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Quantitative and qualitative study of bioactive compounds of essential oils of plant *Lippia citriodora* by use of GC-MS technique

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ABSTRACT: This plant belongs to the verbenaceae family and from genus *Lippia* with scientific name *Lippia citriodora*. The leaves of this plant has effect anti fever, anti nerves pain, anti anemia, carminative. Also this plant were heart booster, and cause low blood sugar and treatment of migraine. In this study essential oils were extracted from aerial parts *lippia citriodora* (branche , leave and flower) via hydrodistillation(HD) method by clevenger set. By using of gas chromatography-mass spectrometry(GC-MS) technique the component chemicals essential oil were identified. About 55 components with the help of GC-MS technique were identified which encompassed 97. 54% of the whole essential oil. The essential oil yields as a result of hydrodistillation 0.70% (weight/weight) was obtained (it has been based on dried materials). In essential oils *Lippia citriodora* cis sabinene hydrate (38.99%), spathulenol (10.4), cuparene (6.81%), α-terpineol (5.05%), geranyl acetate (3.91%), β-pinene (3.46%) and E citral (3.4%) were the major identified compounds. Oxygenated monoterpens group had the most highly percentage of essential oil. Because the type and percentage of plant essential oil compounds in each region is different with other regions, so the purpose of this study was to identify the constituents of essential oils extracted from plants and also to determine the percentage of each compound in the essential oil of *Lippia citriodora* used as drug.

***Keywords*:** *Lippia citriodora*, hydrodistillation, essential oil, sabinene hydrate, GC-MS.

INTRODUCTION

Lemon verbena is a green shrub from *Verbenaceae* family. The genus *Lippia* has approximately 200 species indigenous to southern and central America and Africa. These compounds have wide-spread application in the food, cosmetics and household product industries (Gourama and Bullerman, 1995; Fang et al., 1994). *Lemon verbena* is an evergreen shrub which reaches two meters height. It's leaves sublimate as a results of odor perfumerubbish (Rezaei and Jaymand, 2002). The fresh and dried leaves of this plant are useful for antifever, antinervation pain, anti-anaemia, anti-bloating and so on. It is also used as heart alleviating and anti-migraine. This plant is used as decreasing blood sugar, anti-blood releasing of nose and enteral. The leaves of this plant are also useful for stomache pain, heartbeat, the feeling of doubtful sounds in ear and mental disturbances. The addition tea to *Lemon* is extraordinarily mental alleviating the essential oils of this plant in food industries and is used in making various perfumes (Rojhan, 2000; Momeni and Shahroki, 1998; Santos-Gomes et al., 2005). The essential oils of this plant is used in treatment tumor and cancers especially paratiroids, abdominal lien and polips. The under investigation species plant is *lippia citriodora* the leaves of this plant are linear and are green pale in color and are in triple group forms. The leaves are pale violet and have panicol in stems. Their investigators and researchers from Iran and other

countries have worked on various species of this plant in the past. the chemical composition essential oils of *lemon* from its leaves recognized in two steps in may and September via GC/MS and GC-FID in both two steps the main components included: geranial, neral, limonene which totally compose 66.3%of the whole essential oils (Catherine et al., 2007). Antioxidation and antibacterial activities investigated of the *lemon verbena* plant (Ansari et al., 2012; Bilia et al., 2008). The essential oils chemical components existed in stem and leaves investigated of lemon verbena plant. They worked based on GC/MS and GC method more than 70 components were known. The main components in both stems and leaves were geranial, neral and limonene (Gomes et al., 2006). The main components found in *lippia* (Rezaei and Jaymand, 2002; Santos-Gomes et al., 2005; Alavi et al., 2008; Linde et al., 2009; Bassole et al., 2005) collected by GC / MS technique from Iran and other countries is in accordance with Table 1. Since the kind and the percent of the essential oils components vary from one are to another area, therefore, the aim of this study is identification and extraction of the composed essential oils components and determination of the percent amount in essential oils by GC/MS technique for medical application.

MATERIALS AND METHODS

Collection of plant materials:

In this study, all aerial parts (leave , branche and flower) of *lippia citriodora* were used as sources to extract and identify the essential ingredients, the percentage of essential oil constituents and their surrounding areas in May, 2012, in kermansha,iran province were collected . In the shade and away from sunlight and kept dried after collecting samples for days. Then they were transferred to Lorestan University for more research.

Extraction method of essential oils:

By using a clevenger apparatus and hydro distillation (HD) method oil extraction plant was done. Weighed about 100 of plants lippia citriodora after drying and then added to a balloon and was connected to the clevenger apparatus. They were converted to liquid the resulting vapor after passing through the refrigerants were then collected in another container. Two-phase fluid consisting of water and oil was obtained which was separated by normal hexane the oil. Oil collected in the tube using a special syringe was collected after 3.5 hours. It was dehydrated with sodium sulfate. It was immediately poured into the sample container the aim to prevent the penetration of sunlight with aluminum foil into its close. Then, to perform tests, it was kept in refrigerator.

GC analysis:

GC analysis was performed by using gas chromatograph 17A Shimutzu equipped with a FID and a DB-5 capillary column (30 m × 0.25 mm; 0.25 μm film thickness). The oven temperature was programmed from 40°C to 150 ^{0}C at 3 ^{0}C/min rate. Then held isothermal for 10 min and finally raised to 250 ^{0}C at 10 ^{0}C/min. Other operating conditions were as follows: carrier gas, He with a flow rate of 1.9 ml/min; injector temperature, 250; detector temperature, 260 °C; split ratio, 1:5

GC/MS analysis:

The analysis of the essential oils was performed with gas chromatography 17A shimudzu coupled with mass spectroscopy shimudzu model QP5050. Separating compounds was performed in fused silica capillary DB-5 column(30 m × 0.25 mm inner diameter, with 0.25 µm film thickness). The oven temperature was programmed from 40°C to 150 ^{0}C at 3 ^{0}C/min rate.Then held isothermal for 10 min and finally raised to 250 ^{0}C at 10 ^{0}C/min. The quality of mass spectrometer was quite similar to gas spectrometer and for GC/MS detection an electron ionization system with ionization energy of 70 eV was used. Carrier gas was helium at a flow rate of 1.9ml/min. Mass range was from *m/z* 50–500 amu.

Compounds identified using the technique of GC / MS:

After providing and injection of essential oil to GC system, it was the best condition to separating. Then by using of method coupled gas chromatography with mass spectroscopy (GC-MS) the quantitative and quality of essential

oil components were recognized. The constituent compounds of the essential oils were identified by calculation of their retention indices under temperature-programmed conditions for n-alkanes(C_8-C_{24}) and the oil on a DB-5 column under the same chromatographic conditions. Identification of compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library data GC-MS system (Wiley 229) and with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature (Adams, 1995 and 2001). The relative percentage of each compound was obtained according to it's under peak area in GC chromatogram, without the use of correction factors.

RESULTS AND DISCUSSION

The result of this research was identification of 55 compound in essential oils extracted from aerial parts of *lippia citriodora* which encompassed 97.54% of the whole essential oil. The essential oil yields 0.70% (weight/weight) was obtained (it has been based on dried materials). Studies show that between different groups of monoterpenes, oxygenated monoterpenes has the highest concentration (62.77%) of essential oils. Other groups include: oxygenated sesquiterpene (13.77%), sesquiterpene hydrocarbons (11.22%)and monoterpene hydrocarbons (5.32%) (table 2). cis sabinene hydrate (38.99%), spathulenol (10.4), cuparene (6.81%), α-terpineol (5.05%), geranyl acetate (3.91%), β-pinene (3.46%) and (E) citral (3.4%) were the major identified compounds. Compounds identified in Table 2 are consistent. But also, shows figure(1) GC chromatogram the essential oils of *lippia citriodora*.

Previously the essential oils of several spices of *lippia* were recognized both by Iranian researcher and other countries. The main essential oils components was reported include: E-citral, geranial, neral, limonene, caryophillene oxide, spathulenol, curcumen, borneol, neryl acetate, camphor, carvacrol, beta-caryophillene and para-cymene (Rezaei and Jaymand, 2002; Santos-Gomes et al., 2005; Catherine et al., 2007; Gomes et al., 2006; Alavi et al.,2008; Linde et al., 2009). There are in similarities between the main components essential oils of *lippia* in this study with studied previously. For example, E Citral have reported as one of the main componenets of essential oils of species of *lippia citriodora* (Alavi et al., 2008; Linde et al., 2009). Also, researchers have been reported spathulenol as one of the main essential oil compounds (Rezaei and Jaymand, 2002; Alavi et al., 2008). Furthermore, there are considerable qualitative and quantitative differences between essential oils composition of *lippia* in this study, with those of previously reported from different parts of iran and other countries. In conclusion, chemical differentiation of *lippia* essential oils might be correlated with environmental conditions, geographic, climatic, genetic, plant age, soil, phase of vegetation, anatomical part of plant and harvesting season (Gudaityte and Venskutonis, 2007; Tetenyi, 1987 and 2002; Kokini et al.,1994; Burbott and Loomis, 1967), therefore, there are essential differences between the compounds of plants that have been previously reported with this plant. Because the type and concentration of plant essential oils chemical compounds in each region is different with other regions, therefore necessary perform many study in this case in different place of iran for uses various medicinal.

Table 1. The main components found in the essential oil of Lippia collected from Iran and other countries

entry	Region	Specie	Part of harvesing	The main compounds
1	Iran[3]	Lippia citriodora	and Leave Flower	1,8cineole,limonene,geranial, neral,β-guaiene,spathulenol, caryophillene oxide
2	portegal [6]	vervain	Flower and Leave	in both two: Geranial,neral,limonene
3	Iran[11]	Lippia citriodora	Aerial parts	E-citral,geranial,neral,limonene, caryophillene oxide,spathulenol, curcumen,borneol,neryl acetate, beta-caryophillene, Camphor, carvacrol,para-cymene
4	[12]Southern Africa	verbenaceae	Aerial parts	Citral(E,Z),borneol,camphor,neryl acetate, iso and beta caryophillene,beta-caryophillene oxide,para-cymene
5	Burkina faso[13]	Lippia chevalieri	Leave and flower	In leave :thymol,para-cymene,2-phenyl-ethyl propionate and in flower : βelemene,ethyl cinnamate,α-amorphene piperitol

Table 2. identify compounds of plant essential oils lippia citriodora by GC-MS on the DB-5 column

name of compound	RI	Content(%)
α-pinene	939	0.7
Camphene	954	0.06
β-pinene	979	3.46
Myrcene	991	0.38
2-octanol	995	1.93
α-terpinene	1017	0.28
γ-terpinene	1060	0.44
Cis sabinene hydrate	1070	38.99
Linalool	1097	1.29
Trans sabinene hydrate	1098	2.0
Trans pinene hydrate	1123	0.34
Trans limonene oxide	1142	0.41
Cis sabinol	1143	0.07
Trans verbenol	1145	0.57
Camphor	1146	0.12
Thujol	1169	0.53
Rose furane epoxide	1177	1.19
Terpinene- 4-ol	1177	1.65
α- terpineol	1189	5.05
Pulegone	1237	1.13
Carvone	1243	1.43
piperitone	1253	0.64
E citral	1267	3.4
Butyl heptanoate	1291	0.05
Perilla alchole	1295	0.05
Trans carvyl acetate	1342	0.05
α- cubebene	1351	0.05
unknown	-	0.17
Geranyl acetate	1381	3.91
β- bourbonene	1388	0.98
Metyl eugenol	1404	0.15
arromadendrene	1441	0.4
α- himachallene	1451	1.46
Allo aromadendrene	1460	0.12
Geranyl propionate	1478	0.42
β- selinene	1490	0.52
Zingiberene	1494	0.14
(+)cuparene	1505	6.81
γ-cadinene	1514	0.2
δ-cadinene	1523	0.33
Nerolidol Z	1533	0.35
α- cadinene	1539	0.06
Nerolidol E	1563	0.45
Geranyl butyrate	1564	0.07
Spathulenol	1578	10.4
Hexyl benzoate	1580	0.95
Caryophillene oxide	1583	0.16
Salvial4(14)en-1-one	1595	0.07
β- oplopenone	1608	0.19
Humulene epoxide	1608	0.32
δ- cadinol	1646	0.43
Acorenone B	1698	0.1
Juniper camphor	1700	0.04
Longifolol	1715	1.26
unknown	-	0.82
Monoterpene hydrocarbons		5.32
Oxygenated monoterpenes		62.77
Sesquiterpene hydrocarbons		11.22
Oxygenated sesquiterpenes		13.77
Unknown compounds		0.99
Other compounds		3.47
Total		97.54

RI: retention indices relative to C_8-C_{24} n-alkanes on the DB-5 column.
Content(%): indicates relative area (peak area relative to the total peak area)

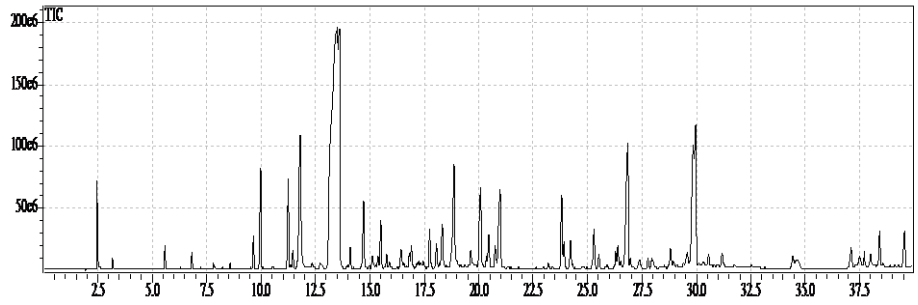

Figure 1. GC chromatogram the essential oil of Lippia citriodora

CONCULSION

The technique discussed in this paper provides an interactive approach in which the decision maker can search for an acceptable solution of the multi-objective optimization problem. The proposed method to solve multiobjective linear programming problem is better than many existing methods as the concept of bound is used in the iteration.

If we substitute some values to a_i , α_i in multi-objective linear programming problem (3.1), it reduces into single objective LPP. This discussion also holds in the case as given by by Kanniappan and Thangavel (1998). The same problem for integer solution was studied by Bhargava and Sharma (2003).

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