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Research Paper

Bud, leaf and stem essential oil composition of *Syzygium aromaticum* from Madagascar, Indonesia and Zanzibar

Gaylor Razafimamonjison [1, 2, 3]*, Michel Jahiel [2, 3, 4], Thierry Duclos [5], Panja Ramanoelina [1], Fanja Fawbush [1], Pascal Danthu [2, 6, 7]

[1] *Université d'Antananarivo, École Supérieure des Sciences Agronomiques, BP 175, Antananarivo, Madagascar*
[2] *DP Forêts et Biodiversité, BP 853, Antananarivo, Madagascar*
[3] *Centre Technique Horticole de Tamatave, BP 11, Tamatave, Madagascar*
[4] *CIRAD, UPR Hortsys, PS4, Boulevard de la Lironde, 34392 Montpellier Cedex 05, France*
[5] *Laboratoire QUIMDIS SAS, 71, rue Anatole France, 92300 Levallois-Perret, France*
[6] *CIRAD, UR 105, Campus de Baillarguet, 34392 Montpellier Cedex 05, France*
[7] *Directeur régional CIRAD Madagascar, Représentant d'Agreenium, BP 853, Antananarivo, Madagascar*
**Corresponding author E-mail: rdinagaylor@yahoo.fr*

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Abstract

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 essential oils 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 chemical composition of Madagascar, Indonesia and Zanzibar bud, leaf and stem essential oils. 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 of Madagascar and Indonesia bud essential oils was eugenol (72.08 – 80.71% and 77.32 – 82.36% respectively). Out of this constituent which was common to Madagascar and Indonesia bud essential oils, significant difference was observed with respect to eugenyl acetate (11.68 – 21.32% vs 8.61 – 10.55% respectively) and β–caryophyllene (2.76 – 6.38% vs 5.34 - 8.64% respectively). Comparing chemical composition of leaf essential oils from Madagascar with those of Indonesia, variation in the contents of main constituent, eugenol (80.87 – 83.58% vs 75.04 – 77.54%), β-caryophyllene (11.65 – 15.02 vs 17.04 – 19.53%) and eugenyl acetate (0.29 – 1.45% vs 0 - 0.06%) was observed. The major constituents of Madagascar, Indonesia and Zanzibar stem essential oils were eugenol (91.81 - 96.65%, 88.76 - 89.28% and 87.52 - 89.47%, respectively) and β-caryophyllene (1.66 - 4.48%, 7.40 - 7.75% and 7.19 - 9.70%). For each plant material, variation in the percentage of the main constituents was observed according to the sample geographic origin.

Keywords: *Anatomic origins; Chemical composition; Essential oils; Geographic origins; Syzygium aromaticum.*

1. Introduction

The clove tree (*Syzygium aromaticum* Merrill & Perry), 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 [20 - 23] carried out on bud, leaf and stem oil 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, α-humulene, caryophyllene oxide and eugenyl acetate, although in different concentrations.

Recently, the effects of phenological stages on yield and composition of essential oil of *S. aromaticum* buds from Madagascar has been studied by Razafimamonjison *et al.* [22]. They found that eugenol was lower in the young bud stage (39.66%) and increased in the subsequent phenological stages to reach maximum in the full fruiting stage (94.89%). In contrast, eugenyl acetate was higher in the young bud stage (56.07%), after which decreased to reach minimum in the full fruiting stage (2.01%).

Other works carried out on the leaf essential oils of *S. aromaticum* show the variability of content of the major constituents. In the essential oils studied by Raina *et al.* [21] and Srivastava *et al.* [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%).

Finally, little literature describes the chemical composition of clove stem essential oils. Gaydou *et al.* [23] 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 oils 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* [24], *Cedrelopsis grevei* [25] and *Cinnamosma fragrans* [26], many factors such as the geographical origin, the plant material, the 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 chemical composition of Madagascar, Indonesia and Zanzibar bud, leaf and stem essential oils.

2. Materials and methods

2.1. Essential oil

S. aromaticum essential oil used in this study were commercial samples provided by either industrial exporting companies of Madagascar, of Indonesia and of Zanzibar (Table 1). The essential oil samples were dried over anhydrous sodium sulfate (Na_2SO_4) and stored in a cool and dark chamber until the analysis.

Table 1: *S. aromaticum* essential oil samples

	Anatomic origins		
Geographic origins	Bud	Leaf	Stem
Madagascar	39	28	27
Indonesia	6	4	2
Zanzibar	-	-	15
Total	45	32	44

2.2. Gas chromatography

GC analyses were performed on a Varian gas chromatograph, model CP-3380, with flame ionization detectors fitted with two silica capillary columns: CP Sil 5 CB low bleed/MS (100% dimethyl polysiloxane phase, Chrompack/Varian, Palo Alto, CA) capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) and Supelcowax 10 (polyethylene glycol), Supelco Inc, Bellefonte, PA) fused capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness); carrier gas, N_2; flow rate, 0.8 ml/min; injection type, split, 1:100 (0.2 µl pure essential oil); injector temperature, 220 °C; detector temperature, 250 °C; temperature programme, 50 - 200 °C at 5 °C/min. The linear retention indices of the components were determined relative to the retention times of a series of n-alkanes and the percentage compositions were obtained from electronic integration measurements without taking into account relative response factors. All GC analyses on the apolar column were performed in triplicate, indicating a reproducibility of at least 3% in the relative percentages.

2.3. 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 [27]. Quantitative analysis of each oil component, expressed in relative percentages of area, was carried out by peak area normalization measurements.

2.4. Statistical analysis

The distribution of the 121 samples was analyzed by Principal Component Analysis (PCA) using the XLSTAT Version 2012 statistical software package. The data set was composed of the values taken by the variables identified by GC and the 121 *S. aromaticum* essential oil samples. PCA was performed as it is among the best-known multivariate analysis methods for correlation variable determination [24, 25].

3. Results and discussion

3.1. Chemical composition of bud, leaf and stem essential oils

Bud essential oils (45 samples), leaf essential oils (32 samples) and stem essential oils (44 samples) were analyzed by GC. Ten constituents were identified and quantified, as shown in table 2. Great variability in the chemical compositions of the three essential oils was observed (Table 2). In all essential oils, eugenol was the major constituent, with increasing percentages from bud (72.08 – 82.36%) to leaf (75.04 – 83.58%) and to stem (87.52 – 96.65%). In bud essential oils this compound is followed by eugenyl acetate (8.61 – 21.32%), while in leaf and stem the latter was detected in considerably lower amounts (0 – 1.45% and 0.07 – 2.53% respectively). In leaf essential oils, the second main compound was β-caryophyllene (11.65 – 19.53%) less represented in bud essential oils (2.76 – 8.64%) and in stem essential oils (1.66 – 9.72%).

Table 2: Chemical composition (Relative percentages, extreme values) of bud, leaf and stem essential oils (In bolt: Discriminate components in each group).

		Group 1	Group 2	Group 3
RI[a]	Compounds	Bud	Leaf	Stem
1176	methyl salicylate	0 - 0.32	tr	0 - 0.56
1241	chavicol	0 - 0.24	0 - 0.13	0 - 0.22
1348	**eugenol**	**72.08 - 82.36**	**75.04 - 83.58**	**87.52 - 96.65**
1375	α-copaene	0 - 0.27	0 - 0.24	0 - 0.27
1387	methyl eugenol	0 - 0.08	0 - 0.24	0 - 0.15
1420	**β-caryophyllene**	2.76 - 8.64	**11.65 - 19.53**	1.66 - 9.72
1453	iso-eugenol	0 - 0.24	0 - 0.24	0 - 0.83
1465	α-humulene	0.34 - 1.04	1.38 - 2.17	0.22 - 1.31
1494	**eugenyl acetate**	**8.61 - 21.32**	0 - 1.45	0.07 - 2.53
1585	caryophyllene oxide	0.06 - 0.37	0.05 - 0.55	0.14 - 0.68

[a] retention indices on CP Sil 5 column. tr: trace

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 (Fig.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).

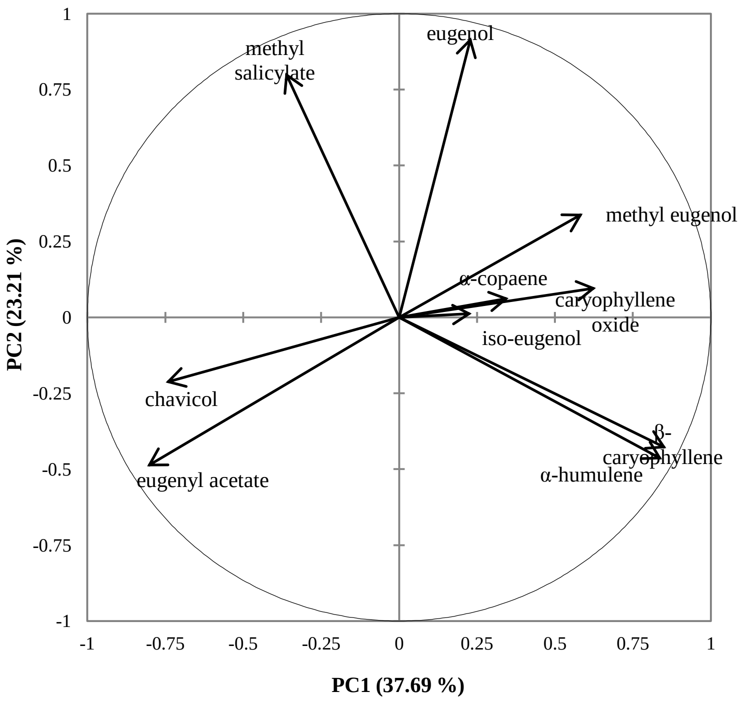

Fig. 1: Circle of correlations of the ten examined variables from bud, leaf and stem essential oils by the first two principal components (PC1/PC2).

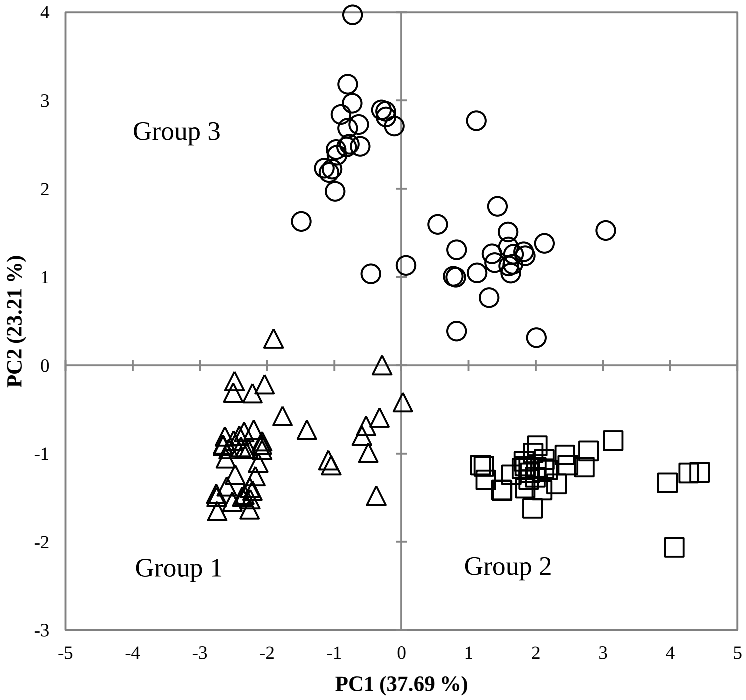

Fig. 2: Graphical representation of the 121 samples of essential oil from *S. aromaticum* using PCA according to PC1/PC2. Bud essential oils are represented by triangle (Group 1), leaf essential oils by square (Group 2) and stem essential oils by circle (Group 3).

The PCA according to planes PC1 and PC2 made it possible to distribute the 121 samples analyzed into three groups (Fig.2 and Table 2).

Group 1 is constituted by bud essential oils which are characterized by lower percentage of eugenol (72.08 – 82.36%) and high content of eugenyl acetate (8.61 – 21.32%). Group 2 is composed of leaf essential oils, mainly containing eugenol (75.04 – 83.58%) and characterized by high content of β-caryophyllene (11.65 – 19.53%). Group 3, constituted by stem essential oils, is characterized by high eugenol content (87.52 – 96.65%).

Taking into account our research, we can affirm that it is possible to use the percentage of eugenol, eugenyl acetate and β-caryophyllene to characterize the essential oil obtained from the different plant parts, bud, leaf and stem.

3.2. Comparison of the chemical composition of Madagascar and Indonesia bud essential oils

Bud essential oils (39 samples from Madagascar and 6 from Indonesia) were analyzed by GC and ten constituents were identified and quantified, as shown in table 3. The major constituent of Madagascar and Indonesia bud essential oils was eugenol (72.08 – 80.71% and 77.32 – 82.36% respectively). Out of this constituent which was common to Madagascar and Indonesia bud essential oils, significant difference was observed with respect to eugenyl acetate (11.68 – 21.32% vs 8.61 – 10.55% respectively) and β–caryophyllene (2.76 – 6.38% vs 5.34 - 8.64% respectively) (Table 3).
To highlight this difference in the chemical composition of Madagascar and Indonesia bud essential oils, PCA were carried out on 45 samples combined, considering ten variables (The ten constituents identified by GC). The statistical analysis results were as follows:

Table 3: Chemical composition (Relative percentage, extreme values) of Madagascar and Indonesia bud essential oils.

		Group 1	Group 2
RI	Compounds	Madagascar	Indonesia
1176	methyl salicylate	0 - 0.32	0.04 - 0.16
1241	chavicol	0 - 0.24	0.13 - 0.18
1348	eugenol	72.08 - 80.71	77.32 - 82.36
1375	α-copaene	0 - 0.11	0.17 - 0.27
1387	methyl eugenol	0 - 0.06	0.04 - 0.08
1420	β-caryophyllene	2.76 - 6.38	5.34 - 8.64
1453	iso-eugenol	0 - 0.20	0.02 - 0.24
1465	α-humulene	0.34 - 1.04	0.65 - 1.04
1494	eugenyl acetate	11.68 - 21.32	8.61 - 10.55
1585	caryophyllene oxide	0.11 - 0.37	0.06 - 0.32

The first two principal components (Fig. 3) extracted by PCA explained 53.98% of the variability of which 38.02% was represented by PC1 and 15.96% by PC2. 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 made it possible to distribute the 45 samples of bud essential oils into two groups. In both groups, eugenol, β–caryophyllene and eugenyl acetate are also the major compounds, although in different amounts (Fig.4 and table 3).
Group 1, constituted by 39 samples from Madagascar was characterized by lower percentage of eugenol and β–caryophyllene (72.08 – 80.71% and 2.76 - 6.38% respectively) and by higther content of eugenyl acetate (11.68 – 21.32%). Group 2 is constituted by 6 samples from Indonesia. In this group, eugenol, β–caryophyllene and eugenyl acetate were detected at 77.32 – 82.36%, 5.34 - 8.64% and 8.61 – 10.55% respectively.

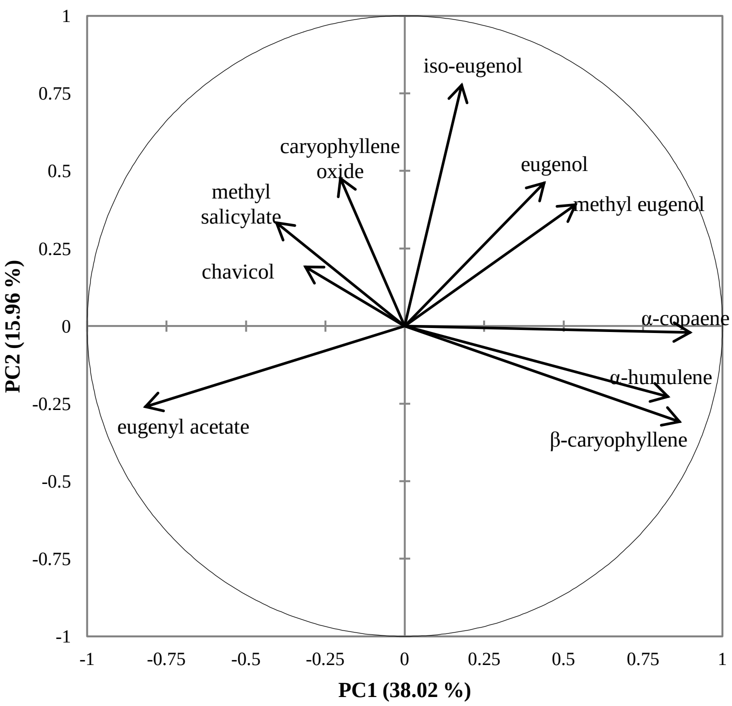

Fig. 3: Circle of correlations of the ten examined variables from bud essential oils by the first two principal components (PC1/PC2).

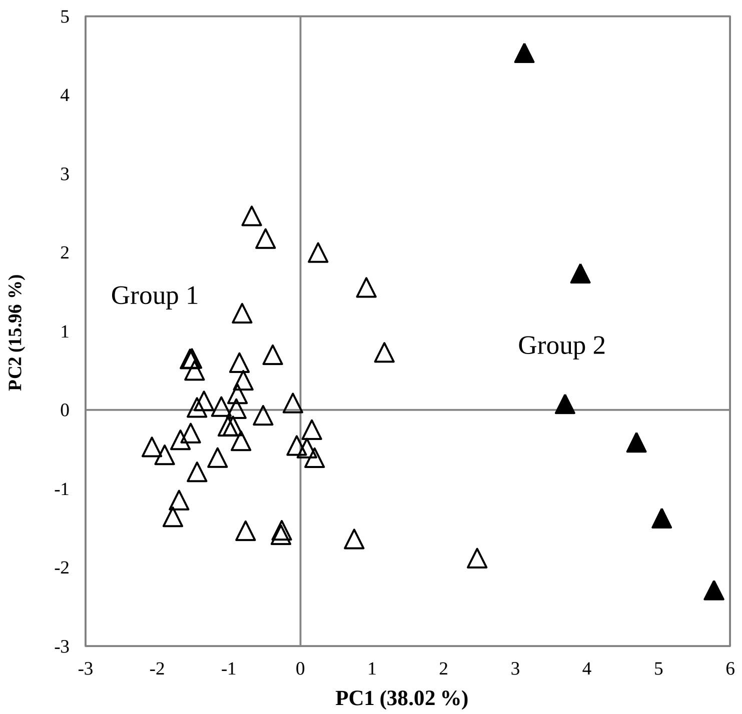

Fig. 4: Graphical representation of the 45 samples of bud essential oils using PCA according to PC1/PC2. Bud essential oils from Madagascar are represented by white triangle (Group 1) and from Indonesia by black triangle (Group 2).

These results are in agreement with those from Srivastava *et al.* [20] while still demonstrating the variability among *S. aromaticum* bud essential oils. They 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 Randriamiharisoa *et al.* [28], Gaydou *et al.* [23] and Hector *et al.* [29] 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%).

3.3. Comparison of the chemical composition of Madagascar and Indonesia leaf essential oils

Leaf essential oils (28 samples from Madagascar and 4 from Indonesia) were analyzed by GC and 10 constituents were identified and quantified (Table 4). Comparing chemical composition of leaf essential oils from Madagascar with those of Indonesia, variation in the contents of main constituent, eugenol (80.87 – 83.58% vs 75.04 – 77.54%), β-caryophyllene (11.65 – 15.02 vs 17.04 – 19.53%) and eugenyl acetate (0.29 – 1.45% vs 0 - 0.06%) was observed.

Table 4: Chemical composition (Relative percentage, extreme values) of Madagascar and Indonesia leaf essential oils.

		Group 1	Group 2
RI	Compounds	Madagascar	Indonesia
1176	methyl salicylate	tr	tr
1241	chavicol	0 - 0.12	0.07 - 0.13
1348	eugenol	80.87 - 83.58	75.04 - 77.54
1375	α-copaene	0 - 0.1	0.16 - 0.24
1387	methyl eugenol	0 - 0.1	0.15 - 0.24
1420	β-caryophyllene	11.65 - 15.02	17.04 - 19.53
1453	iso-eugenol	0 - 0.24	0.08 - 0.13
1465	α-humulene	1.39 - 1.67	1.93 - 2.17
1494	eugenyl acetate	0.29 - 1.45	0 - 0.06
1585	caryophyllene oxide	0.05 - 0.55	0.37 - 0.43

tr: trace

To highlight this variation in the chemical composition of Madagascar and Indonesia leaf essential oils, 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 (Fig. 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 by 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 (Fig. 6 and table 4) made it possible to distribute the 32 samples of leaf oil analyzed into two groups:

Group 1 constituted by 28 samples from Madagascar, contains eugenol, β-caryophyllene and eugenyl acetate at 80.87 - 83.58%, 11.65 - 15.02% and 0.29 - 1.45% respectively. Group 2 comprises 4 samples from Indonesia. This group is characterized by lower percentage of eugenol (75.04 - 77.54%) and higher percentage of β-caryophyllene (17.04 - 19.53%) than that of Group 1.

It was reported earlier that cloves leaf essential oil from Madagascar [20] was characterized by higher content of eugenol 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 [21] 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 [21] matched to a great extent with the leaf oil from south India reported by Gopalakrishnan *et al.* [30] 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.

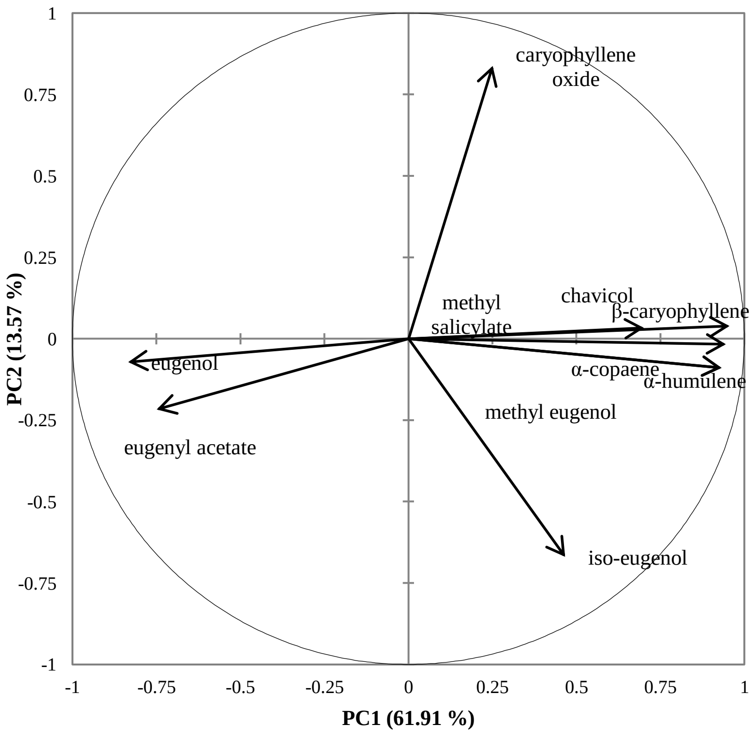

Fig. 5: Circle of correlations of the ten examined variables from leaf essential oils by the first two principal components (PC1/PC2).

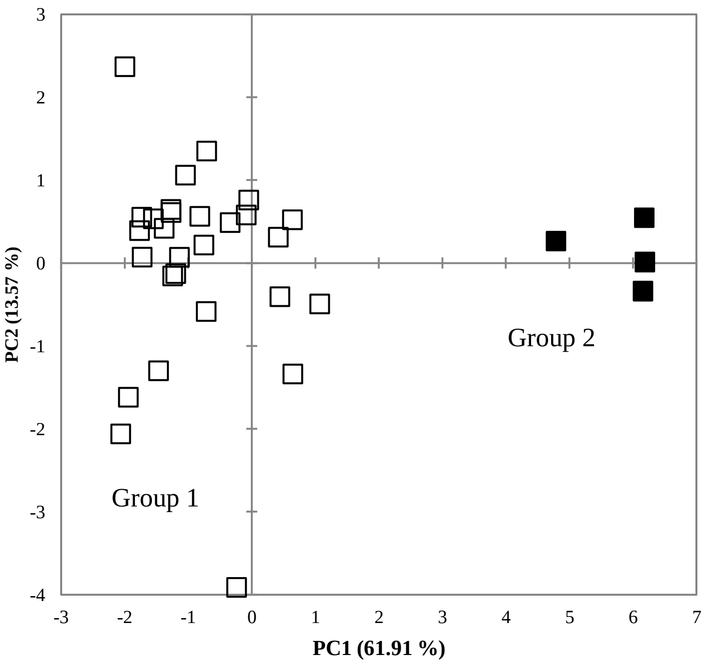

Fig. 6: Graphical representation of the 32 samples of leaf essential oils using PCA according to PC1/PC2. Leaf essential oils from Madagascar are represented by white square (Group 1) and from Indonesia by black square (Group 2).

3.4. Comparison of the chemical composition of Madagascar, Indonesia and Zanzibar stem essential oils

Stem essential oils (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). The major constituents of Madagascar, Indonesia and Zanzibar stem essential oils were eugenol (91.81 - 96.65%, 88.76 - 89.28% and 87.52 - 89.47%, respectively) and β-caryophyllene (1.66 - 4.48%, 7.40 - 7.75% and 7.19 - 9.70%) (Table 5).

Table 5: Chemical composition (Relative percentage, extreme values) of Madagascar, Indonesia and Zanzibar stem essential oils.

		Group 1	Group 2	
RI	Compounds	Madagascar	Indonesia	Zanzibar
1176	methyl salicylate	0 - 0.56	0.06 - 0.20	0 - 0.27
1241	chavicol	0 - 0.22	0	0
1348	eugenol	91.81 - 96.65	88.76 - 89.28	87.52 - 89.47
1375	α-copaene	0 - 0.20	0.07 - 0.16	0 - 0.27
1387	methyl eugenol	0 - 0.15	0.03 - 0.11	0 - 0.15
1420	β-caryophyllene	1.66 - 4.48	7.40 - 7.75	7.19 - 9.70
1453	iso-eugenol	0 - 0.80	0.01 - 0.03	0.01 - 0.10
1465	α-humulene	0.22 - 0.79	0.93 - 1.31	0.75 - 1.08
1494	eugenyl acetate	0.37 - 2.53	0.07 - 0.17	0.55 - 0.88
1585	caryophyllene oxide	0.14 - 0.6	0.20 - 0.26	0.25 - 0.68

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

The first two principal components (Fig. 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 (Fig. 8 and table 5) made it possible to distribute the 44 stem oil samples analyzed into two groups:

Group 1 composed of all samples coming from Madagascar which is characterized by higher percentage of eugenol (91.81 – 96.65%) and lower percentage of β - caryophyllene (1.66 – 4.48%). Group 2, which is constituted by all samples coming from Indonesia and all samples from Zanzibar, is characterized by lower content of eugenol (87.52 – 89.76%) and higher content of β - caryophyllene (7.40 – 9.70%) than the first group.

Little literature describes the chemical composition of clove stem oils. Gaydou *et al.* [23] assumed that clove stem essential oil of is characterized by high eugenol content (up to 70%).

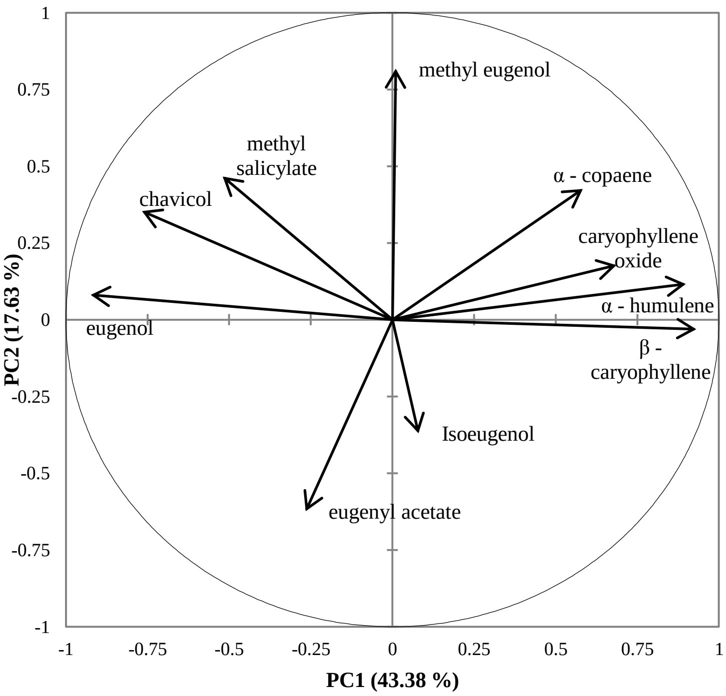

Fig. 7: Circle of correlations of the ten examined variables from stem essential oils by the first two principal components (PC1/PC2).

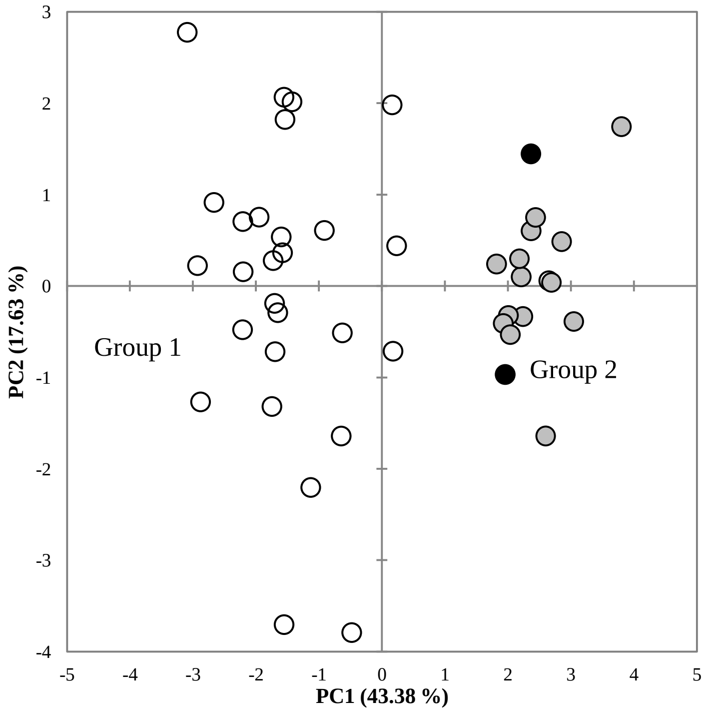

Fig. 8: Graphical representation of the 44 Samples of stem essential oils using PCA according to PC1/PC2. Stem essential oils from Madagascar are represented by white circle (Group 1), from Indonesia by black circle and from Zanzibar by grey circle (Group 2).

4. Conclusion

Taking into account our research, we can affirm that it is possible to use the percentage of eugenol, eugenyl acetate and β-caryophyllene to characterize the essential oil obtained from the different plant parts, bud, leaf and stem. For each plant material, variation in the percentage of these three main constituents was observed according to the geographic origins of sample.

Acknowledgements

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