Research Article

Cost Effective Essential Oil Extraction from Surplus Betel (*Piper betle* L.) Leaves

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ABSTRACT

The betel leaf production in West Bengal, India is facing a serious decline due to recurrent storms and drastic weather fluctuation. A low-cost essential oil extraction process from the decomposed betel leaves could assist the farmers in partial recovery from the huge financial loss. 'Sada Bangla' cultivar of betel leaf collected from betel-vine of South 24 Parganas district was used for betel leaf essential oil (BLEO) extraction. A modified steam distillation process involving Clevenger apparatus was used for increasing oil recovery with different treatments. The process involving pre-grinded leaves in 10% cooking salt-based brine was found best for optimum oil extraction with a solute: Solvent ratio of 1:1 with a continuous time period of 3 hr with a 100°C initial and 80°C constant temperature after boiling of the mixture. The process is effective with both fresh and partially decomposed leaves with oil recovery of 1.82 ml and 1.72 ml respectively from 1 kg betel leaf sample. The removed fresh petioles as well as 10 days old petiole samples yielded 0.91ml /kg and 0.84 ml/kg of oil respectively. The process leads to 9 times reduction of de-oiled leaf-waste showing the viability of the process in profitable agri-waste management and the waste could be reused for further oil extraction. The fragrance and volume of essential oil remain intact for 6 months in airtight amber bottle in room temperature or 4°C chamber of refrigerator. The by-product fragrant water extracted in the process could be utilized as taste enhancer or re-extraction of oil. This technique could be effectively utilized by the betel farming families for additional income generation and development of rural cottage production unit of BLEO.

Key words: Bangla cultivar, Betel leaf essential oil, Income generation, Partially decomposed leaves, Farmer's economy, Steam distillation.

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INTRODUCTION

The erratic weather condition and gradual shift of rainfall pattern of West Bengal is creating hindrance in betel crop cultivation. The recurrent attack of super cyclones in the Bay of Bengal also created negative influence in crop production in the Southern region of Bengal. West Bengal produces very good quality betel leaves and is regarded as the largest producer of this high value crop. The South 24 Parganas district of West Bengal is occupied with small to large betel baroj cultivating diverse landraces. The baroj based cultivation of betel-vine got affected by the devastation made by climatic forces and the farmers are shifting their attention to other crops. In the Amtala, Diamond Harbor region of South 24 Parganas, mainly the 'Bangla Leaf cultivars of *Piper betle* are grown in abundance while the Midnapore region nurtures mainly 'Meetha' cultivar. The perishability of the leaves made this business more vulnerable than the cereal or seed crops and the cultivation loss becomes irreversible.[1]

In such a condition the extraction of oil from the betel leaves from the devastated orchards could aid the farmers to mitigate the loss incurred by the storm. The extraction of oil from the partially decomposed leaves will help the farmers to earn some money and also reduce the environmental pollution arising from the devastated baroj or stacked leaf load. The storage life

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of the Betel leaf essential oil (BLEO) is 3 years or more. This shelf life of the BLEO along with high market price plays a salvaging role for the betel farmers. The betel leaf oil has multipurpose use (Table 1)[2-21] and if the farmers get a proper market for the extracted BLEO then their apparent negativity and ignorance towards betel leaf cultivation under obstinate weather could be reduced and the rich germplasm resource could be saved from absolute elimination.

The betel leaf essential oil has a number of applications. In the food industry apart from use as masticatory, the fragrance was tried in different sweet and dessert preparation. The oil has prospects in the perfumery industry due to the soothing rejuvenating aroma. The oil has medicinal, antibacterial, fungicidal, insecticidal activity.[22] In modern days this oil is used in thin film making for packaging purposes. The oil is used in seed germination experiments and organic disease management purposes in agriculture and plant tissue culture. The fluctuation in the price of betel leaf is another major constraint of betel leaf business. In glut season there is an excessive reduction in the

Table 1: Uses of *Piper betle* Essential Oil.

Sl. No	Type of Use	Organisms	References
1	Anti-bacterial activity	*Mycobacterium smegmatis, Staphylococcus aureus and Pseudomonas aeruginosa*	Madhumita *et al*. 2019[2]
2	Disease control	Avian pathogen *Escherichia coli* by anti-biofilm, anti-adhesion activity	Kulnanan *et.al*. 2022[3]
3	Anti-oxidant	Green-less betel leaf extract or oil could act as natural additive in food	Tagrida, Benjakul, 2020[4]
4	Anti-fungal	*Candida albicans* and *Candida glabrata* based biofilm inhibition	Ali *et al*. 2010[5]
5	Wound healing	healing efficacy of methanol leaf on full-thickness burn and excision wounds in swiss mice	Le *et al*. 2015[6]
6	Anti-diabetic	The kapoori and Sanchi cultivar were beneficial as anti-diabetic natural product	Subramaniam *et al*. 2020[7]
7	Anti-carcinogenic	Hydroxy-chavicol isolated from betel leaves and also the leaf extract exerts apoptosis in CML cell and controls breast cancer proliferation	Roy and Guha, 2020[8]
8	COVID-19	Synergistic Prophylaxis and therapeutic treatment of COVID as herbo-mineral constituent with swarna bhasma	Soni *et al*. 2020[9]
9	Anti-inflamatory	sesquineolignan, neolignans controls inflammation	San *et al*. 202[10]
10	Food preservative	incorporating this oil ranging from 0.005% to 0.50% could preserve diverse food product	Guha and Nandi, 2019[11]
11	Edible film	Edible film preparation from betel leaf extract is a thin layer made from the basic ingredients of corn starch, sorbitol, and HPMC controls canker sore	Tanjung *et al*. 2021[12]
12	Betel-noodles	Hokkien noodles incorporated with betel leaf extract of 15% on flour weight) with a moisture content of 14% showed structural modification of the noodles, a soft product compared to the control.	Nouri *et al*. 2015[13]
13	Topical anaesthetic agent	Both betel and clove essential oil in 100% concentration could give better mean onset	Ronal *et al*. 2021[14]
14	Traditional treatment	Green betel leaf decoction is used as vaginal wash and it maintains the pH, ensures cleanliness	Widowati *et al*. 2020[15]
15	Geothermal power plant maintenance	*Piper betle* leaf extract was able to inhibit the formation of $CaSO_4$ scale with changes in the morphology of the crystal	Santoso *et al*. 2019[16]
16	Eco-friendly dish soap	60 ml of betel leaf extract, 20 g of lime and 10 g of gambier based dish-soap to substitute chemicals	Chandra Halim *et al*. 2020[17]
17	Aqua-farming	antimicrobial agent in some aqua farmer in Indonesia for an alternative method to control fish diseases	Bond, Senggagau, 2019[18]
18	Bio-plastic	Starch-based bioplastic from sago with 20%(v) glycerol and 9%(v) betel leaf extract showed anti-bacterial activity	Nasution *et al*. 2021[19]
19	Bio-fungicide	treatment with *T. asperellum* and betel extract could reduce the incidence of downy mildew, extend the incubation period, and increase the dry weight of corn shoots	Prasetyo *et al*. 2021[20]
20	Integrated Pest Management	Acute toxicity of chemical pesticides could be reduced by plant-derived essential oil on the behavior and development of earthworms, *Eudrilus eugeniae* (Kinberg) and *Eisenia fetida* (Savigny)	Vasantha-Srinivasan *et al*. 2018[21]

price of leaves. In monsoon also the demand of betel leaves decreases but the betel-vine (pan-baroj) yields a sufficient amount of leaves that remains unsold and creates problems for the farmers. The cost of pure betel oil (BLEO) is very high and could effectively help the farmer in additional income generation. In such a condition the training of the farmers in essential oil extraction is inevitable and urgent. If the betel farmers get a clear cut idea about the oil extraction procedure, then they could use the technique and could utilize additional leaves for oil extraction on a daily or weekly basis. The advantage of this technique is that they could store the oil in the same container after subsequent extractions and after accumulation of a judicious amount of oil the farmer could go for selling or using the oil for domestic or agricultural purpose.[23] The female members of the farmer's family could look after the process with their normal household activities and may save the manpower cost and develop a women-led cottage industry. In this paper a cost-effective modified steam distillation method is innovated for extraction of betel leaf essential oil (BLEO) from fresh and partially decomposed leaves of local betel-vines of South 24 Parganas, West Bengal.

MATERIALS AND METHODS

In this present investigation an attempt is taken to estimate and standardize the low cost procedure that could be recommended to the local farmers for betel leaf essential oil extraction. The leaf samples were collected from a betel baroj located at Dakshin Gouripur region of Bishnupur Block I of Amtala area South 24 Parganas of West Bengal, India. The cultivar was identified as 'Sada Bangla' betel leaf. Both fresh and partially affected leaves were collected from the baroj and brought to the Genetics and Plant Breeding laboratory of School of Agriculture and Allied Sciences, The Neotia University. Easily notable morpho-biochemical data (details given in Table 2) of the leaves were recorded before oil extraction.

The leaves were either used fresh or kept in normal room temperature at laboratory for 15 days and included in the trial. The extraction was performed in a 2 L Clevenger apparatus using the method of steam distillation. The experiment was conducted in the month of August, 2021. Both fresh, intact as well as partially decomposed leaves (15 days old) were separately included in the oil extraction process and a comparison was made between the results. The petiole part of the collected leaves was removed and separately used for oil extraction. Different simple treatments were included in the process to increase the oil yield as well as to keep the process simple and adoptable the quantitative analysis was performed using Microsoft Excel (ver. 2011).

RESULTS AND DISCUSSION

Initially the leaves were cut into 1 cm^2 pieces and 500g of leaf pieces were placed inside the round bottom flask of the Clevenger apparatus with 1000 ml of distilled water (Solute: Solvent ratio 1:2). The oil was collected in a container but the 500g leaf sample yielded nominal oil that remained mixed with the water forming emulsion creating hindrance in separation. The oil extraction process was performed for continuous 3hr in a heating mantle of 120V/60 Hz capacity. The initial temperature of the experiment was 100°C but later reduced to 80°C to avoid bouncing of water and overheating of leaf sample. The experiment was repeated 3 times for accurate data collection. The process yielded an average of 1.21 ml of BLEO from 1 kg leaf sample.

In an attempt to improvise the process in the second phase the leaf sample was grinded in a mixer grinder before placing inside the round bottom flask. In the first instance 500 g of leaf samples were grinded in 1000 ml of distilled water in a ratio of 1:2 and the process gave better results. Later to obtain better oil recovery

Table 2: Morpho-biochemical parameters estimated before Oil Extraction.

Leaf Parameter	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	Mean	SD
Lamina Length (cm)	21.8	21.4	20.8	20.6	20.7	20.2	21.2	21.6	21.5	20.6	20.6	20.5	20.96	0.52
Lamina Breadth (cm)	14.2	13.8	13.4	13.3	13.3	13.1	13.7	14.1	14	13.2	13.3	13.3	13.56	0.38
Leaf Weight (g)	5.2	5.1	4.9	4.85	4.81	4.79	5	5.21	5.2	4.89	4.8	4.84	4.97	0.17
Petiole Length (cm)	9.6	9.56	9.48	9.53	9.57	9.48	9.52	9.5	9.6	9.48	9.49	9.47	9.52	0.05
Petiole weight (cm)	1.6	1.56	1.49	1.55	1.5	1.49	1.52	1.57	1.58	1.48	1.49	1.47	1.53	0.04
Leaf Moisture Content (%)	80.2	79.9	79.1	80.4	80.5	80.8	78.2	78.9	80	81.7	80.6	80.6	80.08	0.95
Total Solid Content (TSS) (◦Brix)	2.18	2.13	2.15	2.14	2.19	2.13	2.16	2.13	2.16	2.15	2.09	2.19	2.15	0.03
pH of grinded leaves	5.03	4.95	4.99	4.99	5.03	5.09	5.01	5.02	4.99	5.06	5.04	5.06	5.01	0.03
Moisture content of fresh leaves (%)	88.7	89.0	89.1	89.2	87.9	87.2	88.0	87.6	89.3	86.9	87.2	87,5	88.1	0.89

in less time 1 kg of betel leaf samples were grinded with 1000 ml of distilled water (1:1) and oil extraction was performed. In this second process the oil recovery was better and the oil formed a separate distinguishable layer from the water ensuring better separation. The process yielded an average of 1.52 ml of BLEO from 1kg of grinded leaves with a solute solvent ratio of 1:1. The process was repeated 3 times for accurate data integration.

In another set 1 kg leaf sample was grinded using 10% and 15% NaCl solution and oil extraction was performed with 3 replications for each brine concentration. The process yielded 1.73 ml of BLEO from 1kg leaves with addition of 15% brine water with grinded leaf sample. To make the process simpler and farmer-friendly in another set 1 kg betel leaf sample prepared with 15% cooking salt and water obtained from home water purifier was also operated, yielding more or less same oil recovery of 1.72 ml. The application of 10% NaCl as well as cooking salt gave an oil recovery of 1.82 ml from 1 kg leaf sample.

In another set to study the objective of agricultural waste management the oil extraction was performed with partially decomposed leaves (15 days old) collected from betel-vine with standardized protocol with 10% brine (cooking salt based) yielding more or less equivalent oil amount of 1.72 ml from 1 kg. In third set of experiment the fresh petiole sample of 1 kg also gave 0.9 ml of pure transparent oil recovery and 10 days old petiole gave an oil recovery of 0.84 ml. The result of different treatments with replicated yield trial was tabulated in Table 3. The optimum treatment was shown in Table 4 for recommendation to the betel-farmers.

DISCUSSION

The betel-leaf is highly perishable in nature and in erratic weather condition, the farmers' experience huge loss. The leaves start rotting after 3-4 days after plucking. Being highly perishable lots of measures were taken to conserve plucked betel leaves. Being an aromatic plant, the extraction of betel leaf essential oil is a novel process as the crude oil retains the positive properties of the *Piper betle* plants. The extracted crude oil (BLEO) is costly and is not perishable. The cost of 1 ml betel leaf essential oil is nearly Rs. 200 in West Bengal and from 1 kg partially decomposed betel leaves (oil recovery is 1.72 ml) a farmer could earn nearly Rs. 400 which usually gets perished in baroj or vine adjacent areas.

Table 3: Details of the extracted oil recovered from 'Sada Bangla' leaf sample under different treatment with estimate of left over sample.

Treatment (1 Kg Sample)	Recovered Oil Content (ml)					Colour of Oil	De-oil leaves left after Oil Extraction (g)				
	R1	R2	R3	Mean	SD		R1	R2	R3	Mean	SD
Control (leaf)	1.23	1.2	1.2	1.21	0.01	Yellow	129.5	128.8	128.2	128.83	0.65
Grinded in distilled water (leaf)	1.51	1.52	1.52	1.52	0.005	Yellow	126.4	126.6	126.2	126.4	0.2
Grinded in 10% laboratory brine (leaf)	1.85	1.82	1.83	1.83	0.01	Yellow	123.4	124.0	124.2	123.86	0.41
Grinded in 15% laboratory brine (leaf)	1.73	1.73	1.74	1.73	0.005	Colourless	124.6	124.8	125.0	124.8	0.2
Grinded leaf in 15% cooking salt-based brine (leaf)	1.7	1.73	1.72	1.72	0.015	Colourless	125.1	124.4	124.7	124.73	0.35
Grinded in 15% laboratory brine (petiole)	0.89	0.94	0.89	0.91	0.028	Colourless	134.1	133.9	133.6	133.86	0.25
Grinded in 10% laboratory brine (petiole)	0.82	0.86	0.85	0.84	0.02	Colourless	144.1	140.9	143.6	142.86	1.72
Grinded in 10% laboratory brine (partially decomposed leaf)	1.73	1.71	1.72	1.72	0.01	Yellow	125.3	124.9	125.2	125.13	0.20

Table 4: Recommended Low-Cost Procedure for Betel Leaf Essential Oil Extraction by Steam Distillation.

Amount of Raw Material	Type of Raw Material	Solvent	Quality of Water	Solute: solvent ration	Time	Distillation temperature	Moisture Content of raw material
1 kg fresh or partially decomposed leaf	Grinded	10% cooking salt- based brine	Water from home water purifier	1:1	3 hr constant	100°C initially later reduced to 80°C	80.08%

In this oil extraction process if a farmer purchases a 5L/10L Clevenger Apparatus with an initial expenditure of Rs. 15,000/-, then with a minimum investment for electricity and purchase of some oil collecting bottle, he/she could extract oil and store it for future use or start a side business. In this business investment is not required for the raw material and utilization of the surplus and partially decomposed leaves will reduce labour cost for baroj management.

In this investigation a low-cost, efficient process is presented where in a very short space inside the 2 L round bottom flask of Clevenger apparatus, sizable amount of betel leaves could be accommodated for oil extraction and the oil could be stored in small vial in a very small space for long period of time (Figure 1). If a farmer could purchase a 5L Clevenger apparatus, he/she could extract essential oil on daily or weekly basis and could store it for future use or business. As the farmer usually uses same kind of planting material or landrace, there will be no problem of mixture and the process could be operated for 6 hr also for complete extraction of oil.

In the normal steam distillation process the un-grinded leaf samples are used with a recovery of messy oil-water mixture. The process involves use of a separating funnel for oil recovery. In this investigation the leaves were grinded before placing them inside Clevenger apparatus. This grinding of the leaves to fine particle increases surface contact of the leaf particle with the solvent ensuring greater diffusion of oil facilitating higher oil recovery.[24] For improvisation of the technique the grinding of leaves along with the use of NaCl or cooking salt yields a distinct layer of oil facilitating hassle free separation. The reason may be due to the decreased solubility of essential oil in water because of the existence of NaCl. The NaCl assist in avoidance of emulsion formation of oil as well as it decreases the solubility of some non-polar component to water increasing the quality of extracted oil.[25] In this experiment 10% brine was found more effective than 15% with a recovery volume of 1.82 ml and 1.72 ml respectively.

Figure 1: Cost-effective Steam Distillation process for extracting Betel Leaf Oil (BLEO) from 'Sada Bangla' cultivar.

The fragrant water generated in this process could be re-filtered for separation of additional emulsified oil or could be added in food items for flavour enrichment. In this process 1 kg of leaf sample yields 109g of de-oiled leaf-waste thereby showing a 9 times reduction in the waste load. Additionally, this waste material could be analysed and used for bio-fertilizer production or could be evaluated for utilization in composting processes.[26] The fragrant water generated in this process could be utilized for flavour enhancement of food, air purification or could be filtered and reused for oil recovery.

The accommodation of a small Clevenger apparatus (5L) in a small space is possible within farmer's home and with the use of cooking salt, clean water and waste leaves, could extract BLEO in a 3-hr time-frame. The extracted oil could be stored in an airtight small amber bottle and kept in room temperature or 4°C preservation for at least 3 years. The storing of BLEO is far easier than stacking huge amounts of decomposed leaves in baroj adjoining places. The storage and piling of rotting betel-leaves could generate unpleasant odour and disease-causing pathogens. The consumption of that diseased betel-leaves by bovine animals causes health problem and leads to even death of the animals. The perished leaves could aggravate *Salmonella* infection. *Salmonella* food poisoning could affect the bovine as well as human population and lead to great community health hazard.[27]

Oil extraction is possible from the leaf petioles of betel leaves. The petiole produces good amount of clear transparent oil.[28] The petiole could be stored for 15 days without decomposition but shrinkage of the petioles leads to good amount of oil extraction. In this paper the farmers are recommended to use grinded fresh or partially decomposed betel leaves in 10% cooking salt-based brine for extraction of highest amount of BLEO in a 1:1 solute versus solvent ratio for continuous 3 hr at 80°C constant temperature. This agro-technology ensures a clean and green waste management of vine wastes in time of need along with income generation. The erratic onset of super-clones creates substantial devastation of betel-vines in coastal areas. This process could assist the farmers in partial or complete recovery from their devastation.

Though betel leaves possess some common volatile and non-volatile compounds but every landrace has some specificities. In future the chemo-typing of the extracted oil could reveal the exact use of the BLEO extracted

from 'sada bangla' cultivar. The climatic condition of South 24 Parganas is suitable for the cultivation of commercially desirable cultivars under shade and protected atmosphere inside betel baroj, encouraging production of different biomolecules related to taste and aroma enhancement of betel leaves. Eugenol, chavicol, hydroxyl-chavibetol, safrole, Stearaldehyde, linalool are some of the recognised compounds present and detected in betel leaf oil specifying landraces with authentic identity. Bangla, Meetha, Sanchi, kalibangla, Bagerhati, Jhalpata are distinct clonal variant prevalent in South Bengal.[29]

In a study with Bangla cultivar of betel leaves Madhumita *et al*. 2019, mentioned about the extraction of betel leaf essential oil (BLEO) from fresh and cured leaves and conducted the chemical analysis of the extracted oil. Box-Behnken Design coupled with response surface methodology was employed to estimate the weightage of independent variables towards essential oil production. The study confirmed liquid to solid ratio of (30:1-50:1 mL/g), extraction time (5–7 hr), and particle size (0–20 mesh) as standard for BLEO extraction.[2]

BLEO could be extracted by micro-wave assisted hydro distillation process (MAHD) using different power (watt) and leaf/water (L/W) ratio. The MAHD method shows greater essential oil recovery in less time (90 min) with 500 W power and 0.33 L/W ratio. This method yields 3.48 ml of oil from 1 kg of leaf sample without affecting the quality of the essential oil.[30]

CONCLUSION

In West Bengal several elite cultivars of *Piper betle* are available for inclusion in this oil extraction process. The landrace specific BLEO may show a differential profile and could play significant role in food and pharmacy sector.

Recommendations for farmers are as follows:

1. A 4 ft. X 2 ft. Table with adjoining water inlet/outlet source for operation of the apparatus
2. Initial investment of Rs. 15,000/- for purchase of the Clevenger apparatus and a home Mixer-grinder (Rs. 1,000/-)
3. Extraction of oil from 7-14 days old leaf-sample for less electric consumption after collection of some leaves
4. Grinding with cooking salt (10% brine) and purified water
5. Steam distillation for 3 hr at 80°C constant temperature
6. Collection of oil in air-tight amber bottle
7. Collection of hydro-lysate in separate vessel
8. Use of hydro-lysate in food sector and oil for medical purpose

This method is very simple and could be adopted by small farmers as a safeguard device to mitigate loss under climatic fluctuation and time of market oscillation. The partially decomposed leaves could be used for oil extraction. This farmer-friendly, clean and green, climate resilient agri-waste management technology has immense prospects in income generation for poor farmers and could strengthen the future of Indian cottage industry.

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CONFLICT OF INTEREST

The author declares no conflict of interest and has conceptualized the experiment as a part of her own project, collected the betel-leaf germplasm, leaf samples individually and performed the experiments at her laboratory, recorded and analysed the data and written the manuscript by her own.

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