C_{29}H_{50}O_2$	-	-	-	-	-	2.66	-	-	-
α-gurjunene	$C_{15}H_{24}$	-	-	-	-	-	-	9.20	-	-
α-copaene	$C_{15}H_{24}$	-	-	-	-	-	-	5.52	-	3.19
(23s)ethylcholest-5-en-3. β-ol	$C_{29}H_{50}O$	-	-	-	-	-	-	-	18.66	-
9,12,15-octadecatrien-1-ol,(z,z,z)- or 7,10,13-hexadecatrienoic acid	$C_{18}H_{32}O$	-	-	-	-	-	-	-	7.03	-
3,7,11,15-tetramethyl-2-hexadecene	$C_{20}H_{40}$	-	-	-	-	-	-	-	-	1.52
4-allyl-1, 2-diacetoxy benzene	$C_{13}H_{14}O_4$	-	-	-	-	4.84	-	-	-	-
unknown*		4.20	13.83	61.24	23.24	-	25.48	18.32	33.73	11.15

*Unknown from each species is not the only one compound and may not be the same compound through all species.

** - means not detected

Isoeugenol and chavibetol, isomers of eugenol, compounds of the phenylpropanoid group are some of the primary constituents in the extract of *P. betle*. *Piper maculaphyllum* also contains isougenol or chavibetol (3.24%), though in smaller amounts.

Caryophyllene is an important constituent that was found in all nine of the studied species, though in different quantities: *P. yinkiangense* (39.44%); *P. semiimmersum* contains trans-caryophyllene (15.30%) and α-caryophyllene (5.21%); *P. tricolor* (14.59%); *P. maculaphyllum* (13.44%); *P. submultinerve* (8.56%); *P. betloides* (5.89%); *P. rubroglandulosum* (5.33%); *P. betle* (3.83%) and *P. crocatum* (2.06%).

Sabinene and phellandrene are monoterpene constituents of essential oils and were found in relatively high percentage in all four of the studied species, viz. *P. semiimmersum*, (32.91%); *P. crocatum* (10.17%); *P. betloides* (18.43%); and *P. yinkiangense* (3.65%). Phellandrene is a constituent of the essential oil of Eucalyptus dives [24]. Phellandrenes are used in fragrances because of their pleasant aromas; the odor of β-phellandrene has been described as peppery-minty and slightly citrusy. Sabinene is a natural bicyclic monoterpene, isolated from the essential oils of a variety of plants, including holm oak (*Quercus ilex*) and Norway spruce (*Picea abies*), which are not grown in Thailand. In addition, sabinene is one of the chemical compounds that contributes to the spiciness of black pepper (*Piper nigrum*) and is a major constituent of carrot seed oil. It also occurs in tea tree oil at a low concentration and is present in the essential oil obtained from nutmeg [25].

Germacrene A and germacrene D, sesquiterpenes, are significant components of *P. maculaphyllum* containing 24.26% and 3.78% respectively. The sesquiterpenes are also present in *P. betloides* (13.29%), *P. submultinerve* (12.13%), *P. betle* (5.21%), and *P. crocatum* (4.59%).

The minor common volatile aromatic compounds are found in *P. betloides* (9.42% α-pinene and 4.38% L-linalool), *P. semiimmersum* (3.40% α-pinene), *P. submultinerve* (2.38% L-linalool), and *P. yinkiangense* (6.03% α-pinene). These compounds are components in both branded and imitation perfumes [31]. *Piper rubroglandulosum* contains significantly

high amounts of butylated hydroxytoluene (89.83%) also known as butylhydroxytoluene (BHT) or 2,6-bis (1,1-dimethylethyl)-4-methylphenol. BHT is a lipophilic (fat-soluble) organic compound, a derivative of phenol that is useful against viruses in the herpes family, for its antioxidant properties, as a food additive, and as a prevention for apoptosis in etiolated seedlings. BHT induces large structural changes in the organization of all cellular organelles and the formation of new unusual membrane structures in the cytoplasm [32,33]. This is very interesting, because people are very close to the species. The people in Southern Thailand have been using *P. rubroglandulosum* for chewing (instead of *P. betle*) and for medicinal purposes for long time. This high content compound may be a key active ingredient which should be further examined.

The preliminary study on the chemical constituents of betel-like-scented *Piper* species (screened down to nine species) uses the non-polar solvent hexane for the extraction process, thus isolating the major monoterpenes (sabinene and phellandrene), sesquiterpenes (germacrene A, germacrene D, and caryophyllene), and phenylpropenes (eugenol, isoeugenol, and chavibetol). Even though hexane was used to extract chemical substances for the GC-MS analysis rather than the essential oils, the chemical constituents found tended to be similar, for instance among *P. betle*, *P. nigrum*, and *P. guineense.* A study of *P. nigrum* and *P. guineense* essential oils also provided evidence of the similarity among the chemical constituents [10].

3.3. Uses of Piper Species

Many research studies have been conducted on various *Piper* species, such as *P. nigrum*, *P. guineense* and *P. chaba* to investigate their potential uses. *Piper betle* has been studied for many years, particularly for its compounds and usefulness in treating diseases [10,34,35]. The plants are still of interest among researchers for their pharmacological activities, antimicrobial, and insecticidal properties, however, more information about the other Piper species with a similar aroma could be obtained. The study, therefore, examines whether the eight species identified as aromatic betel-like plants have any important identical chemical substances. The research findings would contribute to the data on aromatic plants and provide the basis for further research investigations.

In addition to eugenol (or isoeugenol, 4-cyclopropyl-2-methoxyphenol or chavibetol) reported in P. betle [15,16,17], caryophyllene is another compound found from this study.

The main compounds, which include β-caryophyllene, germacrene D, limonene, β-pinene, α-phellandrene and α-humulene, are identical to the compounds found in the Piper species from Cameroon [10]. The minor constituents, including δ-carene, β-phellandrene, isoborneol, α-guaiene, sarisan, elemicin, calamenene, caryophyllene alcohol, isoelemicin, T-muurolol, cubenol, and bulnesol, are of great importance for the characteristic pepper odor of these *Piper* species. Additionally, minor common volatile aroma compounds, including α-pinene and L-linalool found in *P. betloides*, *P. semiimmersum*, *P. submultinerve*, and *P. yinkiangense* are components found in both branded and imitation perfumes [31].

4. CONCLUSION

The GC-MS analysis of the hexane crude extracts of different *Piper* species shows the presence of a composition of volatile chemicals from the phenylpropene, monoterpene, and sesquiterpene groups. The identification of economically beneficial chemicals existing in many other plant species is important for industry and is also

beneficial in providing a growing population with supplements from natural sources.

Many plant species in the wild are not exploited or used because they are not known or well characterised. The hardiness, good growth, and abundance of branches of the wild species should be studied for uses in native forms, modified forms, and purified chemicals. The diversity of betel-like-scented species and the chemical components could be useful for industries to produce flavors, fragrances, cosmetics, drugs, perfumes, and food preservatives.

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