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Research Article
Comparative Study on Essential Oil Composition in Various Organs of Sodom Apple (*Calotropis procera*) Grown Wild in Egypt

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Abstract

Background and Objective: The essential oil composition of *Calotropis procera* (*C. procera*) has various biological properties. Different plant organs such as stems, leaves, flowers and fruits are considered to be a natural source of numerous essential oil constituents. The aim of this investigation was to evaluate the *C. procera* essential oil composition extracted from each plant organ (leaf, stem, flower and fruit). **Methodology:** Essential oils of different plant organs were isolated by Soxhlet apparatus and then analyzed by GC and GC-MS to identify the essential oil constituents. The averages of data were statistically analyzed using one-way ANOVA. **Results:** Fruits produced the greatest amounts of essential oil contents that recorded the values of 0.9% or 9.0 g kg^{-1}. The major constituents of essential oil isolated from all organs were E-phytol, myristicin, myristic acid and E,E-farnesyl acetone. Leave essential oil produced the greatest amounts of E-phytol (40.5%), myristicin (28.1%) and myristic acid (15.8%) while fruits oil induced the highest amounts of E,E-farnesyl acetone (8%). The highest amounts of monoterpene hydrocarbons, MCH (5.9%), oxygenated monoterpenes, MCHO (4.9%) and sesquiterpenes hydrocarbons, SCH (4.1%) were recorded with essential oil isolated from fruits while the greatest values of oxygenated sesquiterpenes, SCHO (53.9%) and oxygenated diterpenes, DCHO (40.5%) were recorded with leaves essential oil. **Conclusion:** Plant organs resulted in various changes in essential oil composition of *C. procera* plants as well as *C. procera* grow in Egypt produce high amounts of some active ingredients which have an important role in pharmaceutical and drug industries in Egypt.

Key words: *Calotropis procera*, essential oil, E-phytol, oxygenated sesquiterpenes, oxygenated diterpenes

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Calotropis procera (*C. procera*) is a perennial shrub and belongs to family Apocynaceae; the common name is giant milk wood or Sodom apple[1]. *Calotropis procera* used in various traditional systems of medicine in Asia and Africa. Traditionally, roots and leaves of *C. procera* used for the treatment of cancer, gonorrhea and scorpion bite[1]. *Calotropis procera* latex, roots, leaves and flowers has different biological activities such as anti-inflammatory, antidote, antipyretic, antidiarrhoeal, antibacterial and analgesic[2-8].

Essential oils are secondary metabolites formed in aromatic plants. These oils are volatile, complex compounds have a strong scent, such oils are usually extracted by steam or hydro-distillation. Essential oils have many properties such as antiseptic, bactericidal, virucidal, fungicidal, medicinal as well as their fragrance. They are used in many food industries and in embalment. In nature, essential oils protect the plants from bacteria, fungi and virus. They can attract insects to flavour the dispersion of pollens and seeds, or repel undesirable others[9]. Essential oils (terpenoids) have chemopreventive properties against liver and lung carcinogenesis. In the case of animal colon tumors and gastric cancer, the animals feed with monoterpenes produce a significant increase in apoptosis compared with control diet[10-12].

Singh and Javed[13] investigated the essential oil composition of *Calotropis gigantea* and its antimicrobial activity and reported that the major constituents were linalool (46%), α-terpineol (10%) and pentacosane (3.5%), on the other hand, it was also found that *Calotropis gigantea* essential oil has antibacterial (against *Staphylococcus aureus*, *Escherichia coli*) and antifungal (against *Rhizoctonia solani*) activities.

Previous studies showed that there are a few studies on the constituents of *C. procera* essential oil isolated from different plant organs, so the aim of this investigation was to describe the chemical constituents of *C. procera* essential oil extracted from leaves, stems, flowers and fruits. This study may increase the natural products using in drugs and pharmaceutical industries in Egypt.

MATERIALS AND METHODS

Plant materials: Fresh samples of different *C. procera* plant organs (leaves, stems, flowers and fruits) were collected during the first week of September, 2017 from some tress grow in Nasr City located at North of Cairo, Egypt. Different organs were then air dried and weighted to extract the essential oil.

Essential oil isolation: For solvent extraction, a Soxhlet apparatus was used[14]. Petroleum ether (20-40°C) and ether were used as solvents to extract the essential oil from the all four parts. Ten grams from each replicate were used for this method. Solvent extracts of all the volatile compounds from the samples were collected in a flask. This was the concrete oil, which was further processed to remove any remaining solvent from oil. A distillation process was performed to recover the solvent from the concrete oil (organic solvent and rose) using a rotary evaporator. In this way, all the organic solvent was recovered. Distillation by using rotary evaporator is useful because the active ingredients of oil are not lost. Absolute oil from concrete oil was recovered by adding 1 mL of absolute alcohol in 10 mL of concrete oil. The alcohol removes all the natural waxes present in the essential oil. The oil was filtered and the absolute alcohol was removed by performing distillation with a rotary evaporator. Final traces of alcohol were removed by bubbling nitrogen gas through this oil. In this way, absolute oil by using solvent extraction was obtained. The essential oil (% and g kg^{-1} of plant material) was calculated.

GC and GC-MS conditions: The GC analyses were performed using a Shimadzu GC-9 gas chromatograph equipped with a DB-5 (dimethylsiloxane, 5% phenyl) fused silica column (J and W Scientific Corporation) (60 m×0.25 mm i.d., film thickness 0.25 μm). Oven temperature was held at 50°C for 5 min and then programmed to rise to 240°C at a rate of 3°C min^{-1}. The flame ionization detector (FID) temperature was 265°C and injector temperature was 250°C. Helium was used as carrier gas with a linear velocity of 32 cm sec^{-1}. The percentages of compounds were calculated by the area normalization method, without considering response factors.

The GC-MS analyses were carried out in a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30 m×0.25 mm i.d., film thickness 0.25 Lm), oven temperature was 50-240°C at a rate of 4°C min^{-1}, transfer line temperature 260°C, carrier gas, helium with a linear velocity of 31.5 cm sec^{-1}, split ratio 1:60, ionization energy 70 eV, scan time 1 sec and mass range 40-300 amu.

Identification of volatile components: The components of the oils were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature[15]. Mass spectra from the literature were also compared[15]. Further identification was made by comparison of their mass spectra on both columns with those stored in NIST-98 and Wiley-5 Libraries. The retention indices were calculated for all volatile constituents using a homologous series of n-alkanes.

Statistical analysis: In this experiment, one factor was considered: Plant organs (stems, leaves, flowers and fruits). For each organ there were 3 replicates, the experimental design followed a complete random block design. Data were statistically analyzed using one-way analysis of variance (ANOVA)[16]. Significant values determined according to p-values ($p<0.05$ = Significant, $p<0.01$ = Moderate significant and $p<0.001$ = Highly significant). The applications of that technique were according to the STAT-ITCF program version 10[17].

RESULTS

Essential oil content: Different variations were found in *C. procera* essential oil contents [% or yield (g kg^{-1})] with different plant organs. The highest contents of essential oil were produced from fruits with the values of 0.9% followed by flowers (0.6%) and leaves or stems (0.4%) ANOVA revealed that the changes in essential oil contents were moderate significant ($p<0.01$) for various plant organs.

Essential oil constituents: In this investigation, twenty two components (accounting 99.0-99.6%) were detected by GC-MS analysis of *C. procera* essential oil which extracted from various plant organs i.e., leaves, stems, flowers and fruits (Table 1). E-phytol, myristicin, myristic acid and E,E-farnesyl acetone were observed as the major constituents that recorded the highest percentages of all essential oils isolated from various organs. All essential oil constituents that identified in this study were classified into five groups, sesquiterpene hydrocarbons (SCHO) and oxygenated diterpenes (DCHO) were the major groups, the remaining groups as monoterpene hydrocarbons (MCH), oxygenated monoterpenes (MCHO) and sesquiterpene hydrocarbons (SCH) formed the minor groups (Table 1). The essential oils produced from leaves recorded the highest values of E-phytol (40.5%), myristicin (28.1%) and myristic acid (15.8%) while fruits oil recorded the highest amounts of E,E-farnesyl acetone (8%). The essential oil isolated from fruits produced the highest amounts of MCH, MCHO and SCH with the values of 5.9, 4.9 and 4.1%, respectively. The SCHO and DCHO were the

Table 1: Constituent identified in essential oil isolated from various *C. procera* organs

			Plant organs								
			Stems		Leaves		Flowers		Fruits		
Constituents	RI[a]	RI[b]	M	SD	M	SD	M	SD	M	SD	F-ratio
α-pinene	939	939	0.3	±0.1	0.1	±0.0	0.1	±0.0	0.4	±0.2	5.4*
Sabinene	977	976	0.3	±0.1	0.4	±0.1	0.6	±0.1	0.5	±0.1	5.0*
β-pinene	980	980	0.3	±0.1	0.1	±0.0	0.4	±0.1	1.6	±0.1	30.7***
α-terpinene	1019	1018	0.6	±0.2	0.3	±0.1	0.3	±0.1	2.4	±0.4	55.6***
p-cymene	1026	1026	0.4	±0.1	0.6	±0.2	0.2	±0.1	0.5	±0.1	5.0*
Limonene	1031	1031	0.6	±0.1	0.3	±0.1	0.9	±0.1	0.5	±0.1	18.8***
Linalool	1098	1098	0.6	±0.3	0.2	±0.1	0.4	±0.1	0.4	±0.1	2.7[ns]
Nonanal	1099	1099	0.4	±0.2	0.2	±0.1	0.1	±0.0	1.4	±0.4	20.3***
1S- β-fenchol	1117	1117	0.3	±0.1	0.8	±0.2	0.1	±0.0	0.6	±0.1	19.3***
Terpinen-4-ol	1177	1177	0.4	±0.1	0.6	±0.2	0.1	±0.0	1.5	±0.5	14.5***
β-cyclocitral	1202	1202	0.3	±0.1	0.6	±0.1	0.5	±0.1	0.5	±0.1	4.8*
2-Hydroxy-1,8-cineole	1220	1219	0.4	±0.1	0.6	±0.1	0.4	±0.1	0.5	±0.2	1.6[ns]
β-caryophyllene	1418	1418	0.3	±0.1	0.2	±0.1	1.3	±0.0	1.5	±0.1	15.0***
Geranyl acetone	1453	1453	0.6	±0.1	0.6	±0.1	0.6	±0.1	1.5	±0.1	8.7**
β-ionone	1461	1460	1.2	±0.2	0.6	±0.1	0.4	±0.1	0.5	±0.1	22.1***
β-bisabolene	1510	1509	1.7	±0.2	0.2	±0.1	0.3	±0.1	2.6	±0.1	229.7***
Myristicin	1520	1520	26.4	±0.4	28.1	±0.1	27.9	±0.1	25.9	±0.1	75.1***
Caryophyllene oxide	1581	1581	0.3	±0.1	0.6	±0.1	2.0	±0.1	0.4	±0.2	7.1**
Cedrol	1596	1596	0.8	±0.2	0.3	±0.1	0.1	±0.0	0.5	±0.2	11.9***
Myristic acid	1720	1720	14.5	±0.5	15.8	±0.8	15.1	±0.1	13.6	±0.6	8.3**
E,E-farnesyl acetone	1921	1921	5.9	±0.1	7.9	±0.2	7.4	±0.4	8.0	±1.0	9.0**
E-phytol	1950	1949	37.5	±0.5	40.5	±0.5	39.8	±0.8	34.0	±1.0	48.1***
MCH			2.5	±0.3	1.8	±0.2	2.5	±0.3	5.9	±0.1	18.8***
MCHO			2.4	±0.4	3.0	±0.1	1.6	±0.4	4.9	±0.1	13.8**
SCH			2.0	±0.1	0.4	±0.1	1.6	±0.3	4.1	±0.1	18.7***
SCHO			49.7	±0.7	53.9	±0.9	53.5	±0.5	50.4	±0.4	31.9***
DCHO			37.5	±0.7	40.5	±0.5	39.8	±0.2	34.0	±1.0	66.8***
Total identified			94.1		99.6		99.0		99.3		

[a]RI: Retention index calculated, [b]RI: Retention index from literature, MCH: Monoterpene hydrocarbons, MCHO: Oxygenated monoterpenes, SCH: Sesquiterpene hydrocarbons, SCHO: Oxygenated sesquiterpenes, DCHO: Oxygenated diterpenes, M: Mean, SD: Standard deviation, *p<0.05, **p<0.01, ***p<0.001, values are given as Mean±SD, ns: Non-significant

highest (53.9 and 40.5%) in the essential oil extracted from leaves. Statistical analysis (ANOVA) indicated that insignificant changes were found in linalool and 2-Hydroxy-1,8-cineole for various plant organs. The variations in α-pinene, sabinene, p-cymene and β-cyclocitral were significant ($p<0.05$) for stems, leaves, flowers and fruits. Moderate significant changes ($p<0.01$) were cleared in geranyl acetone, caryophyllene oxide, myristic acid, E,E-farnesyl acetone and MCHO. Highly significant differences ($p<0.001$) were found in β-pinene, α-terpinene, limonene, nonanal, 1S-β-fenchol, terpinen-4-ol, β-caryophyllene, β-ionone, β-bisabolene, myristicin, cedrol, E-phytol, MCH, SCH, SCHO and DCHO.

DISCUSSION

Calotropis procera essential oil (contents and constituents) were changed with different plant organs. These changes in *C. procera* essential and its constituents may be due to the changes in enzyme activity and metabolism of essential oils production in various plant organs[18]. Similar constituents were detected in the essential oil of *C. procera* grown in Nigeria[1], so this study helps pharmaceutical companies of Egypt in not importing *C. procera* plant abroad. The variations in essential oil contents and constituents with different plant organs were confirmed by previous literature of some medicinal and aromatic plants. The essential oil and its major components of oregano, thyme and *Eucalyptus cinerea* were significantly changed with different plant organs[19-25]. On the other hand, the changes in essential oil groups were confirmed by Sipahi *et al.*[26], reported that the values of essential oil groups (aldehydes, aromatic components, oxygenated monoterpenes, oxygenated sesquiterpenes and aliphatic hydrocarbons) of *Sarcopoterium spinosum* plants were changed according to plant organs.

Calotropis procera plant contains different kinds of terpenes, which are composed of essential oils[27-29]. The terpenoids found in the greatest abundance in essential oils are the monoterpenes, sesquiterpenes and the diterpenes[29]. The monoterpenes and diterpenes have antinociceptive, antioxidant and antibacterial properties[29]. Phytol is known to inhibit the growth of *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans* and *Aspergillus niger* to block the teratogenic effects of retinol[30-31]. Phytol produces antinociceptive activity in mice, suggesting central and peripheral effect, without changing the motor function of animals[32]. Phytol has antischistosomal properties[33]. Myristicin has high activity as a Glutathione S-transferase (GST) inducer in the liver and anticarcinogenic natural products[34]. E,E-farnesyl acetone presented moderate antibacterial activity against *Aeromonas* sp.[35]. Myristic acid has antibacterial and antifungal principle for clinical application[36]. Myristic acid most strongly related to the average serum cholesterol rates in humans body[37], meaning acid was positively correlated with higher cholesterol levels as well as raising triglycerides in plasma by some 20% increasing the risk for cardiovascular disease although some research points to myristic acid's positive effects on HDL cholesterol and hence improving HDL (good cholesterol) to total cholesterol ratio[38].

CONCLUSION

It may be concluded that the highest amounts of *C. procera* essential oil were obtained from *C. procera* fruits. The major constituents of *C. procera* essential oil were E-phytol, myristicin, myristic acid and E,E-farnesyl acetone. Essential oil isolated from leaves recorded the highest amounts of E-phytol, myristicin and myristic acid while fruits oil recorded the highest amounts of E,E-farnesyl acetone. Different variations were found in the chemical classes of *C. procera* essential oil. It can be recommended that many studies can carry out on *C. procera* to discover more natural compounds which are required for pharmaceutical and drug industries.

SIGNIFICANT STATEMENT

This study discovers that the essential oil *C. procera* grow in Egypt produce high amounts of some active ingredients which have an important medicinal proprieties. This investigation will encourage the producers of medicinal and aromatic plants as well as pharmaceutical companies to cultivate *C. procera* shrub to produce the essential oil as a natural source of drugs and pharmaceutical industries.

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