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Extraction of leaf essential oil from ‘Khasia’ landrace of *Piper betle* L.: An innovative business input to conventional agroforestry system

Bidisha Mondal

Genetics & Plant Breeding, School of Agriculture & Allied Sciences, The Neotia University, Sarisha, West Bengal, India. E-mail: bidisha.mondal@tnu.in

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Abstract

The ‘Khasia’ betel leaf-based agroforestry involving timber and non-timber crops is a natural non-destructive, chemical free traditional cultivation system adopted by the tribal communities of North Eastern India. The leaves are harvested five times a month and due to perishability the unsold leaves are discarded that sometime creates environmental pollution due to disease contamination in decomposed leaves. The excess leaves could be utilized for extraction of leaf essential oil having a shelf life of more than three years. The ‘Khasia’ betel leaves collected from Tripura state was used for extraction of essential oil with yield of 0.44% on fresh weight basis. The gas chromatography-mass spectroscopy (GC_MS) analysis detected 30 medicinally important components predominated by phenylpropenoid, sesquiterpenes and monoterpenes (90.3 %). The major components were chavibetol (50.4 %), chavicol (7.9 %), chavibetol acetate (6.1 %), chavibetol diacetate (2.3 %) and methyl chavicol (1.5 %). This phenylpropenoid class of aromatic compound are useful in pharmaceutical sector. The government aided training of the tribal communities in clean and green technology of essential oil extraction may open a new avenue of income generation, provide environmental sustainability, ecological equilibrium with articulate management of agroforestry waste.

Key words: community training, essential oil extraction, Khasia betel leaf, post-harvest management.

Introduction

Agroforestry system exhibits a sustainable model of land utilization integrating socially and ecologically acceptable agricultural crops with tree species for increasing overall productivity and providing soil restoration. In the North Eastern forest segment of India, a traditional agroforestry system involving betel-vine (*Piper betle* L.) and other timber or non-timber crop is widely prevalent providing economic benefit to the tribal communities of that region (Das et al. 2016). Several important trees or shrubs with straight bole provide support and shade to the betel creeper, including *Mangifera indica* L., *Areca catechu* L., *Phyllanthus emblica* L., *Termina-*

lia arjuna (Roxb.) Wight & Arn., *Ananas comosus* (L.) Merr., *Musa sapientum* L., *Pongamia pinnata* (L.) Pierre, *Azadirachta indica* A. Juss, *Aegle marmelos* (L.) Corrêa, *Datura innoxia* Mill., *Piper longum* L., *Citrus reticulata* Blanco were found as support plant with vines of betel leaf (Uddin and Mukul 2012). The betel leaf plant is an aromatic medicinal plant and contains important chemical components with widespread application in medicine, pharmacology, food, and flavouring sectors (Guha and Nandi 2019). This agroforestry model is semi-organic in nature and this manure-based organic cultivation produces high-quality toxin free leaf safe for consumption as a masticator or mouth freshener. In betel-vine based agroforestry system in the initial stage of propagation, the upper canopy structure of the support plant is pruned for proper penetration of sunlight to the betel creeper (Nath et al. 2016). Within three to four months after the initiation of the cultivation, the system acquires the phenotype of natural forest system with sufficient canopy growth. The tribal villagers depend on both betel leaf as well as the support plant for their livelihood. The woods, fuel, fruits, betel leaves were harvested and sold for earning money.

For ages, the North Eastern hilly tribes depend on floral diversity for treatment of several ailments (Rahman et al. 2011). The 'Khasia' tribe of North Eastern India holds expertise in ethnomedicinal treatments and use agroforestry plant species for treating more than 40 ailments. The tribal people use green betel leaves for multiple cases including constipation, cough, ear infection, diarrhea, fever, stomach ailment and as aphrodisiac (Rahmatullahet al. 2010). In biodiversity enriched tropical countries the integration of medicinal plants in primary health care system could serve as a cheap, viable and accessible treatment option compared to expensive drugs having side effects. In herbal treatment, the precise knowledge about the potential plant, confirmation of the potent plant part, formulation of the decoction and the accurate dose of the decoctions are priorities for adoption of the folk techniques (Islam and Sarwar 2020).

The 'Khasia' tribes adopts a special technique for the cultivation of betel leaf creepers and a skilled worker plucks nearly 3600 leaves per day from the creepers with local agro-tools and accessories. The leaves are kept in shaded condition in bamboo basket for 3–4 days with intermittent watering for keeping them fresh for sale in the local market. In the next step 350–360 leaves are made into a bundle called 'mora' and sold through middleman in the market (Haider et al. 2017). In North Eastern states the peak season for betel leaf business is considered from June to August. The monsoon month plays pivotal role in the production of highest amount of betel leaves. Contrary to northern and eastern India the betel leaf agroforestry in North East depends only on cow-dung manure and meager use of insecticide and fungicide leading to harvesting of pristine produce both for fresh consumption and post-harvest processing.

The betel leaf is a perishable cash crop with average shelf life of 4–5 days,withers and rot after 7–10 days in ambient conditions decreasing the market value. As the tribes use betel leaf for herbal treatment, the training of the tribes on extraction of leaf essential oil (LEO) could provide them the opportunity to use the LEO for treatment purposes (Uddin and Mukul 2012). It has been proved that in most of the cases minute amount of essential oil (0.1–6.3 %) out competes the aqueous extracts in anti-bacterial activities (Man et al. 2019). The community training of the tribal people about the use of clean and green technologies for extraction of essential oil could enable them to use the forest waste for daily healthcare and also for additional income generation through the utilization of an innovative agro-technology. In this paper ‘Khasia’ betel leaves collected from Tripura state was utilized for extraction of leaf essential oil and subsequent analysis of chemotypic profiling of the LEO was done to investigate the chemical profile and importance of the LEO and in determining its usage in medicinal and aromatic industries.

Material and Methods

Plant material and extraction of essential oil

Specimen betel leaves were collected from Kanchanpur village of Dasda block of North Tripura district of India and brought to the laboratory for further study and analysis. The betel creepers were marked and herbarium of the leaf specimen was preserved in the Genetics & Plant Breeding departmental laboratory. In North Tripura region only ‘Khasia’ cultivar is grown throughout the forest and leaves were collected randomly from 10 creepers and utilized for extraction of essential oil in a Clevenger-type apparatus using the modified method of hydro-distillation (Mondal 2022). The data was analyzed using MS Excel with three replications for each plant.

Analysis and characterization of the essential oil constituents

The LEO extracted from ‘Khasia’ betel leaves were utilized for chemical analysis. The composition of essential oil was detected using gas chromatography-mass spectroscopy (GC-MS) techniques. The identification of individual compounds was carried out using retention index (RI) determined using a homologous series of n-alkanes (C_7 to C_{30}, Supelco Bellefonte, PA, USA) and comparing mass spectra with those of authentic sample, as well as mass spectra library search in NIST and WILEY Mass Spectral Library and by comparing with a mass spectral literature data (Adams et al. 1995).

Result and Discussion

The hydro-distillation technique was able to recover good amount of LEO from the fresh, as well as unsold waste leaves. The LEO yield was found to be 0.44 % on fresh weight basis (Table 1). The colour of the oil is light green with spicy phenolic fragrance. The thrown-away diseased leaf samples where decomposition has just initiated were also included in the trial. The obtained result is analogous to previous study that revealed the betel leaf essential oil recovery from diverse cultivars ranges from 0.09–0.51 % in fresh weight basis (Sankaret al. 1996).

The GC-MS analysis detected the presence of 30 components in the LEO (Table 2). Although 90.3 % of the LEO was characterized, the essential oil is mainly predominated by phenylpropanoid followed by sesquiterpenes and monoterpenes. The ‘Khasia’ leaves contain highest amount of chavibetol (50.4 %) followed by chavicol (7.9 %), chavibetol acetate (6.1 %), chavibetol diacetate (2.3 %) and methyl chavicol (1.5 %), respectively. The LEO contain a noticeable amount of sesquiterpenes compound β-caryophyllene (4.8 %), γ-muurolene (2.9 %)

and germacrene D (2.9 %) that modulates the anti-microbial and insecticidal property of the plants. The phenylpropanoid families of secondary metabolites are profusely found in the plant kingdom providing defense against UV radiation, high intensity light rays, phytoalexins (Deng and Lu 2017). All the components identified in leaf essential oil of ‘Khasia’ cultivar of *Piper betle* possess valuable medicinal properties taking from malarial treatment, hepato-protective (Depiet al. 2020), anti-cancerous activities (Preethy et al. 2017), as well as profound action against drug-resistant tuberculosis (Endriyatno 2022).

Table 1. Morpho-biochemical parameters of betel leaf.

Length of leaves, cm	Breadth of leaves, cm	Petiole length, cm	Weight, g	Oil content, %	pH	Taste	Texture	Colour
14.1 ±1.83	9.8 ±1.11	3.11 ±0.69	3.31 ±0.68	0.44 ±0.05	4.07 ±0.23	Spicy	Rough, pubescent	Green with brown patches

Note: Oil content was calculated relative to fresh weight.

Table 2. Essential oil composition of betel leaf accession.

Type of constituent	RI[a]	RI[b]	Area, %	Molecular weight, $g \cdot mol^{-1}$	Type	Uses
Leaf alcohol	851	851	0.1	-	Alcohol	Sweet aroma, fights diseases
α-pinene	933	932	0.1	136.23	Monoterpene	Treatment of urinary stone, bladder
Camphene	949	946	0.1	136.24	Monoterpene	Anti-inflammatory, anti-oxidant
Sabinene	973	969	t	136.23	Bicyclic monoterpene	Anti-fungal, anti-inflammatory
β-myrcene	990	988	0.1	136.23	Monoterpene	Flavouring agent
p-cymene	1024	1020	t	134.21	Monoterpene	Anti-inflammatory, anti-oxidant
Limonene	1029	1024	0.1	136.24	Cyclic monoterpene	Anti-obesity, anti-cancer, anti-oxidant, anti-fungal
1.8-Cineole	1031	1026	0.8	154.25	Monoterpene	Respiratory, cardiovascular diseases
(Z)-β-ocimene	1035	1032	0.1	136.23	Acyclic monoterpene	Anti-fungal, scent
(E)-β-ocimene	1046	1044	0.5	136.23	Monoterpene	Cosmetics, anti-perspirant

Type of constituent	RI[a]	RI[b]	Area, %	Molecular weight, g·mol^{-1}	Type	Uses
γ-terpinene	1057	1054	t	136.23	Monoterpene	Anti-oxidant
Terpinolene	1088	1086	t	136.23	Monoterpene	Anti-proliferative
Linalool	1099	1095	0.6	154.25	Monoterpene	Insecticide, fragrant
Camphor	1141	1144	nd	152.23	Monoterpene ketone	Antiseptic, antipruritic
Terpinen-4-ol	1177	1174	t	154.25	Terpineol	Antimicrobial, anti-inflammatory
α-terpineol	1191	1186	0.1	154.25	Tertiary monoterpene	Anti-convulsant, anti-nociceptive
Methyl chavicol	1200	1195	1.5	148.2	Phenyl propane	Stomach cramp, muscle tension
Decanal	1204	1201	0.1	156.2	Aldehyde	Anti-fungal
Chavicol	1256	1247	7.9	134.18	Phenyl propane	Headache, toothache
Safrole	1291	1285	t	162.19	Phenyl propanoid	Antibiotic, antigiogenic
α-cubebene	1347	1345	1.9	204.35	Tricyclic sesquiterpene	Flavour and fragrance
Eugenol	1358	1356	0.3	164.2	Phenyl propanoid	Toothache, gastrointestinal
Chavibetol	1376	1370	50.4	164.2	Phenyl propanoid	Antioxidant, fungicide
α-copaene	1377	1374	0.7	204.36	Sesquiterpene	Anti-malarial, anti-cancer
β-bourbonene	1385	1387	0.3	204.35	Sesquiterpenoid	Anti-microbial
β-cubebene	1391	1387	0.5	204.35	Tricyclic sesquiterpene	Citrus like scent, flavor
Methyl eugenol	1405	1403	0.3	178.23	Phenylpropanoid	Flavour, anaesthetic
β-caryophyllene	1421	1417	4.8	204.36	Sesquiterpene	Anti-inflammatory, anti-microbial
β-copaene	1433	1430	0.2	204.35	Tricyclic sesquiterpene	Pesticide, flavouring
α-humulene	1453	1452	1.0	204.35	Monocyclic sesquiterpene	Appetite suppression, weight loss
γ-muurolene	1477	1478	2.9	204.35	Sesquiterpene	Anti-bacterial
Germacrene D	1481	1484	2.9	204.35	Sesquiterpene	Perfume, anti-microbial
Bicyclogermacrene	1495	1500	0.8	204.35	Germacrene	Malaria and tumor
δ-cadinene	1523	1522	1.6	204.35	Sesquiterpene	Malaria, headache, toothache, burn
Chavibetol acetate	1527	1524	6.1	176.21	Benzoate ester	Influenza, fatigue, diuretic

Type of constituent	RI[a]	RI[b]	Area, %	Molecular weight, g·mol^{-1}	Type	Uses
Unknown	1557	-	0.2	-	-	
Chavibetol diacetate	1644	-	2.3	164.20	benzoate ester	Influenza, fatigue, diuretic
α-cadinol	1654	1652	1.0	222.37	Sesquiterpenoid alcohol	Hepatoprotective, drug resistant tuberculosis
Unknown	1744	-	t	-	-	-
Total component			90.3			

Note: RI[a] – calculated Retention Index in Elite-5 capillary columns using a homologous series of *n*-alkanes (C_7-C_{30} hydrocarbons), RI[b] – Retention Index in published library (Adams et al. 1995), t – trace ≤0.1 %, IM – identification method based on the GC-FID results (RI) and mass-spectrometry detection (MS), nd – not detected. Suvaas (*Ocimum basilicum* L.) data was taken from Srivastava et al. (2022).

The 'Khasia' leaf essential oil (EO) is a promising product with multiple utilities and shows a substantially higher shelf life than aqueous extract and could be stored in small vial in any safe place at tribal home occupying small area. The price of an essential oil distillation unit is approximately $ 2450 and price of 50 ml oil is nearly $ 16. The machine works well with nominal electricity cost for 12–15 years. In each day nearly three cycles of extraction could be done with a recovery of more than 300 ml of EO per day. A continuous flow of water is required for the hydro-distillation for three hours but that water is re-cyclable. The unsold leaves could be utilized for oil extraction and has the potential to make a prominence in the medicinal industry. The community training of the tribal villagers on essential oil extraction could support them in livelihood enrichment. Male and female members of 'Khasia' tribe contributes equally to betel leaf-based agroforestry. The cultivation, pruning, mulching, cutting was done by male counterparts, whereas the processing and marketing operations were done by female members. The betel leaf is harvested five times a month and in such a condition the training of betel leaf oil extraction could aid the tribal villagers to utilize excess betel leaves for essential oil extraction. As 'Khasia' betel leaf is grown in a wide area in North Eastern Indian states, the storage and extraction protocol will remain the same and could be adopted by several tribes of that region for large scale Khasia betel-leaf essential oil production.

Conclusions

The betel leaf-based agroforestry is typical to tropical countries mainly covering the South East Asian countries of the world. The betel leaf creeper is a predominant non-timber cash crop of North Eastern (NH) India. The perishable nature of the crop produces huge amount of agro-waste. The waste and unsold leaves of the agro-forestry system could be utilized for essential oil (EO) extraction. The extracted EO is golden in colour with spicy, strong fragrance, chavibetol rich, costly product with a shelf life of more than three years. The Khasia tribe of NH region utilizes betel leaf for daily health care and it is regarded as an important ethno-medicinal plants. The training of the forest people on Khasia betel leaf essential oil extraction technique could support them in the incorporation of simple, clean and green agro-techniques in advancement of traditional agroforestry with waste management. This system could act as an innovative cottage industry and may be adopted by the South East Asian countries for meaningful utilization of the perishable betel leaves and diverse parts of the creepers for essential oil extraction. The framework involving betel leaf germplasm could unwind novel avenues for sustainable utilization of the non-timber agroforestry models with futuristic profitable business strategies along with judicious agro-waste management.

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References

Adams R.P., Chu G.-L., Zhang S.-Z. 1995. Comparisons of the volatile leaf oils of *Juniperus rigida* Mig. from northeastern China, Korea and Japan. Journal of Essential Oil Research 7(1): 49–52. https://doi.org/10.1080/10412905.1995.9698461

Das T.K., Samajdar T., Islam M., Singh N.A., Marak G. 2016. Traditional Farming System: A Case Study of Garo Tribe in West Garo Hills District of Meghalaya, North-Eastern India. International Journal of Agriculture Sciences 8(50): 2140–2145. https://www.researchgate.net/publication/339841516

Deng Y., Lu S. 2017. Biosynthesis and Regulation of Phenylpropanoids in Plants. Critical Reviews in Plant Sciences 36(4): 257–290. https://doi.org/10.1080/07352689.2017.1402852

Depi S., Rusdi S., Misfadhila S. 2020. Review of traditional use, phytochemical and pharmacological activity of *Piper betle* L. Galore International Journal of Health Sciences and Research 5(3): 59–66.

Endriyatno N.C. 2022. Molecular Docking of Betel Leaf (*Piper betle* L.) on Protein Dihydrofolate reductase of Mycobacterium tuberculosis. Science and Community Pharmacy Journal 31(1): 1–10.

Guha P., Nandi S. 2019. Essential oil of betel leaf (*Piper betle* L.): A novel addition to the world food sector. Essential Oil Research: Trends in Biosynthesis, Analytics, Industrial Applications and Biotechnological Production. Springer: 149–196. https://doi.org/10.1007/978-3-030-16546-8_5

Haider M.R., Khair A., Rahman M.M., Alam M.K. 2017. Indigenous management practices of betel-leaf (*Piper betle* L.) cultivation by the Khasia community in Bangladesh. Indian Journal of Traditional knowledge 12(2): 231–239. https://www.researchgate.net/publication/282609075

Islam M.S., Sarwar A.K.M.G. 2020. The Garo tribe’s ethnobotanical knowledge about medicinal plants. Jordan Journal of Natural History 7: 93–107. https://www.researchgate.net/publication/344954277

Man A., Santacroce L., Jacob R., Mare A., Man L. 2019. Antimicrobial activity of six essential oils against a group of human pathogens: A comparative study. Pathogens 8(1), 15. 11 p. https://doi.org/10.3390/pathogens8010015

Mondal B. 2022. Cost effective oil extraction from surplus betel (*Piper betle* L.)

leaves,Asian Journal of Biological Life Sciences 11(1): 100–106. doi. 10.5530/ajbls2020.11.14

Nath A.J., Reang D., Das A.K., Brahma B., Das M. 2016. Traditional practice of Paan-Jhum cultivation among Khasia community in Barak valley, Assam. Journal of Traditional and Folk Practices 02, 03, 04(1): 96–99. https://www.jntbgri.res.in/downloads/jtfp/11.pdf

Preethy T.T., Elsy C.R., Beena C. 2017. Profiling of essential oil in Tirurvettilai (*Piper betle* L.), a group of unique betel vine land races from Malappuram district, Kerala. Journal of Pharmacognosy and Phytochemistry 6(3): 774–778.

Rahman M.H., Rahman M., Roy B., Fardusi M.J. 2011. Topographical distribution, status and traditional uses of medicinal plants in a tropical forest ecosystem of north-eastern Bangladesh. International Journal of Forest Usufructs Management 12(1): 37–56. https://www.researchgate.net/publication/215650517

Rahmatullah M., Haque M.R., Islam S.K., Jamal F., Bashar A.A., Ahmed R., Ahmed I., Jahan R., Ahsan S., Chowdhury M.H. 2010. A survey on the use of medicinal plants by folk medicinal practitioners in three areas of Pirojpur district, Bangladesh. American-Eurasian Journal of Sustainable Agriculture 4(2): 247–259.

Sankar C.R., Sridevi D., Babu M.K. 1996. Studies on essential oil and oil constituents of betel vine cultivars. Andhra Agricultural Journal 43(1): 24–26.

Srivastava S., Lal R.K., Yadav K., Pant Y., Bawitlung L., Kumar P., Mishra A., Gupta P., Pal A., Rout P.K., Chanotiya C.S.2022. Chemical composition of phenylpropanoid rich chemotypes of *Ocimumbasilicum* L. and their antimicrobial activities. Industrial Crops and Products 183(1), 114978. https://doi.org/10.1016/j.indcrop.2022.114978

Uddin M.B., Mukul S.A. 2012. Ethnomedicinal knowledge of Khasia tribe in Sylhet region, Bangladesh. Indian Journal of Tropical Biodiversity 20(1): 69–76. https://www.researchgate.net/publication/268575047