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Research Article

GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS AND *IN VITRO* ANTIOXIDANT ACTIVITIES OF *RUTA GRAVEOLENS* L. FROM WESTERN GHATS REGION - SOUTH INDIA

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ABSTRACT

Objective: The present study examines the chemical composition of essential oil of *Ruta graveolens* and antioxidant activity of petroleum ether and methanol extracts of dry leaves of *R. graveolens* which belongs to *Rutaceae* family found in many parts of the India. There are few reports are available for its essential oil in India.

Methods: The fresh leaves were hydrodistilled by Clevenger type apparatus, the obtained essential oil was analyzed using gas chromatography-mass spectrometry (GC-MS). The petroleum ether and methanol extracts were tested for antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) methods.

Results: The GC-MS results showed that 2-nonanol (34.25), 2-nonanone (32.15) and nonyl ester (15.43) are the major components and some of the other minor compounds are 2-dodecanone (2.77), tridecanone (2.54) and tumeronol (2.16). The preliminary phytochemical investigation of petroleum ether and methanol extracts showed the presence of carbohydrates, phenolic compounds, flavonoids, furanocoumarins, fatty acids and alkaloids. Hence, an attempt was made to find the possible sources for future novel antioxidants in food and pharmaceutical formulations.

Conclusions: GC-MS studies indicated that 2-nonanone (32.15) and nonyl ester (15.43) are the major components. The methanol extract showed highest inhibitory concentration values for DPPH (19.50±1.25 µg/ml) and ABTS (48.32±0.9 µg/ml) and petroleum ether extract showed moderate activity, both results are comparable with standard employed for each method.

Keywords: *Ruta graveolens, Rutaceae*, Gas chromatography-mass spectrometry, Essential oil, antioxidant, 2,2-diphenyl-1-picrylhydrazyl, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid).

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INTRODUCTION

In Indian system of traditional medicine from ancient to the current period, most of the rural families mainly depend on plant-based products for their medical needs. Especially, in hilly areas, 90% of the disease can be resolved by nearly available plants. In this view, our present chemical investigation on *Ruta graveolens* L. is an aromatic and medicinally important plant belongs to the family *Rutaceae* commonly known as Rue and Aruvatham Pachai in Tamil and originated from Europe, which is widely distributed in the Mediterranean Region, India, China, Brazil and throughout the world. There are two important *Ruta* species, namely, *R. graveolens* and *Ruta chalepensis* are available in India, in which *R. graveolens* is one of the promising specie due to its biological importance [1,2]. In the traditional system of medicine whole parts of the plant is used as stimulant, emmenagogue, diuretic, abortifacient, resolvent, anti-inflammatory, eye problems, dermatitis and anti-rheumatic medicine and for the treatment of hypertension, skin illness phototoxic, bacteriostatic and anthelminthic activities and rhinitis [3-7]. The presence of secondary metabolites such as coumarins, alkaloids, terpenes flavonoids and other compounds in *R. graveolens* has been reported [8]. There are many reports are available for its volatile oil composition and it exhibited various biological activities such as hepatotoxicity, anti-oxidant, anti-inflammatory, cytotoxic, antitumor, antimicrobial, antifungal, anti-conceptive and anti-fertility activity [9-16] of various extracts of *R. graveolens* in different origins. In India, Western Ghat Region is one of the hotspot region and most promising area for its different agroclimate and enormous wealth of economically important medicinal plants. Most of the plants in this region are unexplored for its chemical composition. The leaves and aerial parts of this aromatic plant are used for skin diseases, headaches, stomach ache and indigestion for children by local people and also its unpleasant odor is used to repel insects and mosquitoes. Hence, the main purpose of this investigation is to determine the chemical composition of essential oil of fresh leaves and *in vitro* antioxidant activity of petroleum ether and methanol extracts of *R. graveolens* using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays. There are only a few reports are available for the chemical composition of *R. graveolens* in India, but no reports are available from the Western Ghat region.

MATERIALS AND METHODS

Plant materials

The plant materials were collected from Vallparai Hills, Western Ghat Region, Tamil Nadu; it was identified and authenticated by Dr. A. Logamadevi, Assistant Professor, Department of Botany, NGM College and voucher specimen (16CHE001) were preserved in the same department.

Isolation of essential oil

The fresh leaves of *R. graveolens* (300 g) were washed with tap water and cut into small pecies, then taken into 1 L round-bottom flask with water and subjected to hydrodistillation using clevenger type apparatus for 3 hrs. The pale green volatile oil obtained was separated from the water by anhydrous sodium sulfate and stored in a sealed container which is kept in the freezer until analysis.

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis of the essential oils of *R. graveolens* was carried out using thermo GC - trace ultraversion: 5.0 coupled with thermo MS DSQ II instrument. Compounds were separated on DB-35, MS capillary standard non-polar column (30 m × 0.25 mm), film thickness 0.25 µm. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 70°C and held for 2 minutes and the temperature of the oven was raised to 260°C for 10 minutes and raised 6°C/minute and final temperature was 300°C for 10 minutes. The sample of 100 ml was dissolved in 1 mL of acetone and injected with splitless mode. Mass spectra were recorded over 50-500 amu range with electron impact ionization energy 70 eV, while injector and MS transfer line temperature were set at 230°C and 280°C, respectively.

Identification of essential constituents

The components were identified by comparison of their mass spectra with those of NIST mass spectral library version 2.0d, as well as on comparison of their retention time either with those of authentic compounds or with literature values.

Extraction process

The plant (200 g) was shade dried and coarse powdered material was defatted with petroleum ether by cold maceration and was subjected to vacuum distillation to yield a greenish residue (4.5 g). The defatted plant leaves was again extracted with methanol using a Soxhlet apparatus for 4 hrs, then the solvent was distilled using rotary evaporator to yield a brownish residue (11 g).

Preliminary phytochemical screening

The preliminary phytochemical analysis was done for both petroleum ether and methanol extracts [17,18]. Small quantity of both residues were dissolved with appropriate reagents for detection of secondary metabolites such as sterols (chloroform and sulfuric acid), alkaloids (Dragendroff' reagent), flavonoids (sodium hydroxide), carbohydrates (Fehling A and B), terpenoids (Salkowski test), saponins (by distilled water), phenolic compounds (3% lead acetate solution) and coumarins (1 ml of 10% sodium hydroxide).

***In vitro* antioxidant activity**

DPPH radical scavenging assay

The free radical scavenging capacity of the petroleum ether and methanol extracts of *R. graveolens* was determined using DPPH method described by Brand-Williams [19] with small modifications. DPPH (200 µm) solution was prepared in 95% methanol. Both extracts (1.0 mg/ml) were diluted to final concentrations of 20, 40, 60, 80 and 100 µg/ml in ethanol was taken in five test tubes and one ml of freshly prepared 0.3 mm DPPH solution was added and incubated with both extracts and standard ascorbic acid used as reference. After 30 minutes, decrease in absorbance was taken at 518 nm using spectrophotometer and percentage of DPPH activity was expressed by the following formula.

$$AA\ \% = 100 - \{ [(ABS_{SAMPLE} - ABS_{BLANK}) \times 100]/ABS_{CONTROL}\}$$

$ABTS^{+}$ radical-scavenging activity

ABTS radical-scavenging activity of both extracts was determined according to Arnao *et al.* [20]. Both plant extracts were diluted with concentrations of 20, 40, 60, 80 and 100 µg/ml and 1 ml of ABTS solution was added, a decrease in absorbance was recorded at 734 nm using spectrophotometer after 5 minutes. The percentage inhibition of ABTS radical was calculated using the following formula:

$$\text{ABTS scavenging activity (\%)} = (A_0\text{-}A_1)/A_0 \times 100$$

Where, A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Statistical analysis

The experiment was conducted 3 times for independent results and was expressed as means±standard. Analysis of variance was evaluated by one-way ANNOVA method. Significant differences in $p<0.05$ were analyzed by Dunnett's test.

RESULTS

GC-MS analysis

Hydrodistilled essential oil was pale green about 0.5% (v/w) of yield. Its volatile composition was analyzed by GC-MS. A total of 20 components were identified and represent 99% of the detected oil composition. The results were given in Table 1 and GC-MS profile of essential oil was given in Fig. 1.

Preliminary phytochemical screening

The preliminary phytochemical screening evaluated for petroleum ether and methanol extracts revealed that the petroleum ether extract

Table 1: GC-MS analysis of volatile composition of essential oil of *R. graveolens*

S.No.	Name of the compound	RT	(%) Composition
1	2-Nonanone	6.56	32.15
2	Methyl nonyl ester	8.20	15.43
3	2-Nonanol	8.85	34.25
4	2-Undecanone	9.49	0.29
5	2-Dodecanone	10.44	2.77
6	3-Tert-butylcatechol	11.08	3.42
7	2-Tridecanone	12.16	2.54
8	4-Hydroxy-3-pentyl-cyclohexanone	14.31	0.24
9	Hedycaryol	14.87	1.41
10	2-Pentyl-cyclohexane-1,4-diol	16.06	0.05
11	Gamma eudesmol	16.98	0.09
12	4-Amorphen-11-ol	17.66	0.18
13	2-Phenyl1-1,4-(ethylene-1',2'-diyl) cyclohexane	18.81	0.54
14	Tumeronol B	21.33	2.16
15	9,12,15-octadecatrienal	22.31	0.04
16	Phenanthrene	22.83	0.14
17	Phytol	25.20	0.31
18	(2E)-7-(3',4'-methylenedioxyphenyl)-2-heptenoic acid	27.29	0.81
19	Trans-4-anisylcinnamic acid	29.77	1.95
20	1-Hexadecanol	31.05	0.05

GC-MS: Gas chromatography-mass spectrometry, *R. graveolens: Ruta graveolens*

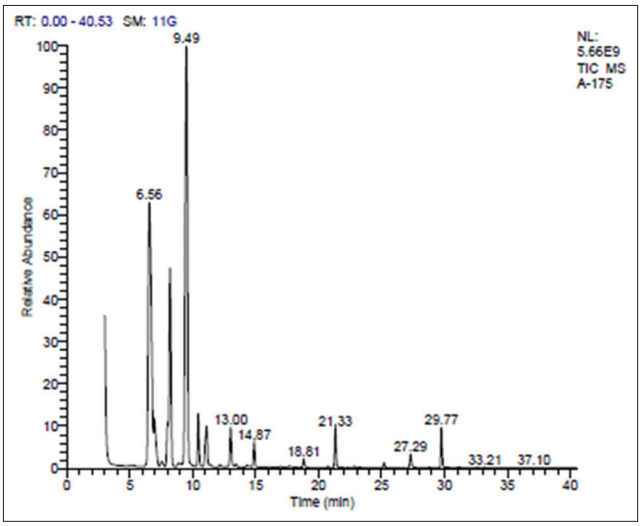

Fig. 1: Gas chromatography-mass spectrometry chromatogram of essential oil of *Ruta graveolens*

containing fatty acids, carbohydrates and steroids. The methanol extract showed the positive results tested for flavonoids, alkaloids, furanocoumarins and terpenoids. The results were given in Table 2.

***In vitro* antioxidant activity**

There are several assays have been employed for the evaluation of the antioxidant capabilities of plant extracts and synthetic compounds for clinical trials, we employed DPPH and $ABTS^{+}$.

DPPH radical scavenging activity

It is very quick, easy, reliable and reproducible method for *in vitro* antioxidant for plant extracts. It has nitrogen-centered free radical when DPPH is added to the test extracts purple color changes to yellow color depending on the reducing power of plant extracts to decrease in absorbance at 518 nm. Both petroleum ether and methanol extracts were prepared in the concentration range of 20-100 µg/ml and the antioxidant potential of both extracts increases with increasing concentration (Fig. 2). The inhibitory concentration (IC_{50}) values for petroleum ether, methanol extracts and the standard employed Ascorbic acid were found to be 74.46±0.01, 19.50±0.03 46.34±0.05, respectively. The methanol extract showed highest percentage of inhibition compared to petroleum ether extract and standard ascorbic acid.

ABTS radical scavenging activity

ABTS assay is employed for the screening of antioxidant potential of both petroleum ether and methanol extracts of *R. graveolens*. In this method, ascorbic acid is used as a standard compound. Fig. 3 showed the concentration dependant activity of both extracts and standard. The IC_{50} values for petroleum ether, methanol and standard were found to be 79.05±0.01, 48.32±0.01 and 52.67±0.01, respectively, the results were shown in Table 3.

DISCUSSION

The major compounds identified were 2-nonanol (34.25%), 2-nonanone (32.15%) and nonyl ester (15.43%). The other important components were 2-dodecanone (2.77%), tri decanone, (2.54%) and tumeronol (2.16%). The phytochemical analysis of essential oil of aerial parts of *R. graveolens* grown in different parts of the world has shown varied chemotype and the majority of the essential oil has 2-undecanone, 2-nonanone and 2-nonanol was the predominant compound. Similar results were found in *R. graveolens* found in Tunisia [21], Egypt [22,23], Ethopia [24,25] and Brazil [26]. In Iran, the plant essential oil showed that 2-undecanone (33.9%), 2-heptanol acetate (17.5%) and 1-dodecanol (11.0%) were major compounds [27]. The major identified compounds were 2-nonanone (37.13%), undecanal (34.69%), 2-acetoxydodecane (5.0%) and 2-decanone (3.31%) [28]. However, their composition of oil varies greatly, due to the geographical origin and climatic variation will affect the composition of essential oil. There are few reports available in India for chemical composition of essential oil of *R. graveolens* showed the varied composition of essential oil contents compared to our results. The predominant components of essential oil tested in North India were n-hex-4-en-3-one (55.06%), n-pent-3-one (28.17%) and n-hex-3en-2-one (14.7%) [2], 2-undecanone (44 to 51.30%) and 2-nonnone (15.88-20.33%) were the major compounds found in West India [29]. In contrast to published results, the essential oil of *R. graveolens* grown in the Western ghat region revealed that 2-nonanol, 2-nonanone and nonyl ester were the important major compounds but 2-undecanone 0.29%) found in trace amounts which is the major compounds of *R. graveolens* grown in different locations.

The preliminary phytochemical analysis revealed the presence of important secondary metabolites from both petroleum ether and methanol extracts of dry leaves of *R. graveolens*. The presence of carbohydrates, phenolic compounds, alkaloids, flavonoids, sterols, triterpenoids and coumarins has been already reported [8,29]. These secondary metabolites have great biological importance and show a broad range of pharmacological activities such as antioxidant activity, cytotoxicity, anti-inflammatory, etc., [1,15]. The methanol extract of *R. graveolens* contains flavonoids and furanocoumarins in high amount

Table 2: Phytochemical profile of petroleum ether and methanol extracts of *R. graveolens*

S.No.	Phytochemicals	Petroleum ether	Methanol extract
1	Steroids	+	-
2	Triterpenoids	+	+
3	Flavonoids	-	+
4	Alkaloids	-	+
5	Furanocoumarin	+	+
6	Phenolic compounds	-	+
7	Saponins	+	-

R. graveolens: Ruta graveolens

Table 3: IC_{50} values for petroleum ether, methanol extracts in DPPH and ABTS method

S.No.	Test/standard solution	DPPH method	ABTS
1	Petroleum ether extract	74.46±0.01	79.05±0.01
2	Methanol extract	19.50±0.03	48.32±0.01
3	Standard	46.34±0.05	52.67±0.01

All values were expressed in mean±SEM (n=3), DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS: 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), SEM: Standard error of the mean, IC_{50}: Inhibitory concentration

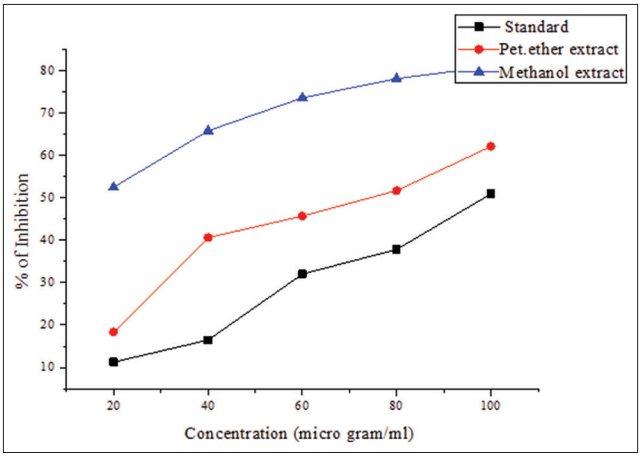

Fig. 2: Free radical scavenging activity of petroleum ether, methanol and standard by 2,2-diphenyl-1-picrylhydrazyl method

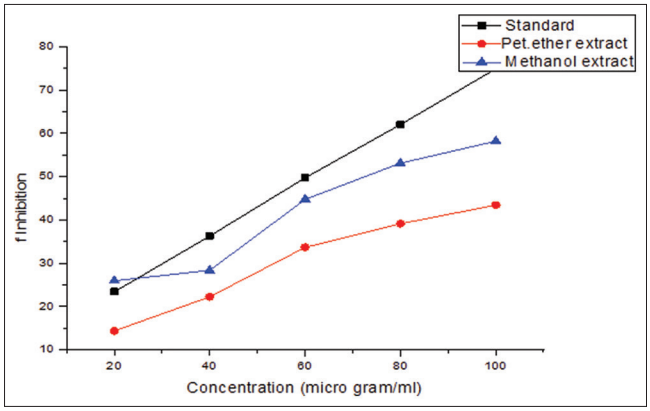

Fig. 3: Free radical scavenging activity of petroleum ether, methanol extracts and standard by 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) method

which have excellent antioxidant properties and protect cells against oxygen free radicals and oxidation [30]. The Ruta plant is less explored for its phytoconstituents and biological activities in the Western Ghat Region, South India. *In vitro* antioxidant potential of both petroleum ether and methanol extracts of *R. graveolens* and showed concentration dependant activity on DPPH and ABTs and ABTS. DPPH radical have stable nitrogen that can accept a hydrogen atom or electron from the scavenger molecule, i.e., antioxidant, results in reduction of unpaired valence electron at one atom of nitrogen bridge in DPPH leading to the change of purple color to yellow with a decrease in absorbance at 515 nm. The color change indicates the scavenging potential of the crude extracts. From the results, methanol extract has highest DPPH radical scavenging activity than the petroleum ether extract and standard compound was due to the presence of flavonoids which was shown in Table 3.

Both extracts of *R. graveolens* were tested with $ABTS^{+}$, that is green, the assay involves reduction of the color intensity of ethanolic solution containing preformed radical of ABTS, generated by oxidation of ABTS with potassium persulfate due to the radical scavenging activity of antioxidants. The change in color intensity is proportional to the antioxidant efficiency of the compound. Both extracts of *R. graveolens* showed concentration-dependent activity against ABTS radicals. However, the methanol extract exhibited excellent scavenging activity compared to petroleum ether extract.

Moreover, antioxidant properties of *R. graveolens* were evaluated by two different methods; free radical scavenging using DPPH and inhibition of lipid peroxidation by the ferric thiocyanate method [30,31,34]. The IC_{50} value of the methanol extract in DPPH inhibition was 200.5 µg/ml which was acceptable in comparison with BHT (41.8 µg/ml). From the results, *R. graveolens* exhibit good antioxidant activity against all the three methods. Further, the essential oil composition of the *R. graveolens* showed variation with already published data. These chemical differences may be due to seasonal, genetic, geographical variation and most probably the existence various chemotypes [32-35]. The present study provides the scientific evidence and strengthens the candidature of *R. graveolens* used in the traditional medicine for combating many diseases.

CONCLUSION

The chemical composition of essential oil of *R. graveolens* grown in Western Ghat Region showed different components compared to other parts of India. The methanol extract exhibited highest antioxidant activity against DPPH and ABTS methods. This may be due to the presence of high content of phenolic compounds, flavonoids and furanocoumarins which enhances the overall activity of the extract. These findings may provide scientific evidence for the traditional uses of the plant. The isolation of active phytoconstituents is under progress.

REFERENCES

1. Benazir JF, Suganthi R, Devi MR, Suganya K, Monisha K, Nizarahamed KP, *et al*. Phytochemical profiling, antimicrobial and cytotoxicity studies of methanolic extracts from *Ruta graveolens*. J Pharm Res 2011;4(5):1407-9.
2. Malik AA, Mir SR, Ahmad J. *Ruta graveolens* L. Essential oil composition under different nutritional treatments. Am Eurasian J Agric Environ Sci 2013;13(10):1390-5.
3. Bently R, Trimen H. Medicinals Plants. 1st ed. New Delhi: Asiatic Publishing House; 2004. p. 44.
4. Chopra RN, Nayer SL, Chopra IC. Glossary of Indian Medicinal Plants. 3rd ed. New Delhi: Niscair; 2002. p. 217.
5. Iserin P. Encyclopédie Des Plantes Médicinales. Paris: Larousse; 2001. p. 335.
6. Petit-Paly G, Ramawat KG, Chenieux JC, Dean M. Xxvi. *Ruta graveolens*: *In vitro* pro-ri-duction of alkaloids and medicinal compounds. Biotechnol Agric Forestry 1988;7:488-505.
7. Miguel ES. Rue in traditional Spain: Frequency and distribution of its medicinal and symbolic applications. Econ Bot 2003;57(2):231-44.
8. Kostova I, Ivanova A, Mikhova B, Klaiber I. Alkaloids and coumarins from *Ruta graveolens*. Monatsh Chem 1999;130:703-7.
9. el Agraa SE, el Badwi SM, Adam SE. Preliminary observations on experimental *Ruta graveolens* toxicosis in Nubian goats. Trop Anim Health Prod 2002;34(4):271-81.
10. Sailani MR, Moeini H. Effect of *Ruta graveolens* and *Cannabis sativa* alcoholic extract on spermatogenesis in the adult wistar male rats. Indian J Urol 2007;23(3):257-60.
11. Ratheesh M, Helen A. Anti-inflammatory activity of *Ruta graveolens* Linn on carrageenan induced paw edema in wistar male rats. Afr J Biotechnol 2007;6:1209-11.
12. Réthy B, Zupkó I, Minorics R, Hohmann J, Ocsovszki I, Falkay G. Investigation of cytotoxic activity on human cancer cell lines of arborinine and furanoacridones isolated from *Ruta graveolens*. Planta Med 2007;73(1):41-8.
13. Preethi KC, Kuttan G, Kuttan R. Anti-tumour activity of *Ruta graveolens* extract. Asian Pac J Cancer Prev 2006;7(3):439-43.
14. Ivanova A, Mikhova B, Najdenski H, Tsvetkova I, Kostova I. Antimicrobial and cytotoxic activity of *Ruta graveolens*. Fitoterapia 2005;76(3-4):344-7.
15. Meepagala KM, Schrader KK, Wedge DE, Duke SO. Algicidal and antifungal compounds from the roots of *Ruta graveolens* and synthesis of their analogs. Phytochemistry 2005;66(22):2689-95.
16. Al-Mahmoud MS, Elbetieha A, Al-Muhur RA. Anticonceptive and antifertility activities of various *Ruta graveolens* extracts in femaie rats. Acta Pharm Turc 2003;45:203-13.
17. Claus FD. Phamacognosy. 4th ed. London: Henry Kumpton; 1961.
18. Kokate CK. Practical Pharmacognosy. New Delhi, India: Vallabh Prakashan; 2005. p. 107-11.
19. Brand-Williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. Lebensm Wiss U Technol 1995;28(1):25-30.
20. Arnao MB, Cano A, Acosta M. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem 2001;73:239-44.
21. Fredj MB, Marzouk B, Chraief I, Boukef K, Marzouk Z. Analysis of Tunisian *Ruta graveolens* L. oils from Jemmel. J Food Agric Environ 2007;5(1):52-5.
22. El-Sherbeny SE, Khalil MY, Hussein MS, Aly MS. Effect of sowing date and application of foliar fertilizers on the yield and chemical composition of Rue (*Ruta graveolens* L.) Herb. Herba Pol 2008;54(1):48-55.
23. Aboutabl EA, Elazzouny AA, Schmidt FJ. The essential oil of *Ruta graveolens* L. growing in Egypt. Sci Pharm 1988;56:121-4.
24. Amabye TG, Shalkh TM. Phytochemical screening and evaluation of antibacterial activity of *Ruta graveolens* L. - A medicinal plant grown around Mekelle, Tigray, Ethiopia. Nat Prod Chem Res 2015;3(6):1-4.
25. Hilawie M, Unnithan CR, Muuz M, Gebremedhin D, Reddy DN, Birhan A, *et al*. Extraction, isolation and chemical composition of the essential oil of *Ruta graveolens* L of Mekelle, Northern Ethiopia. Int J Pharmacother 2015;5(1):5-7.
26. Orlandaa JF, Naascimentab AR. Chemical composition and antibacterial activity of *Ruta graveolens* (*Rutaceae*) volatile oils from Saoluis Maranhao. Braz South Afr J Bot 2015;99:103-6.
27. Soleimani M, Aberoomand AP, Saber-Tehrani M, Rustaiyan A. Volatile composition of *Ruta graveolens* L. of North of Iran. World Appl Sci J 2009;7:124-6.
28. Al-Shuneigat JM, Al-Tarawneh IN, Al-Qudah MA, Al-Sarayreh SA, Al-Saraireh YM, Al-Sharafa KY. The chemical composition and the antibacterial properties of *Ruta graveolens* L. essential oil grown in Northern Jordan. Jordan J Biol Sci 2015;8(20):139-43.
29. Kengar AA, Paratkar GT. Evaluation of essential oil of fresh and dry leaves of *Ruta graveolens* L. Int J Curr Res Biosci Plant Biol 2015;2(9):33-7.
30. Ahmed OM, Moneim AA, Yazid IA, Mahmoud AM. Anti hyperglycemic, anti hyperlipidemic and antioxidant effects and the probable mechanisms of action of *Ruta graveolens* infusion and rutin in nicotinamide-streptozocin induced diabetic rats. Diabetol Croat 2010;39(1):15-35.
31. Motamed SM, Bagherzadeh SS, Forouz SA, Tafazoli H. Evaluation of antioxidant activity of *Ruta graveolens* L. extract on inhibition of lipid peroxidation and DPPH radicals and the effects of some external factors on plant extract's potency. Res J Pharmacogn 2014;1:45-50.
32. Kathirvel P, Ravi S. Chemical composition of the essential oil from basil (*Ocimum basilicum* Linn.) and its *in vitro* cytotoxicity against HeLa

and HEp-2 human cancer cell lines and NIH 3T3 mouse embryonic fibroblasts. Nat Prod Res 2012;26(12):1112-8.
33. Poonkodi K. Phytoconstituents from *Richardia scabra* L. and its biological activities. Asian J Pharm Clin Res 2016;9(6):168-71.
34. Reddy LJ, Jose B. Chemical composition and antibacterial activity of the volatile oil from the bark of *Gliricidia sepium*. Int J Pharm Pharm Sci 2010;2(3):177-9.
35. Hassanein HD, Hah SA, Abdelmohsen MM. Antioxidant polyphenolic constituents of *Satureja montana* L. growing in Egypt. Int J Pharm Pharm Sci 2014;6(4):578-81.