Bud, leaf and stem essential oil composition of clove (*Syzygium aromaticum* L.) from Indonesia, Madagascar and Zanzibar

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Abstract

Cloves (*Syzygium aromaticum*) essential oil is widely used in dental care, as an antiseptic and analgesic and is effective against a large number of bacteria. The major component of clove oil is usually considered to be eugenol, with β-caryophyllene and eugenyl acetate, being present although in lower concentrations. A review of published results reveals a great variability in the chemical composition of clove essential oils. The purpose of this study is to compare the bud, leaf and stem essential oil compositions from *S. aromaticum* and then to evaluate the correlation of the results with anatomic and geographical origins of the essential oil.

121 commercial essential oils isolated from bud, leaf and stem were used in this work. The oils were analyzed by GC and ten constituents were identified from the whole. The major constituent in bud, leaf and stem oils was eugenol, with increasing percentages from bud (72.08 - 82.36%) to leaf (75.04 - 83.58%) and stem (87.52 - 96.65%). In the clove bud essential oil eugenyl acetate is the second major component (8.6 - 21.3%) while detected in considerably lower amount in the leaf (0 - 1.45%) and stem (0.07 - 2.53%)

In leaf essential oil, the second main compounds were β-caryophyllene (11.65 - 19.53%) and α-humulene (1.38 - 2.17%), less represented in bud essential oils (2.76 - 8.64% and 0.34 - 1.04% respectively) and in stem essential oils (1.66 - 9.7% and 0.22 - 1.31% respectively).

For each plant material, variation in the percentage of the main constituents was observed according to the sample geographic origin.

Key words:

Syzygium aromaticum; bud, leaf and stem oils; β-caryophyllene; chemical composition; eugenol; eugenyl acetate; geographic origins

Introduction

The clove tree [*Syzygium aromaticum* (L.) Merril and Perry.; syn. *Eugenia caryophyllus* (S.) Bullock and S.G. Harrison, Myrtaceae] is a perennial tropical plant which grows to a height ranging from 10 to 20 m, having large oval leaves and crimson flowers in numerous groups of terminal clusters. The clove tree is native to Moluccas Island (Indonesia) [1]. Two major products are available and marketed from clove tree: the clove which is the unopened green fully-grown buds, upon drying, and the essential oil extracted either from bud, leaf or stem [2].

In the early eighteenth century, the clove tree was introduced in different parts of the world: Zanzibar, India and Madagascar [3 - 5]. Today the most important producers of cloves are Indonesia (which is also, the main consumer), Tanzania (Zanzibar and Pemba islands) and Madagascar, which is the first world exporter with annual average exported quantities of 11000 tons for cloves and 1500 tons for essential oils [6].

Cloves can be used in cooking, either whole or in ground form. The spice is used throughout Europe and Asia and is smoked in cigarettes also known as “kreteks” in Indonesia. Cloves are also an important incense material in Chinese and Japanese cultures [7].

The essential oil is widely used and well known for its medicinal properties. Traditional uses of clove oil include use in dental care as an antiseptic and analgesic [7], [8]. The oil is active against oral bacteria associated with dental caries and periodontal diseases [9] and is effective against a large number of other bacteria: *Escherichia coli*, *Salmonella enteric* [10] and *Staphylococcus aureus* [11-13]. Previous studies have reported antifungal [14], anticarcinogenic [15], antiallergic [8], antimutagenic [16], antioxidant [17] and insecticidal [18], [19] properties. The chief constituent of clove oil is eugenol, which is used as a starting material for the production of vanillin [20].

Research studies carried out on bud oil [7], [20 - 31], leaf oil [20], [30], [32], [33] and stem oil [25] of *S. aromaticum*, from different parts of the world showed that the major component of these three types of clove oils is usually considered to be eugenol, followed by β-caryophyllene and in lesser amounts, α-humulene, caryophyllene oxide and eugenyl acetate, although in different concentrations.

A comparison of the published results reveals a great variability in the chemical compositions of the clove essential oils. E.M. Gaydou, R.P. Randriamiharisoa. (1987) [25] and R.J. Hector, J.E. Simon (2004) [26] found in bud essential oil the content of eugenol to be 77.10% and 74.4%, respectively, with β-caryophyllene at 11.20% and 7.5% and eugenyl acetate at 7.38% and 15.8%; whereas G. Fichi, G. Flamini, F. Giovanelli, D. Otranto, S. Perrucci (2007) [23] found that the content of eugenol was 59.3%, with the concentration of β–caryophyllene to be 24.9% and eugenyl acetate at 4.2%. The major constituents of Portugal [29] and Cuba [30] bud oil were eugenol (85.3 vs 69.9% respectively) and β -caryophyllene (0.9 vs 13%). Out of the two major constituents which were common to Portugal and Cuba bud oil, significant differences were observed with respect to eugenyl acetate (0 and 16.1%) and α-humulene (6.8 vs 0.6%).

Other works carried out on the leaf essential oils of *S. aromaticum* show the same variability of content of the major constituents. In the essential oils of V.K. Raina, S.K. Srivastava, K.K. Aggarwal, K.V. Syamasundar, S. Kumar (2001) [33] and A.K. Srivastava, S.K. Srivastava, K.V. Syamsundar (2005) [20], the principal constituent was eugenol (94.4 vs 82%, respectively), followed by β-caryophyllene (2.9 vs 13%), α-humulene (0.3 vs 1.5%) and eugenyl acetate (0 vs 0.4%).

Little literature describes the chemical composition of clove stem oils. E.M. Gaydou, R.P. Randriamiharisoa, (1987) [25] assumed that eugenol (80.80%), β-caryophyllene (10.5%), α-humulene (1.26%) and eugenyl acetate (4.40%) were the main compound of stem essential oil of *S. aromaticum* from Madagascar.

In all cases, papers demonstrated a great variability of chemical composition of *S. aromaticum* essential oil, without studying the determinants of this variability. As reported in the literature, for others species, such as *Ravensara aromatica* [34], *Cedrelopsis grevei* [35] and *Cinnamosma fragrans* [36], many factors such as the geographical origin, the plant material, genetic factors and the season at which the plants were collected may be responsible for the chemical composition of the essential oil.

The purpose of this study is to compare the composition of bud, leaf and stem essential oils from *S. aromaticum* and then to evaluate the correlation of anatomic origins of the essential oil and their geographic origins.

Experimental

Essential oil

The clove essential oils of *S. aromaticum* used in this study were commercial samples provided by either industrial exporting companies of Indonesia (12 samples), of Madagascar (94 samples), and of Zanzibar (15 samples) (Table 5). The essential oil was obtained by a steam- distillation method by using an industrial type distiller for 12 hours. The essential oil samples were dried over anhydrous sodium sulfate (Na_2SO_4) and stored in a cool and dark chamber until the analysis.

Analysis

Gas Chromatography

The 121 essential oil samples were analyzed by gas chromatography (GC). Analyses were performed on a Hewlett-Packard gas chromatograph, model GC 6890, with flame ionization detectors fitted with two silica capillary columns: HP-INNOWax(100% Polyethylene Glycol/ Agilent 19091N-113) capillary column (30 m . 0.32 mm i.d., 0.25 µm film thickness) and HP-5 (5% Phenyl Methyl Siloxane / Agilent 19091J-413) capillary column (30 m . 0.32 mm i.d., 0.25 μm film thickness); carrier gas, He; constant flow, 1.9 ml/min; injection type, split, 1:55 (0.2 μl pure essential oil); injector temperature, 250 °C; detector temperature, 250 °C; temperature was programmed, 65 - 250 °C at 5 °C/min. All GC analyses were performed in triplicate, indicating a reproducibility of at least 3% in the relative percentages.

Identification and quantification of components

Component identification was carried out by comparison of the retention data (determined relatively to the retention times of a series of n-alkanes) with those of the data library [38]. Quantitative analysis of each oil component, expressed in relative percentages of area, was carried out by peak area normalization measurements.

Data analysis

The distribution of the 121 samples was analyzed by principal component analysis (PCA) using the XLSTAT Version 2011 statistical software package. The data set was composed of the values taken by 10 variables identified by GC and the 121 clove oil samples. PCA was performed as it is among the best-known multivariate analysis methods for correlation variable determination [34], [35]. Only the first two principal components were considered since they can explain up to 50% of the total variance.

Results and Discussion

The clove bud essential oil (45 samples), leaf essential oil (32 samples) and stem essential oil (44 samples) of *S. aromaticum* were analyzed by GC. Ten constituents were identified and quantified, as shown in table 1. Great variability in the chemical compositions of the three essential oils was observed. In all oils of *S. aromaticum*, eugenol was the major constituent, with increasing percentages from bud (77.81%) to leaf (81.21%) and to stem

(91.83%). In the bud essential oil this compound is followed by eugenyl acetate (15.15%), while in the leaf and stem the latter was detected in considerably lower amounts (0.82% and 0.74% respectively). In the leaf essential oil, the second main compounds were β-caryophyllene (13.99%) and α-humulene (1.57%) less represented in the bud essential oil (4.44% and 0.54% respectively) and in the stem essential oil (5.24% and 0.66% respectively).

To highlight this variability in the chemical compositions of clove bud, leaf and stem essential oils, PCA were carried out on all 121 samples considering 10 variables (the 10 components identified by GC). The results of the statistical analysis were as follows:

The first two principal components (Figure 1) extracted by PCA explains 60.90% of the variability of which 37.69% was represented by PC1 and 23.21% by PC2. PC1 is principally structured by α-humulene, β-caryophyllene and caryophyllene oxide with a positive correlation (0.88. 0.87 and 0.67 respectively) and one variable with negative correlation eugenyl acetate (- 0.80). PC2 is principally structured by eugenol with a positive correlation (0.94).

The PCA according to planes PC1 and PC2 of the figure 2 and table 1 made it possible to distribute the 121 samples analyzed into three groups called respectively G_I, G_{II} and G_{III}:

- G_I (represented by circles) is constituted by all stem essential oil samples (n = 44). This group is characterized by high eugenol content (91.83%).
- G_{II} (represented by squares) is composed of all leaf essential oil samples (n= 32), mainly containing eugenol (81.21%) and is distinguished from G_I and G_{III} in its high content of β-caryophyllene (13.99 vs 5.24 and 4.44%) and α-humulene (1.57 vs 0.66 and 0.54%).
- G_{III} (represented by triangles) is constituted by all samples of clove bud essential oil (n= 45). This group differs to G_I and G_{II} by lower percentage of eugenol (77.81%) and high content of eugenyl acetate (15.15 vs 1.45 and 0.74%).

Our results are in agreement with what have been found in other studies while demonstrating the differences between clove bud, leaf and stem essential oils using the percentage of eugenol, β-caryophyllene, eugenyl acetate and α-humulene [20], [25], [30], [37].

In this section, we compare the chemical composition of *S. aromaticum* essential oils from Indonesia, Madagascar and Zanzibar for each plant material.

Clove bud oil

Clove bud oil (39 samples from Madagascar and 6 from Indonesia) were analyzed by GC and 10 constituents were identified and quantified (Table 2).

The major constituents of Madagascar and Indonesia bud oils were eugenol (77.50 and 79.87% respectively) and β–caryophyllene (4.06 and 6.91% respectively). Out of these two constituents which were common to Madagascar and Indonesia bud oil, significant difference was observed with respect to eugenyl acetate (16.01 vs 9.56% respectively).

To highlight this difference in the chemical composition of clove bud essential oils from Madagascar and Indonesia, PCA were carried out on all 45 samples combined, considering 10 variables which are the 10 components identified by GC. The statistical analysis results were as follows:

The first two principal components (PCs) extracted by PCA explained 53.98% of the variability of which 38.02% was represented by PC1 and 15.96% by PC2. On figure 3 we observe the projection of the ten variables of our study on the first two PC axes. PC1 is principally structured by eugenyl acetate with a negative correlation (-0.82) and one variable with positive correlation β-caryophyllene (0.84).

The PCA according to planes PC1 and PC2 in figure 4 and table 2 made it possible to distribute the 45 samples of bud oil analyzed into two clusters called respectively Cl_1B and

Cl_2B. In both clusters, eugenol, β–caryophyllene and eugenyl acetate are also the major compounds, although in different amounts.

- Cl_1B (unfilled triangles) is constituted of 39 samples from Madagascar. In this cluster, eugenol, β–caryophyllene and eugenyl acetate were detected at 77.50, 4.06, and 16.01 respectively.
- Cl_2B (filled triangles) constituted of 6 samples from Indonesia was characterized by eugenol (79.87%), β–caryophyllene (6.91%) and eugenyl acetate (9.56%).

These results are in agreement with those from A.K. Srivastava, S.K. Srivastava, K.V. Syamsundar (2005) [20] while still demonstrating the variability among clove bud oils. The authors mentioned above compared the composition of essential oils from Madagascar with those from India using eugenol (82.6 vs 70.0%), β- caryophyllene (7.2 vs 19.5%) and eugenyl acetate (6.0 vs 2.1%) contents. The comparison of our results using bud oils from Madagascar with those earlier reported by R.P. Randriamiharisoa, E.M. Gaydou (1987) [31], E.M. Gaydou, R.P. Randriamiharisoa (1987) [25] and R.J. Hector, J.E. Simon, (2004) [26] for Madagascar bud oils, clearly showed similarity to a certain extent in the percentage composition of the main constituents, eugenol (80.6, 77.10 and 74.7 %), β- caryophyllene (10.5, 11.20 and 7.5%) and eugenyl acetate (7.38, 6.6 and 15.8%).

Clove leaf oil

Clove leaf oil (28 samples from Madagascar and 4 from Indonesia) were analyzed by GC and 10 constituents were identified and quantified (Table 3). While comparing chemical composition of Madagascar leaf oil samples with those of Indonesia, variation in the contents of main constituent, eugenol (81.84 vs 76.78%), β-caryophyllene (13.40 vs 18.09%) and eugenyl acetate (0.93 vs 0.04%) was observed.

To highlight this variation in the chemical composition of clove leaf essential oils, from Madagascar and Indonesia, PCA were performed on the 32 samples combined considering 10 variables (the 10 components identified by GC). The statistical analysis results were as follows:

The first two principal components (Figure 5) extracted by PCA explains 71.47% of the variability, of which 61.91% was represented by PC1 and 13.57% by PC2. PC1 is principally constituted of eugenol (-0.84) and eugenyl acetate (-0.75) with negative correlations and one variable, β-caryophyllene with positive correlation (0.96).

The PCA according to planes PC1 and PC2 of figure 6 and table 3 made it possible to distribute the 32 samples of leaf oil analyzed into two clusters called respectively Cl_1L and Cl_2L:

- Cl_1L (unfilled squares) constituted by 28 samples from Madagascar, contains eugenol, β-caryophyllene and eugenyl acetate at 81.84, 13.40 and 0.93% respectively.
- Cl_2L (filled squares) comprises 4 samples from Indonesia. This cluster is characterized by lower eugenol content (76.78%) and higher β-caryophyllene content (18.09%) than that of Cl_1L.

It was reported earlier that clove leaf essential oil from Madagascar [20] was characterized by higher eugenol content than from Indonesia [2] (82.0 and 71.0% respectively). β-caryophyllene and α-humulene were detected at 14.0 and 1.75%, respectively in the leaf oils from Indonesia [2], whereas in the leaf oil from Madagascar [20], these compounds were at 13.0 and 1.5%. It is interesting to note that eugenyl acetate was present in substantial quantity (0.4%) in the oil from Madagascar, while this constituent was either absent or present only in traces in the leaf oil from Indonesia.

Similarly, leaf oil from Little Andaman [33] was quite different from those from Indonesia [2] in respect to its eugenol (94.4 vs 71.0%), β-caryophyllene (2.9 vs 14.0%) and α-humulene (0.36 vs 1.75%) contents, respectively. On the other hand, leaf oils from Little Andaman [33] matched to a great extent with the leaf oil from south India reported by M.

Gopalakrishnan, C.S. Narayanan, A.G. Mathew (1988*)* [37] in its eugenol content (94.4 and 95.2%). β-caryophyllene (2.9%) and caryophyllene oxide (0.67%) were present in the oils from Little Andaman, while either absent or in trace amount in the leaf oils from south India. Eugenyl acetate (1.5%) was present only in the oils from south India.

Clove stem oil

Clove stem oil (27 samples from Madagascar, 2 samples from Indonesia and 15 samples from Zanzibar) were analyzed by GC and 10 constituents were identified and quantified (Table 4). In all stem oils, eugenol was the principal constituent, with decreasing percentages from Madagascar (93.91%), Indonesia (89.02%) to Zanzibar (88.46%). This compound is followed by β-caryophyllene with increasing contents (3.38, 7.58 to 8.27 % respectively).

To highlight this variation in the chemical composition of clove stem essential oils, from Madagascar, Indonesia and Zanzibar, PCA were performed on all 44 samples considering 10 variables (10 components identified by GC). The statistical results were as follows:

The first two principal components (PC1/PC2, Figure 7) extracted by PCA explains 61.02% of the variability, of which 43.38% was represented by PC1 and 17.63% by PC2. PC1 is principally structured by eugenol (-0.93), with a negative correlation and one variable, β-caryophyllene with positive correlation (0.95).

The PCA according to the PC1 and PC2 planes in figure 8 and table 5 made it possible to distribute the 44 stem oil samples analyzed into two clusters Cl_1S and Cl_2S respectively:

- Cl_1S (unfilled circles) contains 27 samples from Madagascar. This cluster is characterized by high eugenol content of (93.91%) and lower content of β–caryophyllene (3.38%).

-	Cl_2S (filled circles) comprises 2 samples from Indonesia and 15 samples from Zanzibar (grey filled circle) which contains eugenol (89.02%, 88.46% respectively)and β – caryophyllene (7.58, 8.27% respectively)

Little literature describes the chemical composition of clove stem oils. E.M. Gaydou, R.P. Randriamiharisoa (1987) [25] assumed that stem essential oil of *S. aromaticum* is characterized by high eugenol content (up to 70%).

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Tables

Table 1: Chemical composition (relative percentages, extreme values and means) of clove bud, leaf and stem essential oils of S. aromaticum

	Bud			Leaf			Stem		
Component	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
eugenol	72.08	82.36	77.81	75.04	83.58	81.21	87.52	96.65	91.83
β-caryophyllene	2.76	8.64	4.44	11.65	19.53	13.99	1.66	9.70	5.24
α-humulene	0.34	1.04	0.54	1.38	2.17	1.57	0.22	1.31	0.66
eugenylacetate	8.60	21.30	15.15	0.00	1.45	0.82	0.07	2.53	0.74
caryophyllene oxide	0.06	0.37	0.22	0.05	0.55	0.35	0.14	0.68	0.33
α-copaene	0.00	0.27	0.07	0.00	0.24	0.07	0.00	0.27	0.08
methyl eugenol	0.00	0.08	0.02	0.00	0.24	0.07	0.00	0.15	0.07
iso eugenol	0.00	0.24	0.04	0.00	0.24	0.07	0.00	0.80	0.06
chavicol	0.00	0.24	0.17	0.00	0.13	0.04	0.00	0.22	0.05
methyl Salicylate	0.00	0.32	0.14	0.00	0.00	0.00	0.00	0.56	0.29

Table 2: Chemical composition (relative percentage, extreme values and means) of bud essential oil from Madagascar and Indonesia

	Madagascar			Indonesia		
Component	min	max	mean	min	max	mean
eugenol	72.08	80.71	77.50	77.32	82.36	79.87
β-caryophyllene	2.76	6.38	4.06	5.34	8.64	6.91
α-humulene	0.34	1.04	0.50	0.65	1.04	0.83
eugenylacetate	11.68	21.30	16.01	8.60	10.55	9.56
caryophyllene oxide	0.10	0.37	0.22	0.06	0.32	0.19
α-copaene	0.00	0.11	0.05	0.17	0.27	0.21
methyl eugenol	0.00	0.06	0.02	0.04	0.08	0.06
iso eugenol	0.00	0.20	0.04	0.02	0.24	0.08
chavicol	0.00	0.24	0.17	0.13	0.18	0.15
methyl salicylate	0.00	0.32	0.15	0.04	0.16	0.10

Table 3: Chemical composition (relative percentage, extreme values and means) of leaf essential oil from Madagascar and Indonesia

	Madagascar			Indonesia		
Component	min	max	mean	min	max	mean
eugenol	80.87	83.58	81.84	75.04	77.54	76.78
β-caryophyllene	11.65	15.02	13.40	17.04	19.53	18.09
α-humulene	1.39	1.67	1.51	1.93	2.17	2.00
Eugenyl acetate	0.29	1.45	0.93	0.00	0.06	0.04
caryophyllene oxide	0.05	0.55	0.34	0.37	0.43	0.40
α-copaene	0.00	0.10	0.05	0.16	0.24	0.20
methyl eugenol	0.00	0.10	0.05	0.16	0.24	0.20
Iso Eugenol	0.00	0.24	0.06	0.08	0.13	0.11
chavicol	0.00	0.12	0.03	0.08	0.13	0.11
methyl Salicylate	0.00	0.00	0.00	0.00	0.00	0.00

Table 4: Chemical composition (relative percentage, extreme values and means) of stem essential oils from Madagascar, Indonesia and Zanzibar

	Madagascar			Indonesia			Zanzibar		
Component	min	max	mean	min	max	mean	min	max	mean
eugenol	91.81	96.65	93.91	88.76	89.28	89.02	87.52	89.47	88.46
β-caryophyllene	1.66	4.48	3.38	7.40	7.75	7.58	7.19	9.70	8.27
α-humulene	0.22	0.79	0.48	0.93	1.31	1.12	0.75	1.08	0.92
eugenyl acetate	0.37	2.53	0.80	0.07	0.17	0.12	0.55	0.88	0.72
caryophyllene oxide	0.14	0.60	0.26	0.20	0.26	0.23	0.25	0.68	0.47
α-copaene	0.00	0.20	0.06	0.07	0.16	0.12	0.00	0.27	0.123
methyl eugenol	0.00	0.15	0.07	0.03	0.11	0.07	0.00	0.15	0.07
iso eugenol	0.00	0.80	0.06	0.01	0.03	0.02	0.01	0.10	0.07
chavicol	0.00	0.22	0.08	0.00	0.00	0.00	0.00	0.00	0.00
methyl salicylate	0.00	0.56	0.33	0.06	0.2	0.13	0.00	0.27	0.22

Table 5: S. aromaticum essential oil samples

	Nb. of samples		
Origins	Bud	Leaf	Stem
Indonesia	6	4	2
Madagascar	39	28	27
Zanzibar	-	-	15
Total	45	32	44

Figures

Figure 1: Circle of correlations of the 10 examined variables by the first two principal components (PC1/PC2)

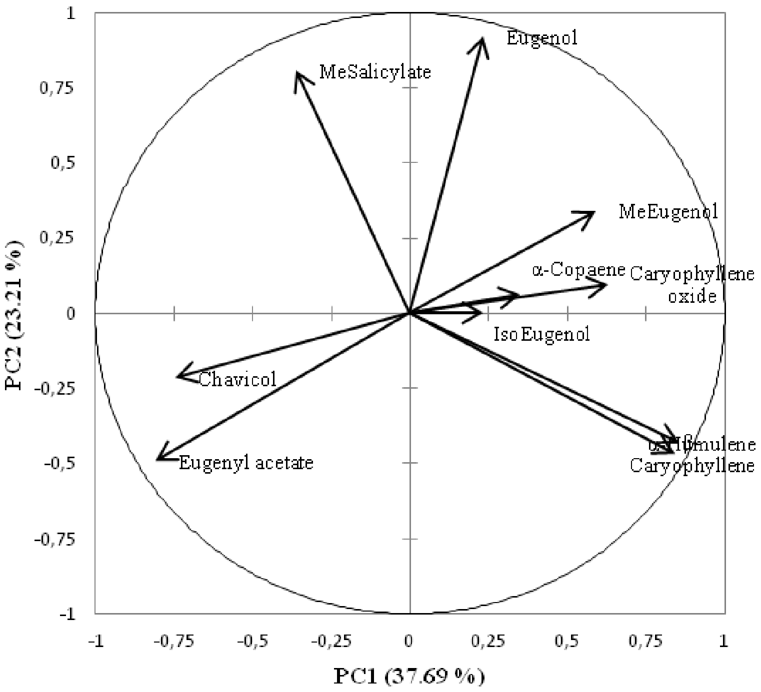

Figure 2 : Graphical representation of the 121 samples of essential oil from *S. aromaticum* using PCA according to PC1/PC2. Bud essential oils are represented by triangle (GI), leaf essential oils by square (GII) and stem essential oils by circle (GIII)

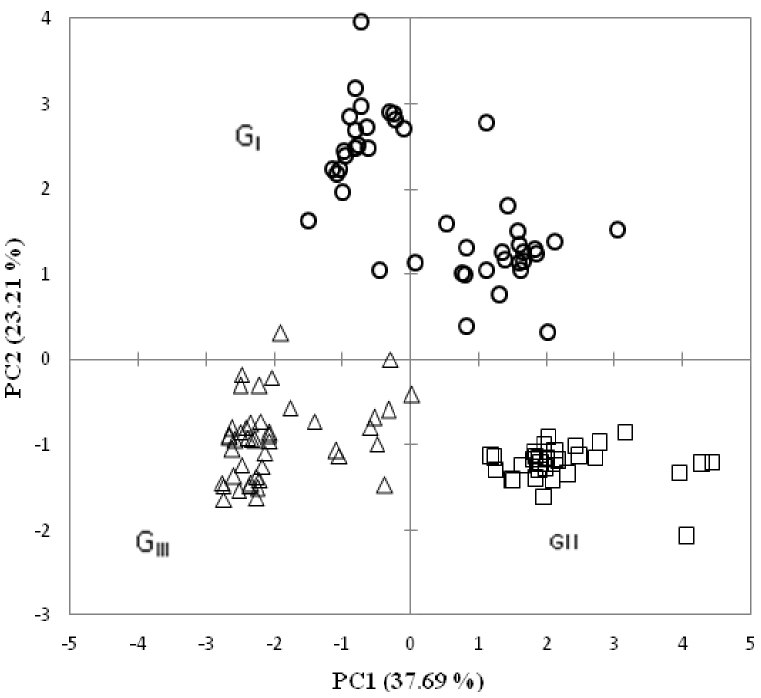

Figure 3 : Circle of correlations of the 10 examined variables of bud oil by the first two principal components (PC1/PC2)

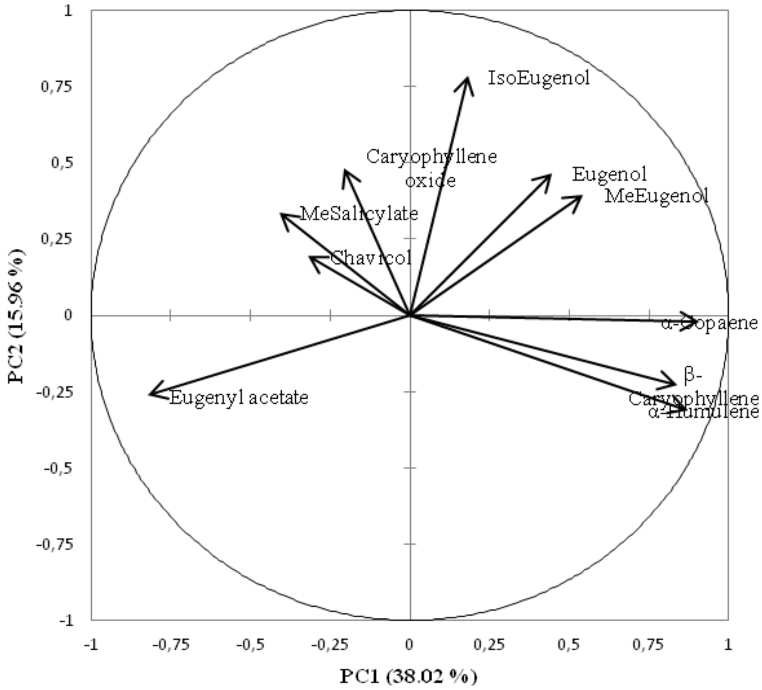

Figure 4 : Graphical representation of the 45 samples of bud essential oil using PCA according to PC1/PC2

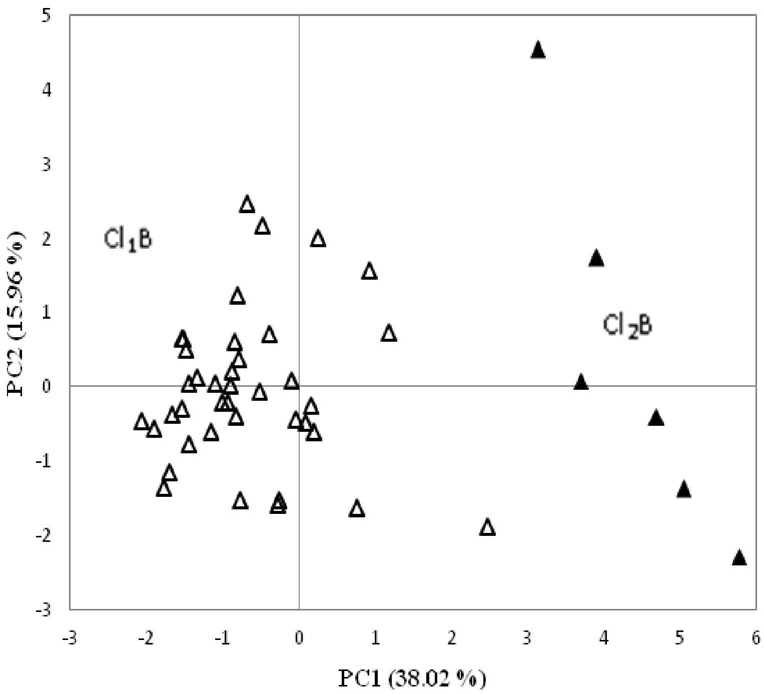

Figure 5 : Circle of correlations of the 10 examined variables of leaf oil by the first two principal components (PC1/PC2)

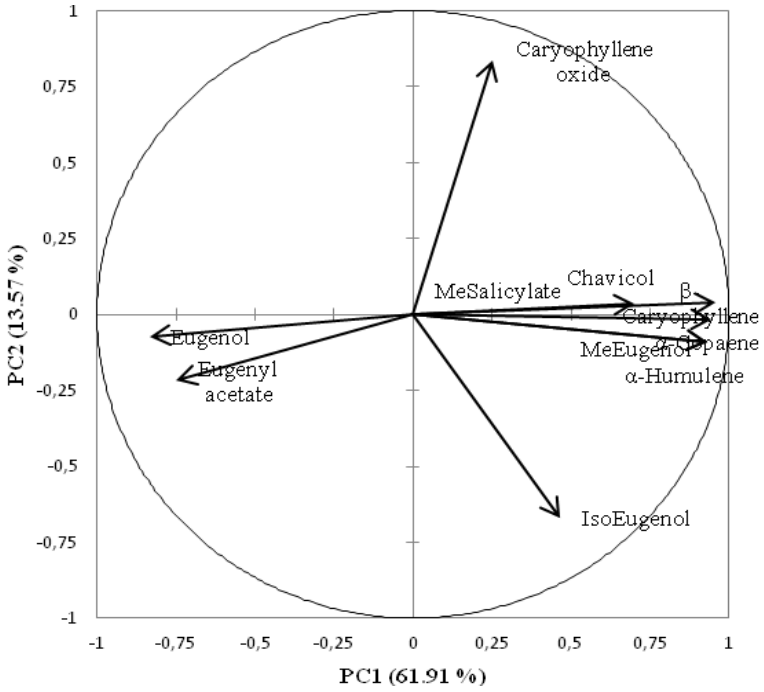

Figure 6 : Graphical representation of the 32 samples of leaf essential oil using PCA according to PC1/PC2

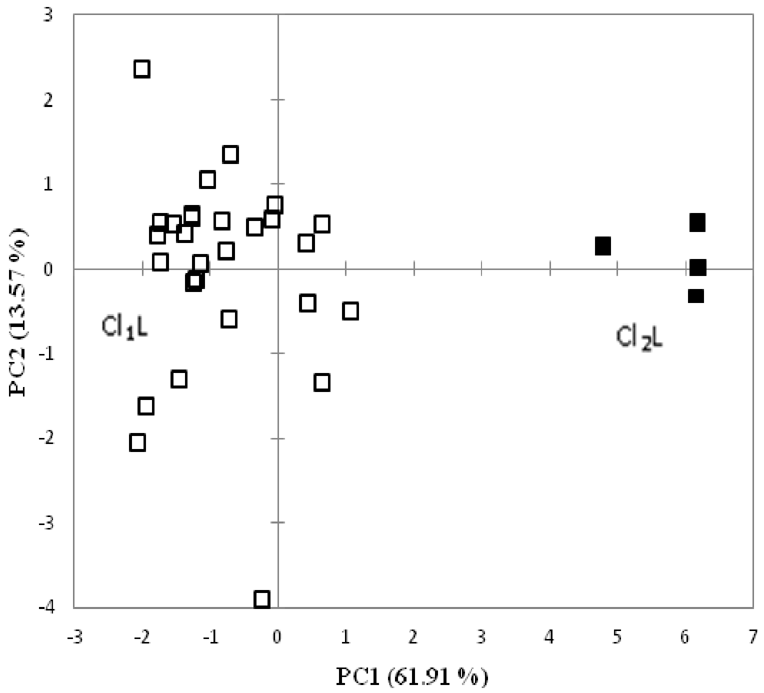

Figure 7 : Circle of correlations of the 10 examined variables of stem oil by the first two principal components (PC1/PC2)

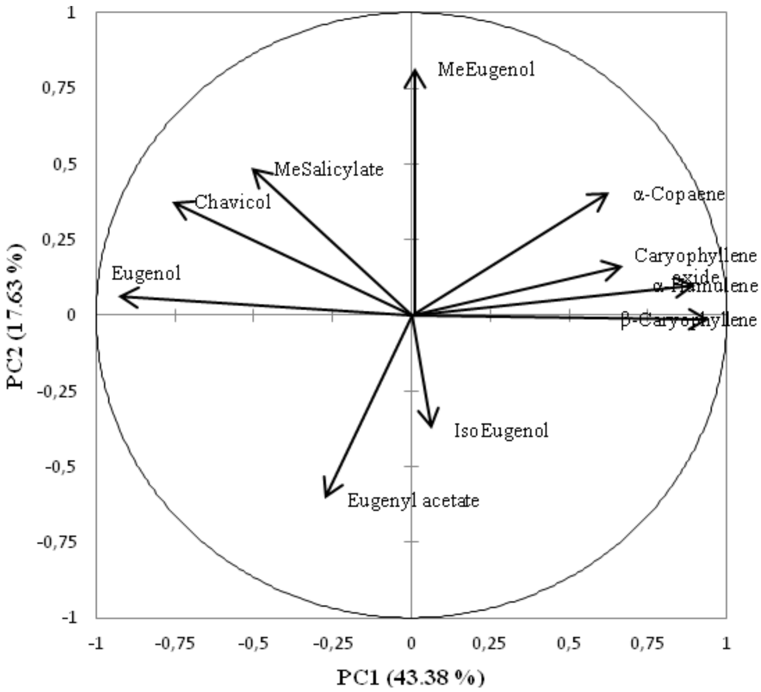

Figure 8 : Graphical representation of the 44 samples of stem essential oil using PCA according to PC1/PC2