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Development of Quality Assurance Methods for Medicinal Plants Using Gas Chromatography Analysis

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A method for the quality assurance of medicinal plants using thermal desorption (TD) coupled with gas chromatography (GC) analysis of the volatile oils is described and is demonstrated using the volatile oils from fresh and dried leaves of *Blumea balsamifera*, Linn. (sambong) as an example. The volatile oil obtained using steam distillation (SD) and TD were compared. Using the relative retention times and % relative peak areas of the components, profiles of the GC chromatograms of the volatiles were prepared. Comparison of SD and TD showed that the latter method gives superior results.

Keywords: quality assurance; gas chromatography; thermal desorption; volatile oil; *Blumea balsamifera,* Linn. (sambong)

INTRODUCTION

Herbal medicines are experiencing a revival in interest in both Asian and Western societies. However despite the rapid rise in their use, there is inadequate attention given to quality assurance methods for herbal products. Herbal medicines contain many compounds in a plant cell matrix whereas Western drugs are usually made up of one or a few compounds in a simpler pharmaceutical preparation. Because of the intrinsically different nature of herbal products, it is inappropriate to use the same analytical procedures for both herbal products and standard drug formulations. Therefore, appropriate quality assurance methods must be developed for herbal medicines which can provide information on product quality.

The main chromatographic techniques used for the analysis of the organic composition of medicinal plants are GC, thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) [1–2]. These methodologies are referred to in various literature on the analysis of herbal medicines which are found in research papers, official herbal pharmacopeias from various countries, and government analytical procedures for the determination of authenticity and adulteration. However, what is needed is a convenient procedure which can be used for quality assurance purposes.

Quality assurance refers to the aggregate of methods used to ensure the quality of the product. One important component of quality assurance of herbal medicines is the chemical analysis of the product, in particular the determination of chemical structure and/or quantification of the compounds present. One approach to the quality assurance of herbal plants is to use the identities and relative amounts of compounds as indicators of plant identity and quality. A convenient group of compounds to focus on is the volatile oil. "Volatile oil" refers to a type of essential oil which is obtained through the process of steam distillation [3–4]. In this method, the volatile compounds are vaporized along with steam and condensed using a Clevenger apparatus [5]. The volatile compounds are collected in an organic solvent, dried and injected into the GC. An alternative method is TD using a pyroprobe directly attached to the GC. In the technique of TD, the leaf sample is heated in a sample tube to release the volatile compounds which are then directly introduced into the GC column. Because the TD apparatus is attached directly to the GC injection port, there is more efficient and reproducible introduction of volatile compounds into the GC. TD also minimizes sample cleanup and eliminates the need for solvent [6].

The GC analysis of the volatile oil has a number of advantages. First, the GC of the volatile oil gives a reasonable "fingerprint" which can be used to identify the plant. The composition and relative concentration of the organic compounds in the volatile oil are characteristic of the particular plant and the presence of impurities in the volatile oil can be readily

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detected. Second, the extraction of the volatile oil is relatively straightforward and can be standardized and the components can be readily identified using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The relative quantities of the components can be used to monitor or assess certain characteristics of the leaves. For example, it might be expected that older or dried leaves will lose the more volatile components. Changes in composition of the volatile oil can also be used as indicators of oxidation, enzymatic changes or microbial fermentation.

In a previous paper, we described the method of TD-GC analysis [7]. This study explores the development of a quality assurance method using GC analysis of the volatile oil of *B. balsamifera*, Linn. locally known as sambong. This plant is a shrub that is widely distributed in the Philippines, India, and Southern China, through Malaya and Moluccas [8]. The leaves are traditionally used to cure fever, headache, abdominal pain, and gaseous distention. A number of studies have been reported on the biologically active constituents of sambong [9–10]. In the volatile oil, *l*-borneol and *l*-camphor were reported to account for close to 25% and 75% of the total, respectively, along with trace amounts of cineol, limonene, β-camphene, myrcene and phenol phloracetophenon-dimethyl ether) [12]. In 1987, the National Integrated Research Program on Medicinal Plants (NIRPROMP) recommend that sambong be used to treat hypertension because of its diuretic property [11]. In 1989, clinical studies proposed its use as an anti-urolithiasis agent [12].

Despite the widespread use of sambong as an herb, there is no systematic way of analyzing its constituents for quality assurance. This work attempts to establish the GC profile and the identity of the major compounds of the volatile oil and to develop a quantitative method of using this profile as a tool for quality assurance. Since commercial herbal drugs are usually made using dried leaves, it is important that the drying process be standardized and a method be developed to check on the quality of the dried leaf material.

Two methods were used to obtain the volatile oil: SD using a Clevenger apparatus and TD. Preliminary identification of the major components of the volatile oils was carried out by GC-MS. In some cases, confirmation of identity was done by coinjection using a standard. Both fresh and dried sambong leaf samples were analyzed.

EXPERIMENTAL

Plant material. Fresh and dried leaves of sambong were supplied by Pascual Laboratories from its farm in Sta. Rosa, Nueva Ecija, and by the Philippine Institute of Traditional and Alternative Health Care (PITAHC-DOH) from its farms in Tuguegarao and Quezon. Some samples were also purchased from stalls in Quiapo. See Table 1 for list of sambong samples used in this study.

Table 1. Sambong samples used in the study.

Type	*Source*	*Sample Code*	*Extraction Method**
Fresh leaves	Sta. Rosa, Nueva Ecija	SPF #1	TD
		SPF #2	SD and TD
		SPF # 3	SD and TD
		SPF #4	TD
	Quiapo, Manila	Quiapo #1	TD
		Quiapo # 2	TD
Dried leaves	Sta. Rosa, Nueva Ecija	SPD #2	SD
		SPD #3	SD and TD
		SPD #4	TD
	Tuguegarao, Cagayan	Tug #1	SD and TD
		Tug #2	SD and TD
	Quezon	Quezon	SD and TD

*SD = Steam Distillation; TD = Thermal Desorption

Instrumentation. A Shimadzu 14B gas chromatograph equipped with a flame ionization detector was used for quantitative analysis. The column was a 50-m × 0.2-mm Carbowax 20M capillary column. GC-MS analysis was performed using a Hewlett-Packard 5890 gas chromatograph and a Finnigan MAT 95ST mass spectrometer with the 62k NIST MS library. MS identification was carried out using the reverse-fit algorithm and only matches with rfit values above 900 were considered. The TD procedure was carried out using a CDS 1500 Valved Interface and a CDS 1000 Pyroprobe controller. The pyroprobe was set at 80°C. The sample was packed inside a quartz tube 79-mm in length with a 8-mm diameter.

Chemicals and other materials. Solvents (methanol and isooctane) and standards (α-pinene, limonene, camphor and borneol) were reagent grade.

Extraction using SD. About 5 g of macerated leaves (fresh or dried) were placed separately in a 100 mL round bottom flask. Enough distilled water (~50 mL) was added to cover the leaves. The collection arm of the Clevenger apparatus was cooled with circulating chilled water through a jacket. The arm was filled with water and then ~2 mL of isooctane was added on top of the water to act as the extracting solvent. The volatile oil was obtained by steam distillation for 1 h. The isooctane distillate solution was dried over sodium sulfate and was analyzed by injection into the GC.

Extraction using TD. About 0.3 to 0.7 g of leaves (fresh or dried) was packed into the quartz tube with anhydrous sodium sulfate. Both ends of the quartz tube were plugged with glass wool. The sample tube was placed inside the TD apparatus. The volatiles were thermally released at 80°C and carried into the GC column by helium gas. The volatilized organics were cryotrapped in a loop of the capillary column which was dipped in liquid nitrogen (-196°C). After a 3-min thermal extraction, the loop was allowed to warm and the GC oven was heated using the temperature program described below. This was set as zero time.

GC analysis. The volatile oils obtained from SD and TD were analyzed using the following GC conditions: injector temperature (220°C); detector temperature (240°C); split (50 mL/min); purge (5 mL/min); column program-initial temperature (60°C); initial time (1 min); and program rate (5°C/min); final temperature (220°C); final time (27 min). The same conditions were used for both GC-FID and GC-MS analyses.

RESULTS AND DISCUSSION

Figure 1 to Figure 4 show the chromatograms of the volatile oil from sambong using the SD and TD methods. Although numerous peaks are detected, only peaks with heights above 2% relative height were considered in the analysis. Each sample was analyzed at least twice. Some general observations can be made for both fresh and dried leaves analyzed using SD or TD: 1) The various chromatograms consistently showed 9 major peaks (>2% relative intensity). The fresh leaves gave more peaks than the dried leaves; 2) The sample obtained by SD contains a large solvent peak below around 6 min. Because of this, no GC results can be obtained before this time; 3) Because of the different methods of sample introduction, there is a slight variation in the absolute retention times of volatile oil samples obtained by SD and those obtained by TD which were injected with a syringe. However, the general pattern of the GC peaks is the same.

Identification of the volatile components of sambong. Nine major compounds were found in the volatiles of sambong. Preliminary identification of the peaks was carried out by mass spectral matching using the MS library. For some of the compounds, further confirmation of identity was done by coinjection with a standard compound. For compounds without standard, the identity was rationalized by analysis of the fragmentation patterns as proposed by McLafferty [13]. Seven of the peaks were identified through MS peak matching. Of these, four were confirmed by coinjection with standards (Table 2). The following components consistently gave peaks above 2% intensity in the fresh and dried leaves: α-pinene,

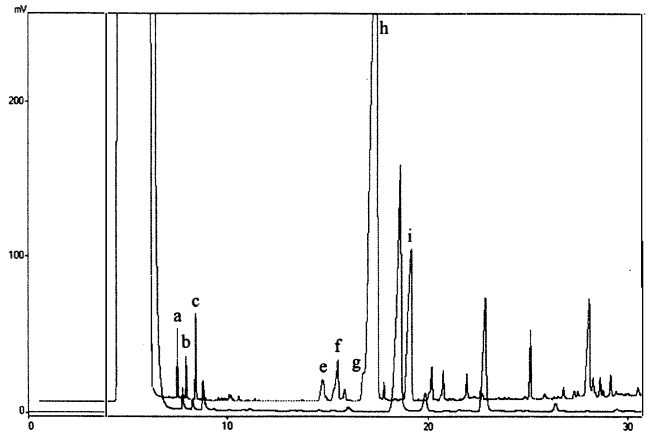

Fig. 1. Examples of GC chromatograms of the oil obtained from fresh sambong leaf samples by SD. (Top: Quiapo #1; Bottom: Quezon).

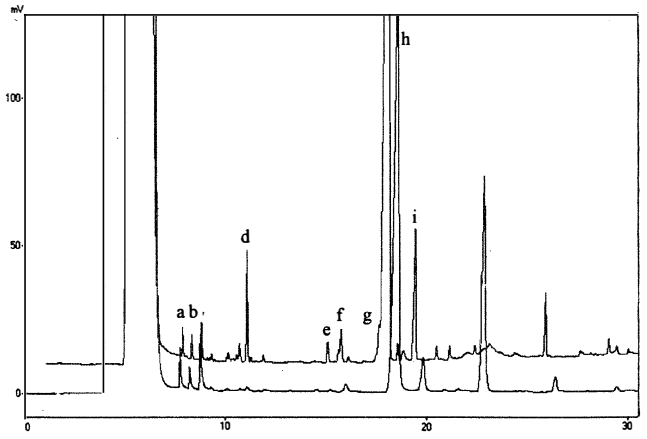

Fig. 2. Examples of GC chromatograms of the oil obtained from dried sambong leaf samples by SD. (Top: Quiapo #2; Bottom: Tuguegarao #1).

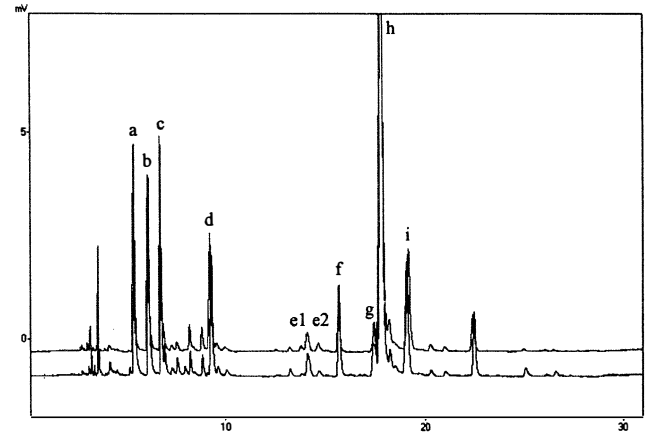

Fig. 3. Examples of GC chromatograms of the oil obtained from fresh sambong leaf samples by TD. (Top: SPF #2; Bottom: SPF #3).

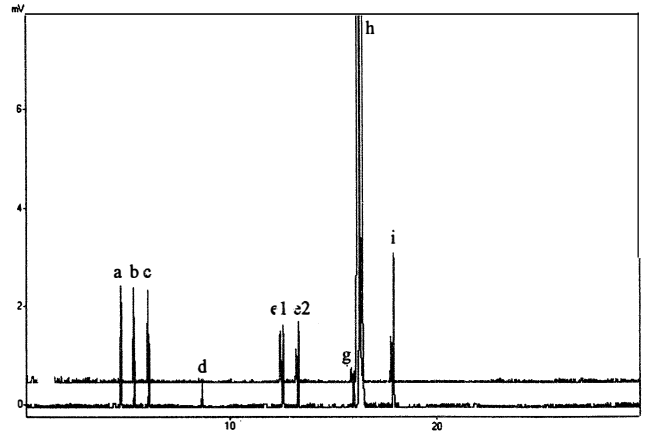

Fig. 4. Examples of GC chromatograms of the oil obtained from dried sambong leaf samples by TD. (Top: SPF # 2; Bottom: SPF #4).

Table 2. The identity of volatile components of the leaves of *Blumea balsamifera*.

Peak	*Assignment*	*Structure*	*Approximate Retention Time (min)**	*Comments***
a	α-pinene		By injection: 7.0 By TD: 5.1	rfit = 983 Identity was confirmed by injection with standard α-pinene.
b	camphene		By injection: 7.5 By TD: 5.7	rfit = 979
c	β-pinene		By injection: 8.0 By TD: 6.4	rfit = 978
d	limonene		By injection: 10.3 By TD: 8.8	rfit = 973 Identity was confirmed by injection with standard limonene.
e	3-carene		By injection: 14.4 By TD: 13.0	rfit = 949
f	not identified		By injection: 15.1 By TD: 14.2	
g	not identified		By injection: 16.2 By TD: 16.6	
h	camphor		By injection: 17.4 By TD: 16.9	rfit = 935 Identity was confirmed using standard camphor.
i	borneol		By injection: 18.8 By TD: 18.3	rfit = 924 Identity was confirmed by injection with standard borneol.

*GC conditions: Column: 50-m × 0.2-mm Carbowax 20M. Injector temperature: 220°C; detector temperature: 240°C. Column program: initial temperature: 60°C; initial time: 1 min; program rate: 5°C/min; final temperature: 220°C; final time: 27 min.
**MS match was carried out using the 62,000 NIST MS library using reverse fit (rfit) algorithm. Highest value=1000.

camphene, β-pinene, limonene, carene, camphor and borneol. The compound occurring in highest amount in the volatile oil was camphor. Two compounds could not be identified.

GC profiles. The GC chromatograms can be compared using a plot of the % relative peak areas of the components (y-axis) versus the relative retention times (x-axis). (See Figs. 5 to 10). The % relative peak area and relative retention time for each peak were computed based on the largest peak, h (camphor). That is, the relative retention time for peak x was calculated based on the retention time of peak h as follows:

Relative retention time = [(retention time of peak x)/(retention time of peak h)].

The % relative peak areas of the various components were likewise compared against the peak area of h which is the largest peak. The reference peak, h (camphor), therefore has a relative retention time and % relative peak area of 1.0 and 100%, respectively. The bars represent the standard deviation of the values obtained from at least eight GC runs. This plot represents the GC profile of a particular sample.

It can be seen from the GC profiles that the standard deviation for the relative retention times of the peaks ranges from 0 to 0.01. This small variation in the relative retention time indicates that this is a reliable indicator for the identity of the components.

In contrast, there was a larger variation observed in the % relative peak areas, and that these values varied consistently according to type sample and mode of extraction of the volatile compounds. For peaks g (unidentified) and i (borneol), there were bigger standard deviations observed. In the case of peak g, this is due to the fact that this peak is not always well resolved from peak h (camphor). On the other hand, the relatively large variation in the % relative peak area of borneol (peak i) may be due to the tendency of borneol to be oxidized to camphor (peak h). This should be studied further.

Assuming that these samples represent standard fresh sambong material, this profile can be used as a reference against which other sambong samples can be compared. This profile gives the range for which the GC profile of any given "good" sambong sample should fit.

Extraction by SD and GC analysis. Figure 1 shows the GC chromatograms of the fresh leaf samples extracted using SD and the corresponding GC profile is shown in Figure 5, while Fig. 2 and Fig. 6 show the corresponding chromatograms and profiles for the dried leaves. The same components were found in both the fresh and dried leaves.

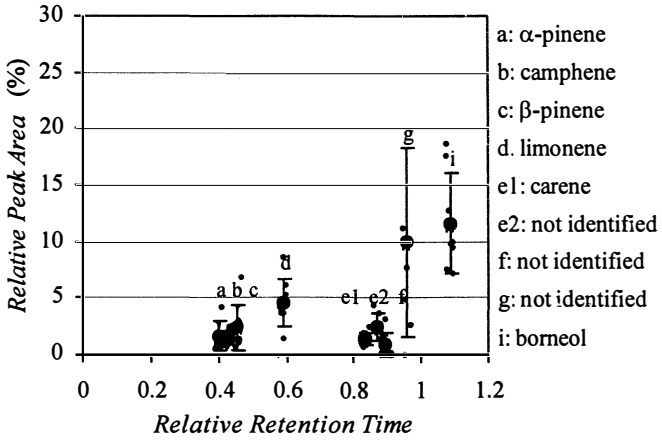

Fig. 5. GC profile for the volatile oil obtained from fresh sambong leaves by SD. The GC profile is generated from eight samples. See Fig. 1 for corresponding GC chromatograms. Camphor (h) *is the reference compound with % relative peak area of 100% and relative retention time of 1.0. It is out of range and is not seen in the plot.*

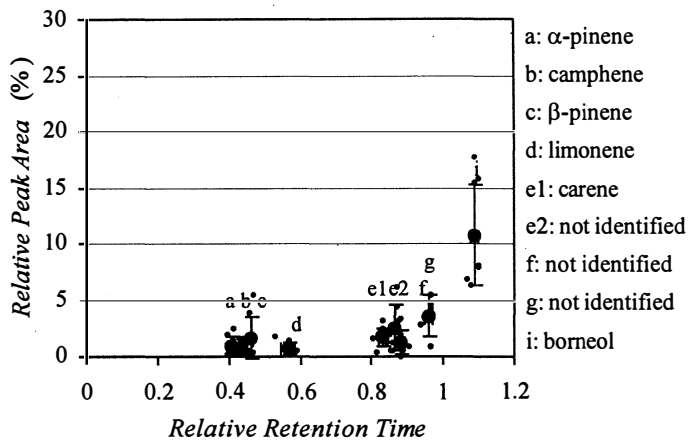

Fig. 6. GC profile for the volatile oil obtained from dried sambong leaves by SD. See Fig. 2 for corresponding GC chromatograms.

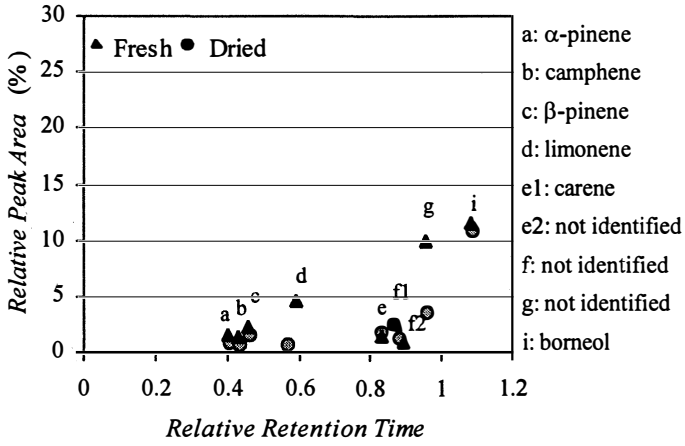

Fig. 7. Comparison of the GC profiles for the volatile oils obtained from fresh (s) *and dried* (l) *sambong leaves by SD* (Fig. 5 and Fig. 6, respectively). *Note that there is only a slight difference in the GC profiles of the volatile oils obtained from fresh and dried sambong leaves by SD.*

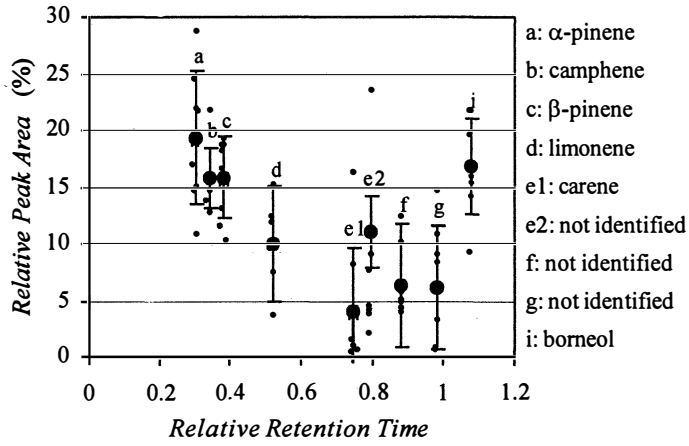

Fig. 8. GC profile for the volatile compounds obtained from fresh sambong leaves byTD. See Fig. 3 for corresponding GC chromatograms.

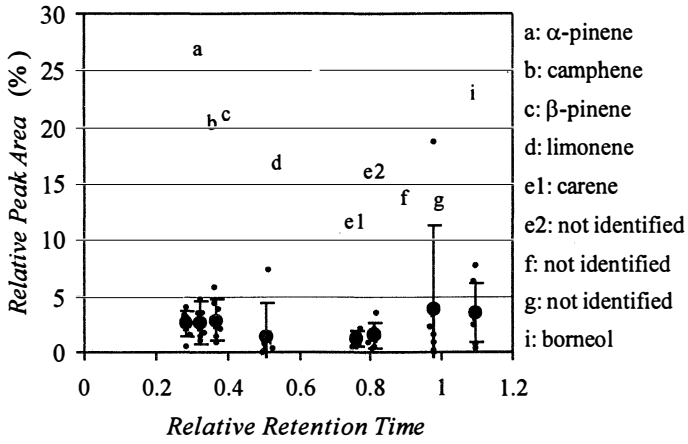

Fig. 9. GC profile for the volatile compounds obtained from dried sambong leaves by TD. See Fig. 4 for corresponding GC chromatograms.

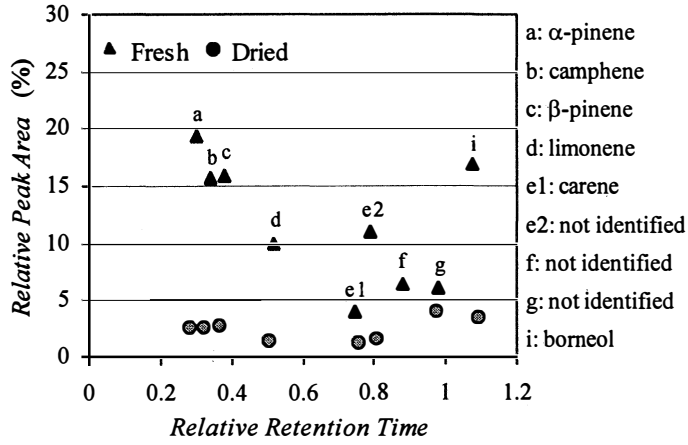

Fig. 10. Comparison of the GC profiles of the oil obtained from fresh (s) *and dried* (l) *sambong leaves by TD* (Fig. 8 and Fig. 9, respectively). *Note the significant differences in the profiles for the volatiles from fresh and dried sambong leaves obtained by TD.*

Table 3. Range of % relative peak areas as determined from the GC analysis of volatile compounds from sambong using SD. The range of values was determined from eight determinations involving four leaf samples.

Compound	% Relative Peak Areas Using Steam Distillation			
	Fresh Leaves		*Dried Leaves*	
	Range of Values	*Mean*	*Range of Values*	*Mean*
α-pinene	0.52–4.25	1.61	0.20–2.45	0.86
camphene	0.70–2.42	1.46	0.28–1.53	0.72
β-pinene	0.78–6.83	2.38	0.24–5.41	1.57
limonene	1.41–8.57	4.56	0.23–1.74	0.83
3-carene	0.72–2.37	1.40	0.37–3.16	1.66
Camphor (ref.)	100	100.00	100	100.00
borneol	7.21–18.68	11.60	6.84–17.76	10.16

Table 4. Range of % relative peak areas as determined from the GC analysis of volatile compounds from sambong using TD. The range of values was determined from eight determinations involving four leaf samples.

Compound	% Relative Peak Areas Using Thermal Desorption			
	Fresh Leaves		*Dried Leaves*	
	Range of Values	*Mean*	*Range of Values*	*Mean*
α-pinene	10.94–28.80	19.36	0.59–9.29	3.88
camphene	13.87–21.73	15.77	1.0–6.0	3.25
β-pinene	10.35–19.22	15.85	0.92–14.12	4.12
limonene	3.72–15.33	10.02	0.04–4.09	0.92
3-carene	1.59–16.33	5.71	0.07–1.71	1.10
Camphor (ref.)	100	100.00	100	100.00
borneol	9.31–21.82	16.87	0.43–7.68	4.26

Table 3 summarizes the % relative peak areas of the components using steam distillation and Fig. 7 compares the GC profiles of the fresh and the dried leaves. The comparative plot shows that the % relative peak areas of the components in the dried leaves samples are only slightly lower than those in the fresh leaves, and the difference is not significant. Thus, SD is not an effective method for distinguishing fresh from dried leaf samples.

Extraction by TD and GC analysis. Figures 3 and 4 show the GC chromatograms of the fresh and dried leaf samples using TD and Fig. 8 and Fig. 9 plot the corresponding GC profiles. Peaks e1, e2 and f could not be identified. The GC chromatogram shows that a higher relative yield of the more volatile components (peaks a-d) is obtained with TD compared with SD. This may be due to the higher loss of volatile compounds in the SD due to the extended heating time and solvent extraction. Also, the TD method is expected to have a more efficient transfer of volatile components into the GC.

Table 4 summarizes the % relative peak areas of the components using TD and Figure 10 compares the GC profiles of the fresh and the dried leaves.

SD versus TD. Tables 3 and 4 summarize the results obtained from the GC analysis of volatile compounds from sambong using SD and TD and a visual comparison of the profiles for the volatile compounds from fresh and dried sambong leaves obtained by TD is presented in Figure 10. The comparison shows that TD is able to differentiate between the fresh and the dried leaves due to the large difference in their relative peak areas. Thus the TD method appears to be sensitive to the state of the plant material.

CONCLUSION

The results of the GC analysis can be summarized as follows: The identities of the components can be readily confirmed using their relative retention times. There is a small difference in the GC profiles of the volatile oil of fresh and dried plant material obtained by SD. Therefore, SD is not a sensitive method of extraction for this purpose. In contrast, the TD method can clearly differentiate among the different states of the sample, such as between fresh and dried samples. Since no solvent is used with the TD method, interferences due to solvent are not encountered.

The following major components were identified in the fresh and dried leaves using both methods of SD and TD: α-pinene, camphene, β-pinene, limonene, carene, camphor and borneol. The compound occurring in highest amount in the volatile oil is camphor. Because all of the major volatile components are monoterpenes, its composition is sensitive to the status and age of the plant material. Using the method of TD, the relative amount of borneol was observed to be lower in the dried leaves as compared with the fresh leaves. Since borneol can be oxidized to camphor, this might account for the lower relative amount of borneol in the dried leaves. This suggests the possibility of using the borneol:camphor ratio as an indication of leaf condition. This should be further studied. SD, on the other hand, did not yield any significant difference in the relative amount of borneol in the fresh and dried leaves.

This work illustrates the potential of the use of TD for generating the GC profile of the volatile compounds in fresh and dried leaf samples for quality assurance.

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REFERENCES

1. Ajura, S. *Am. Chem Soc.* (Washington, D.C., 1992).
2. Watson, C.A. (editor). *Official and Standardized Methods of Analysis*, 2nd edition. (The Royal Society of Chemistry, 243, 1994).
3. Lewis, R.J. (editor). *Hawley's Condensed Chemical Dictionary*, 13th edition. (Van Nostrand Reinhold, 454, 1997).
4. The United States Pharmacopoeia, 22nd revision. (Supplement 3, Rockville, MD, 20852, 2395 1990).
5. *1981 Annual Book of ASTM Standards, Part 29*, "Standard Test Method for Volatile Oil in Rosin." (American Society for Testing and Materials, 1916 Race St., Philadelphia, PA 19103, 982 1981).
6. SIS Thermal Desorption: http://www.sisweb.com
7. Dancel, M.C.A., Niñonuevo, M.R., Teotico, and D.G., Dayrit, F.M. *Kimika.* 16 (2), 19 (2000).
8. Quisumbing, E. *Medicinal Plants of the Philippines*, (Katha, Quezon City, 1978) 964.
9. Fujimoto, Y. and Agustein, S. *J. Plant Biochem.* 1017 (1987).
10. Kong, Y.C., Hu, S.Y., Lau, F.K., Che, C.T., Yeung, H.W., Cheung, S, and Hwang, J. *Amer. J. Chinese Med.* 4, 105 (1976).
11. NIRPROMP, Philippine Council for Health Research and Development, DOST, unpublished research report (1987).
12. Urlanda, R.V. *Philippine PANORAMA.* (Manila, 1994) 10.
13. McLafferty, F.W. *Interpretation of Mass Spectra*, 3rd edition. (W.A. Benjamin, Inc., Massachusetts, 1973).