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# Total Phenolic Compound, Antioxidant Activity of Cultivated Ethiopian *Ruta Chalepensis* Crude Extract and its Essential oils.

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***Abstract***
*In this work essential oils of Ruta chalepensis was extracted using Clevenger hydro steam distillation and its chemical composition were identified using Gas chromatography- Mass spectroscopy (GC-MS). Major components of essential oils were 2-undecanone (31.74), 1-dodecene(13.591), Tridecene (13.189), 2-Nonanone(9.573),2-tetradecanol (4.856).Total phenolic compounds (TPC) and antioxidant activity of Ruta chalepensis crude extract of ethanol, aqueous, methanol, diethyl ether, hexane were determined using the method of Kirby and Schmidt ,calculated using the Gallic acid calibration curve of $y = 1.318x + 0.0892$ with Regression coefficient of $R^2=0.9959$ and evaluated as Milligram Equivalent of Gallic acid (MEGA)/Gram of Dry Weight (GDW. DPPH (2, 2-diphenyl-1-hydraine) was used as free radical scavenging ability for antioxidant determination. TCP is 15.674 MEGA/ GDW for aqueous extract, 13.74 MEGA/ GDW for methanol extract, 10.81 MEGA/ GDW for ethanol extract, 9.686 MEGA/ GDW for diethyl extract and 4.127 MEGA/ GDW for hexane extract. Methanolic crude extract has maximum antioxidant activity followed by ethanolic crude extract in maximum inhibition effect and $IC_{50}$.Hexane crude extract has antioxidant activity than that of aqueous extract, diethyl ether and extract in terms of $IC_{50}$.*

***Key words:*** *Ruta chalepensis, antioxidant, total phenolic compounds, $IC_{50}$, inhibition effect, GC-MS, calibration curve, Regression coefficient*

## Introduction

Essential oils are volatile, liquid aroma compounds and natural products obtained from plants .They refer to any concentrated, hydrophobic, typically lipophilic liquid of plants that contains highly volatile aroma compounds and carries a distinctive scent, flavor, or essence of the plant (Rubiolo *et al*., 2010). They act as antibacterial, antiviral, antifungal, antioxidant, insecticides and widely used in perfumes, cosmetics, food and drink flavoring, for scenting incense, in household cleaning products, and for medicinal purposes. As a result, essential oils have recently been gaining a growing popularity and scientific interest. The chemical composition of essential oils depends on factors like climatic, seasonal and geographic conditions, harvest period and extraction technique. Knowledge of the chemical composition of essential oils is a very important quality criterion for their marketing and contributes to their valorization (Marotti, 1994).

*Ruta chalepensis* belongs to the Rutaceae family, named by local people as *"Tenadam"*. It is commonly known as rue and used in the traditional medicine of many countries for the treatment of a variety of diseases (Neuwinger, 2000). The medicinal value of this plant for the treatment of nervous diseases was emphasized by Dioscorides (Stuart, 1979).The plant is prescribed in the Indian system of medicine for the treatment of dropsy, neuralgia, rheu-matism and menstrual and other bleeding disorders (Foucaud, 1953). In Saudi Arabia, a decoction of its aerial parts is used as an analgesic and antipyretic and for the treatment of rheumatism and mental disorders (Iauk *et al*., 2003). The leaves *Rutac halepensis* infused with vinegar are given to children for the treatment of convulsion and other nervous disorders. Ethanol extract of the aerial parts of *Ruta chalepensis* produced a significant central nervous system depressant activity in mice (Ganzalez-Trujano *et al*., 2006). The aqueous extract of its leaves had a spermotrophic action demonstrated by the increase in sperm count, motility, living percent, and decrease in encountered sperm abnormalities. The hormonal profile was also influenced since the testosterone and follicle stimulating hormone levels were significantly increased with no change in the leutinizing hormone and prolactin levels (Al Qarawi, 2005).

Naturally occurring antioxidants has considerably increased in various products to replace synthetic antioxidants which are being restricted due to their carcinogenicity (Nahed Fakhfakh *et al*., 2012).Antioxidants can be natural or synthetic various substances with different chemical characteristics and they offer protection against lipid oxidation, react with free radicals, reduce oxidative stress, protect both the biologically important cellular components and stop low density lipoproteins (LDLs) or bad cholesterol from being oxidized . The common known synthetic antioxidants are butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), Ascorbic acid and tertiary butyl hydroquinone (TBHQ) (Fasseas *et al*., 2007).However, due to their toxicological effects there is an interest in developing natural antioxidants from plants (Rababah,T. *et al*, 2004). Phenolic compounds are among natural compounds which act as antioxidant and it is found in different aromatic and medicinal plant extract .It can be determined using Folin-Ciocalteu colorimetric assay with gallic acid as reference standard. Its basic mechanism is redox reaction in which the oxidation of phenols by Folin-Ciocalteu reagent yields a colored product with λ max at 765 nm. One methods of measuring Antioxidants is radical scavenging free radical of 2,2-Diphenyl-1-picrylhydrazl (DPPH) and measuring decrease in absorbance with plant extract at 517 nm (Miguel, M.G, 2011)

Previous work indicates that aqueous extract of *Ruta chalepensis* contains the Total phenolic compounds (TPC) of 1328.8 mg GAE/100g (Nahed Fakhfakh *et al*., 2012). But published literature rarely report the TPC and antioxidant activities of only limited solvents of other countries and not yet that of cultivated Ethiopian *Ruta chalepensis* including its essential oils. That is why this work is aimed to determine TPC, its antioxidant capacity of cultivated Ethiopian *Ruta Chalenpensis* (locally; "Tenadam") using different solvent along with its essential oils.

## Materials and Methods

### *Materials*

*Raw Materials*

The plant material used for this project was cultivated *Ruta chalepensis*. The detailed information of plant species and its botanical description was obtained from Wando Genet Agricultural Research center (WGARC) .The center, WGARC, is one of the fourteen Federal research center under Ethiopian Institute of Agricultural Research and it is found in Southern Nation, Nationalities and People of Ethiopian (SNNPE) regional state and located at a distance of 267 km from Addis Ababa. WGARC is known by Aromatic, Medicinal, and Bio energy as center of excellence in Ethiopia. Some of the representative sample was taken from this center and the other was purchased from local people who cultivate this plant and bring it to the local market available at Addis Ababa on morning time. The harvested plant was identified into different parts as stems, leafs, aerial parts including leaf and stems extension found on the upper part of the stems including the flowers parts which are about to flower. The dirty substances harvested along with all the plant part is removed and washed by the tap water. Then all aerial parts of the plant were identified and allowed to be dried at room temperature for about three weeks to prevent the exposure of these plant parts to the open sun light. Then the dried aerial parts were reduced in size to the desired particle size of (0.25-0.75mm) by Cross beater Mill.

*Chemicals*

The chemical used for this work were: hexane, ethanol, distilled water, methanol, diethyl ether, DPPH (2,2-diphenyl-1-picryrazyl) ,ascorbic acid, gallic acid , sodium carbonate, folin-Ciocalteu reagent. All of these chemical were analytical grade (more than 98% minimum assay).

*Essential Oil Isolation Procedure*

Aerial parts of *Ruta chalepensis* plants used were collected at flowering stage in a morning time and air-dried at room temperature. About 100 g of the sample was subjected to hydro distillation for 5 hours in a Clevenger-type apparatus. The yield was calculated as the percentage of the ratio of weight of the essential oils collected over the water to the weight of the sample fed apparatus. Then essential oils were dried overran hydrous sodium sulfate and stored at 5 °C until analysis.

*Extractions of Ruta chalepensis crude extracts*

The dried powder of the *Ruta chalepensis* leaves (30 g) was Soxhlet-extracted with 300ml of ethanol, methanol, hexane and diethyl ether for 8 hours at their respective normal boiling points. Then the solvent was evaporated using a rotary evaporator and the crude extracts were concentrated. Finally, extract was kept in the fridge at $4^0$C until further analysis. For the aqueous extract, the powdered *Ruta chalepensis* leaves (30 g) were mixed with 300 ml distilled water at $60^o$C temperature for 12 hours using incubator shaker at a constant stirring rate of 250 rpm. Afterwards, the solids residue was separated by filtration and the solution was freeze-dried to remove water.

*Gas Chromatography–Mass Spectroscopy (GC-MS) Analysis of Ruta chalepensis essential oils*

GC-MS analysis was performed using an Agilent Technologies 7820A Gas chromatograph system equipped with a HP-5 capillary column (30m x0.25;coating thickness, 0.25 8m) and a Agilent technologies 5977E Mass spectroscopy ion trap detector. Analytical conditions were as follows: injector and transfer line temperature, 220 and 240 $^O$C, respectively; oven temperature, programmed from 60 to 240$^o$C at 3$^o$ C/min; carrier gas, helium at 1mL/min; injection, 6µL(10 % hexane solution);and split ratio, 1:30. Qualitative identification of the constituents was based on comparison of elution or the retention time on the HP-5MS column keeping minimum quality of the each component 90% and Quantitative analysis was done using electronic integration of peak area percentages. Mass\

hunter\ library\ NIST11.L and mass\ hunter\ library\ W9N11.Llibrary database system and previous work were used for analysis and its comparison.

*Total Phenolic Compounds (TPC) Determination of Ruta chalepensis Crude Extract.*
TPC determination involves identification of chemicals, instrumentation, reagents and solution preparation, generation of standard Gallic acid calibration curve and preparation of saturated carbonate solution. The materials and chemicals required were: extracted sample, Folin-ciocalteu Phenol (FC) stored in the dark of not visibly green in color, saturated sodium carbonate ($NaCO_3$), distilled water, 100 volumetric flasks, vortex mixer, Gallic acid calibration standards, micro pipettes and whatman No1filter paper. Instrumentation required was UV-Vis high performance Spectrophotometer which can measure absorbance of the solution at 765nm. For reagents and solution preparation deionized or distilled water was used in all recipes and protocol steps and the procedures of TPC determination was based on the method developed by Singleton and Rossi Singleton and Rossi (1965) which involves three parts as follows.

I) Preparation of different standard concentration of Gallic acid: 0.05g of Gallic acid was dissolved in 1 ml methanol and diluted to 10 ml with water (5g/liter of final stock). From this stock solution, standards of 5, 50, 75, 100, 125, 150,200 and 250 of mg/liter (ppm) were prepared in properly washed, cleaned and dried test tubes. Then, solutions were stored at $4^oC$ in refrigerator.

II) Preparation of saturated sodium carbonates solution and Folin-ciocalteu phenol reagents (FC): 20g of anhydrous sodium carbonate was dissolved in 80 ml distilled water and brought to boil (saturated). After cooling, few crystals of sodium carbonate were added and it was sat for 24 hours at room temperature. Then the solution was filtered through whatman No.1 filter paper and distilled water was added till it reaches 100ml label. Finally it was stored indefinitely at room temperature. Folin-ciocalteu phenol (FC) reagent was prepared taking 1 ml of its stock solution and diluted with 10 ml of Methanol .Then the color of the solution was noted whether it was visibly green or yellow since the solution should be yellow in color for its full potency.

III). Generation of Calibration curve for Gallic acid and determination of TPC: **A**bsorbance of standard concentration of gallic acid prepared on step I was measured using High performance UV-Vis near Infrared spectrophotometer at 765nm and its calibration curve was generated. Then, 0.05 mg of each crude extract was taken and dissolved in 1 ml of methanol solution. From this solution more diluted solution were prepared to search for the concentration of the extracted sample to which the exact value of the maximum possible concentration was included in the interval of calibration curve of standard Gallic acid. In each of the concentration taken 1ml of Folin-Ciocalteu phenol was added to the sample, standard and blank before test tubes were incubated for 8 minutes after they were mixed by Vortex mixer .Then 1ml of Saturated sodium carbonate was added to each of incubated test tubes for 8 minutes and the mixture in the test tubes were filled to 10ml with distilled water and again incubated for about 90 minutes at room temperature. Finally the TPC for each extract was measured as milliequivalents of gallic acid after its maximum absorbance were measured within the standard limits of gallic acid.

*Antioxidant Determination of Ruta Chalepensis Crude Extract.*
The antioxidant activity of *Ruta Chalepensis* crude extracts were evaluated using the method of Kirby and Schmidt (1197). This method uses DPPH as radical scavenging which has strong absorption maximum at 517 nm (purple) and the colour turns from purple to yellow in the presence of antioxidants.

First, 0.004% DPPH was prepared by measuring 0.01g of DPPH in 250ml methanol in volumetric flask and about 3 mg/ml of standards of Ascorbic acid was prepared by dissolving 3mg of ascorbic acid in 1ml methanol and for successive uses, the standard solution was further dissolved by measuring 150 mg of ascorbic acid in 50ml keeping the ratio stated in the Kirby and Schmidt method.

Secondly, 10 mg of extract/ml of methanol solution was prepared as stock solution. Different concentrations of extracts from 0.2-2 mg/ml in methanol where made with total of 1 ml. Then 2ml of 0.004% DPPH solution was added to the test tubes and mixed with vortex mixer for20s.

Thirdly, all the test tube was incubated for about 30 minutes in the dark at room temperature to allow the reaction between the extract and DPPH and to prevent the absorbance of light by DPPH that may interfere with the actual absorbance of the sample and DPPH solution.

Fourthly, scavenging capacity was read spectrophotometrically by monitoring the decrease in absorbance at 517nm using high performance UV-Vis spectrophotometer and inhibition of free radical DPPH in percent (I %) was calculated in the following ways.

$$I = \left(\frac{\text{Acontrol} - \text{A sample}}{\text{A sample}}\right) x100$$

*Where: $A_{control}$ is the absorbance of the control reaction), $A_{sample}$ is absorbance of the test compound.*

Finally the extents of the scavenging will be carried out in doublet and the results were reported as a mean average of the two parallel measurements plus or minus their standard deviation along with their $IC_{50}$ value.

**Result and Discussion**

*Comparative study of Ruta chalepensis L. essential oils with previous results*

The yield of essential oils of cultivated Ethiopian *Ruta chalepensis* is found to be 1.018±0.07 and it is below the yield evaluated by some of the previous works. For instance the yield of the *Ruta chalepensis's* essential oils collected from Tunisia and investigated was reported as 2.32±0.2 (Nahed Fakhfakh *et al*., 2012). The volatile compounds of essential oils identified from cultivated Ethiopian *Ruta chalepensis* were shown in table 1. Some of these compounds can be separated on the basis of their chemical structures into ketones, esters, acids, alcohols, aldehydes, hydrocarbons and diterpenes. From table 1, 2-undecanone (methyl nonyl ketone) (31.74), 1-dodecene (α-Dodecene; Dodec-1-ene (13.591), Tridecene (13.189), 2-Nonanone (methyl heptyl ketone) (9.573) and 2-tetradecanol (4.856) are the major components. These compounds possess dominantly the functional group of ketones, alkenes, and alcohols which are similar with previous work, but only on their percentage and this might be due to geological variation and weather condition that alter the components of essential oils. For example, essential oils of aerial parts of *Ruta chalepensis* plants grown at different locations have shown wide range of variations even in their major constituents. However, almost all of them are dominated by 2-undecanone. However, essential oil of the plant from Italy showed that 2-undecanone and 2-nonanone were major compounds. Moreover, 2-undecanone (26 to 44%) and 2-nonanol (28 to 40%) were found to be the main compounds in essential oils of growing wild *Ruta chalepensis* from Tunisia and the occurrence of a new chemotype of *Ruta chalepensis* growing of Tunisia containing octyl acetate (28.5 to 33.16%), 2-undecanone (22.6 to 23.8%) and 2-nonanone (14.1 to 16.97%) as the major constituents of the essential oil. In addition, other functionalized compounds such as Isomaturnin was detected for the first time in the *Ruta chalepensis* essential oil of Tunisia which was not found in Ethiopian cultivated *Ruta chalepensis* (Baser *et al*., 1996; Rustaiyan *et al.*, 2002; Bagchi *et al*., 2003; Ntalli *et al*., 2011; Saidani-Tounsi *et al*., 2011), but Ficusin (6-Hydroxy-5-Benzofuranacrylic acid d-lactone) (4.186) which belongs to group of furanocoumarins occurring naturally in Rutaceae plant species is the other components exist in maximum amount next to 2-tetradecanol (4.856) in this study. This compound has various medicinal applications and none of the previous work discussed above shows the existence of this compound.

**Table 1.** The chemical composition of cultivated Ethiopian *Ruta chalepensis*

| Compounds | Retention time (minutes) | (%) of Total | Minimum quality |
|---|---|---|---|
| (E)-2-Hexenal | 4.070 | 0.06 | 98 |
| 3-(Z)-Hexenol | 4.117 | 0.018 | 91 |
| α-Pinene (2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene) | 4.322 | 0.004 | 97 |
| Camphene | 6.469 | 0.02 | 97 |
| Benzaldehyde | 6.807 | 0.014 | 96 |
| Isopropyl tiglate (2-methyl-, 1-methylethyl ester) | 7.246 | 0.019 | 92 |
| β-Pinene (4,7,7-trimethylbicyclo[3.1.1]hept-3-ene) | 7.337 | 0.03 | 95 |
| Octanal | 8.205 | 0.024 | 90 |
| Anisole (1-methoxy-2-methylbenzene) | 8.457 | 0.039 | 97 |
| p-Cymene (4-Isopropyltoluene,2,3diol (2,3-dihydroxy-4-isopropyl-1-methyl- benzene) | 8.987 | 0.011 | 93 |
| D-limonene | 9.136 | 0.056 | 98 |
| 1,8-Epoxy-p-menthane (Eucalyptol) | 9.239 | 0.087 | 99 |
| beta-ocimene (3,7-dimethylocta-1,3,6-triene) | 9.896 | 0.015 | 92 |
| γ-Terpine (methyl-4-propan-2-ylcyclohexa-1,4-diene) | 10.296 | 0.013 | 95 |
| 1-octanol | 10.772 | 1.0701 | 91 |
| 2-Nonanone (methyl heptyl ketone) | 11.728 | 9.573 | 97 |
| Nonal (Nonanaldehyde) | 12.161 | 0.378 | 91 |
| 1,7,7-Trimethyl-bicyclo(2,2,1)heptan-2-one (TMPH) | 13.803 | 0.093 | 98 |
| 2-methoxy -3-isopropyl-(5 or6)-methyl pyridine | 14.515 | 0.019 | 93 |
| 1-Nonanol | 15.017 | 0.187 | 90 |
| 3-Cyclohexen-1-ol (L-4-terpineneol) or 4-methyl-1-(1-methylethyl) | 15.238 | 0.029 | 91 |
| 2-Decanone (Methyl octyl ketone) | 15.974 | 2.066 | 97 |
| Estragole (1-methoxy-4-(2-propenyl)-benzene) | 16.179 | 0.040 | 99 |
| 2-tridecanol(tridecan-2-ol) | 16.323 | 0.125 | 90 |
| Decanal (Decyl aldehyde, caprinaldehyde) | 16.52 | 0.074 | 91 |
| 1-dodecene (α-Dodecene; Dodec-1-ene; Dodecene-1; Adacene 12; Dodecylene) | 18.04 | 13.591 | 90 |
| 2-undecanone (methyl nonyl ketone) | 18.796 | 31.74 | 90 |
| Cyclodecane | 19.512 | 0.056 | 93 |
| pregeijerene (1,5-dimethylcyclodeca-1,5,7-triene) | 20.082 | 1.856 | 97 |
| 2-tetradecanol | 20.824 | 4.856 | 90 |
| 4-ethenyl-1 2-dimethoxybenethiol | 21.794 | 0.606 | 96 |

| | | | |
|---|---|---|---|
| Ethyl ,ester | 22.724 | 0.033 | 97 |
| 2-dodecanone | 24.716 | 1.754 | 96 |
| Tridecene | 26.436 | 13.189 | 90 |
| trans-β-Ionone (4-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-3-buten-2-one) | 28.406 | 0.056 | 98 |
| 2-Tridecanone | 28.859 | 1.669 | 97 |
| 1-undecene | 29.12 | 0.325 | 94 |
| α-Farnesene | 29.352 | 0.029 | 90 |
| Cis -α-Bisabolene | 30.682 | 0.058 | 99 |
| Cyclohexa methanol | 30.894 | 0.051 | 91 |
| 4-(3,4-methylenedioxyphenyl)-2-butanone | 32.668 | 0.38 | 98 |
| 1-Tridecene | 34.081 | 0.42 | 93 |
| Linolenic acid | 35.406 | 0.052 | 93 |
| 1,3-Benzodioxole-5-Propanoic acid, ethyl esther | 36.373 | 0.049 | 90 |
| Tetradecanal | 37.124 | 0.056 | 91 |
| Phenanthrene | 38.951 | 0.042 | 96 |
| Ficusin (Psoralen) | 40.688 | 4.186 | 97 |
| Hexadecanoic acid (Methyl ester) | 44.405 | 0.023 | 96 |
| And Others compounds of minimum quality less < 90% and which are not reported as essential oils | | | |

*Results on Total Phenolic Compounds (TPC) Determination.*

Gallic acid was taken as standard since it is the most known synthetic organic compound possessing maximum amount of TPC and natural TPC form plants extracts are evaluated using it as standard and Folin-Ciocalteu reagent. The Folin-Ciocalteu reagent is sensitive to reducing compounds including poly-phenols, there by producing a blue color upon reaction. This blue color is measured spectrophotometrically and its TPC content can be determined ( Savitree M.*et al*,2004). For standard calibration curve different concentration taken and absorbance measured by spectrophotometer at 725nm should give a curve of the form y=bx+a with minimum regression coefficient of $R^2$=0.933 where y is absorbance in nanometer (nm), b is the slope and x is the variable represents Gallic acid in millimole(mM). Then equation of the curve is used to find the TPC by measuring the maximum possible absorbance of the plant extract within the interval of absorbance of the standard as shown in table below.

**Table 2.** Absorbance of Standard Compound (Gallic Acid) at different concentration

| **Concentration (mg/ml)** | **Absorbance at ( λmax=725 nm)** | | |
|---|---|---|---|
| | The first trial | The second trial | Average ±SD |
| **0.01** | 0.0970 | 0.0730 | 0.0850±0.0170 |
| **0.05** | 0.1167 | 0.1850 | 0.15805±0.031 |
| **0.1** | 0.2300 | 0.2404 | 0.2352±0.0034 |
| **0.15** | 0.3089 | 0.2713 | 0.2901±0.0188 |
| **0.2** | 0.3450 | 0.3645 | 0.3548±0.0098 |
| **0.25** | 0.4052 | 0.4033 | 0.4043±0.0100 |
| **0.3** | 0.6223 | 0.5089 | 0.5656±0.0560 |
| **0.4** | 0.7305 | 0.6448 | 0.6877±0.0433 |
| **0.5** | 0.7560 | 0.7049 | 0.7305±0.0256 |

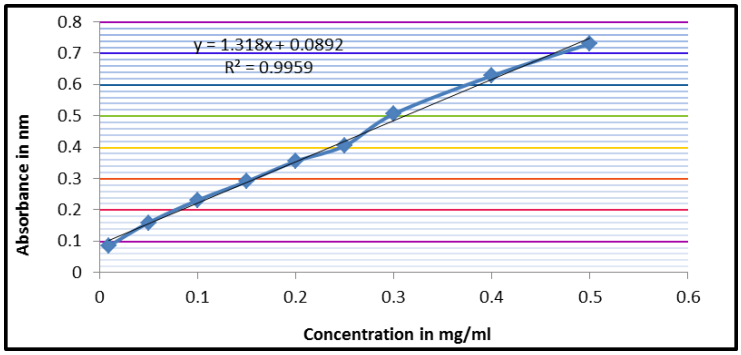


**Fig 1** .Calibration curve of Gallic acid as standard

From the Figure 1 we deduced that absorbance versus concentration of the calibration curve is straight line having equation of the curve y = 1.318x + 0.0892 with Regression coefficient of $R^2$ = 0.9959. This large number of $R^2$ shows the Absorbance and

concentration are linearly related, the calibration curve is successfully obtained and we can use it for determining the TPC of *Ruta Chalepensis* extract finding maximum absorbance of each extract with the limit of the standard (0.085-0.73045). Accordingly, Gallic acid was used as calibration standard, and results were calculated as Gallic acid equivalents (GAE) per gram of dry weight basis (mg/g) and values for two replicates as mean ± standard deviation were given in table below

**Table 3.** The maximum absorbance of different *Ruta chalepensis* crude extract, its essential oils and their corresponding TFC**.**

| Crude extract *Ruta Chalenpensis* | Maximum absorbance measured (nm) | TPC in milligram equivalent of Gallic acid MEGA /gram of dry weight (GDW) |
|---|---|---|
| **Ethanol** | 0.5094±0.015 | 10.81±0.0130 |
| **Methanol** | 0.6254±0.026 | 13.740±0.0023 |
| **Aqueous (distilled water)** | 0.7089±0.0032 | 15.674±0.003 |
| **Diethyl ether** | 0.4650±0.0012 | 9.686±0.0045 |
| **Hexane extract** | 0.2452±0.005 | 4.127±0.0750 |
| **Essential oils** | 0.000±0.000 | 0.000±0.0000 |

From Table 3, we can see that TFC of *Ruta Chalepensis* extract differ from one another based on solvent used for extraction and it is maximum for polar solvents and minimum for non-polar solvent. Aqueous crude extract of *Ruta Chalepensis* have greater TPC, hexane extract has less TPC and there is no significant TFC in its essential oils. As a result, we can understand that TPC found in *Ruta Chalepensis*is polar in nature and they are highly extracted by the Polar solvents. The TPC of aqueous and methanol crude extract of *Rutac halepensis* for this specific study is closer with that of the one evaluated by previous work which has reported TPC of methanol extract to be 13.288mg GAE/g of dry weight, 21.18 for aqueous extract (Alali, F. *et al*., 2007) and 11.58 for ethanol extract (Nahed Fakhfakh *et al*., 2012). From this specific study, we understand that aqueous extract have maximum TPC than ethanol and methanol extracts. Diethyl ether which is known by having intermediate polarity between polar and non-polar solvents shows maximum TPC than hexane. Based on the data of Table 3, we can arrange the *Ruta chalepensis* extract as the following decreasing order: Aqueous extract>Methanol extract>Ethanol extract>Diethyl extract>hexane extract.

*Antioxidant Activity of Ruta chalepensis Crude Oil Extract.*

Antioxidant activity of *Ruta chalepens is* extract was evaluated using Ascorbic acid as standard. It is one of the greatest antioxidant compound known by scavenging the stable radical of DPPH. Antioxidant activity of *Ruta chalepensis* extract were evaluated taking the all amount of ascorbic acid used for determining its antioxidant activity. Antioxidant activity of ascorbic acid increase in the first phase until its maximum inhibition effect of 96.025wasreached and decreased with further addition which was the same for *Ruta chalepensis* extract differencing with maximum inhibition effect. The Inhibition effect versus concentration of the sample get increase in the first phase until it reached the maximum value and starts slight decrease with further addition of the sample extract as shown in figure 2 which were taken from experimental data shown in Table 6. The effect of antioxidant on DPPH radical scavenging was thought to be due to their hydrogen donating ability. When a solution of DPPH is mixed with antioxidants, it is reduced to non-radical form of DPPH which results the change from violet color to yellow at maximum inhibition effect. Further addition of the extract sample can't get any more unstable radical of DPPH to stabilize and cause increase in absorbance that decrease the Inhibition effect.

**Table 4.** Inhibition effect of crude extract of *Ruta chalepensis* of different solvent and ascorbic acid evaluated using on DPPH assay**.**

| Concentrations | Inhibition effects of *Ruta chalepensis* crude extract and ascorbic acid | | | | | |
|---|---|---|---|---|---|---|
| | **Ethanol Extract** | **Methanol Extract** | **Aqueous extract** | **Diethyl ether extract** | **Hexane extract** | **Ascorbic acid** |
| **0.2** | 14.27±0.02 | 20.88±0.01 | 14.18±1.73 | 14.07±2.46 | 6.05±0.02 | 27.93±2.70 |
| **0.3** | 24.94±0.28 | 38.65±1.04 | 21.03±0.45 | 32.17±1.03 | 12.8±0.25 | 50.57±0.01 |
| **0.6** | 51.37.±1.61 | 57.56±0.49 | 29.81±0.03 | 44.25±0.04 | 36.71±0.67 | 67.39±1.81 |
| **0.9** | 62.41±0.99 | 84.34±0.57 | 45.15±0.27 | 47.17±1.00 | 49.95±0.28 | 87.93±0.02 |
| **1.2** | 80.04±1.82 | 88.78±0.07 | 56.51±0.02 | 54.91±0.46 | 71.86±0.35 | 94.5±0.035 |
| **1.5** | 86.79±1.12 | 79.79±0.72 | 62.92±0.77 | 61.27±0.13 | 73.98±0.98 | 96.65±1.10 |
| **1.8** | 87.98±0.10 | 79.13±0.31 | 80.80±0.03 | 68.95±0.23 | 72.36±2.35 | 95.88±0.04 |
| **2** | 86.31±0.25 | 78.55±0.65 | 77.19±0.09 | 60.69±0.84 | 69.83±0.43 | 95.32±0.08 |

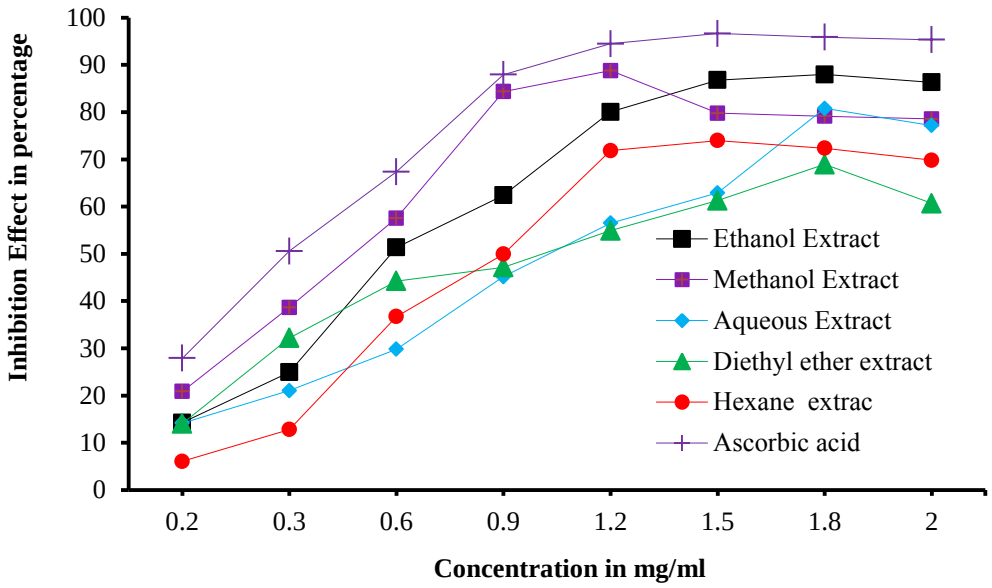


**Fig 2.** Inhibition effect of different extract of *Ruta chalepensis* crude extract

Antioxidants of DPPH scavenging activity can be presented by $IC_{50}$ value, defined as the concentration of the antioxidant needed to scavenge 50% of DPPH present in the test solution. Accordingly, extract concentrations $IC_{50}$were calculated using the data plotted in Figure 3.1.Lower $IC_{50}$ value reflects better DPPH radical-scavenging activity and has strong antioxidant potential. The maximum inhibition effect, concentration of IE and $IC_{50}$of different crude extract of *Ruta chelepensis* were given below.

**Table 5.** Maximum Inhibition effect and $IC_{50}$ of different crude extract oils of *Ruta chalepensis*

| Crude extract | Maximum IE in percentage | Maximum IE Concentration in mg/ml | $IC_{50}$ concentration In mg /ml |
|---|---|---|---|
| **Ethanol extract** | 87.98±0.10 | 1.8±0.11 | 0.720±0.23 |
| **Methanol extract** | 88.78±0.07 | 1.2±0.17 | 0.520±0.34 |
| **Aqueous extract** | 80.8±0.03 | 1.8±0.12 | 1.000±1.46 |
| **Diethyl ether extract** | 68.95±0.23 | 1.8±0.09 | 0.102±0.35 |
| **Hexane extract** | 73.98±0.98 | 1.5±1.30 | 0.960±1.07 |
| **Ascorbic acid** | 96.65±1.10 | 1.04±0.12 | 0.280±2.39 |

**Table 6.** Absorbance (A) measured and Inhibition effect (IE) of *Rutachalepensis* crude extract using different solvent measured at 517nm

| Concentration (mg/ml) | Ethanol Extract | | Methanol extract | | Aqueous extract | | Diethyl ether extract | | Hexane extract | | Ascorbic acid | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | IE (%) | A | IE (%) | A | IE (%) | A | IE (%) | A | IE (%) | A | IE (%) |
| **Blank** | 0.8546 | 0.00 | 0.6780 | 0.00 | 0.8287 | 0.00 | 0.7047 | 0.00 | 0.7996 | 0.0 | 0.8633 | 0.0 |
| **0.2** | 0.7118 | 14.28 | 0.5414 | 20.14 | 0.7011 | 15.40 | 0.6177 | 12.34 | 0.7510 | 6.07 | 0.6368 | 26.23 |
| **0.2** | 0.7121 | 14.25 | 0.5314 | 20.14 | 0.7213 | 12.96 | 0.5933 | 15.80 | 0.7513 | 6.04 | 0.6075 | 29.63 |
| **AVG.** | 0.7120 | 14.27 | 0.5364 | 20.88 | 0.7112 | 14.18 | 0.6055 | 14.07 | 0.7512 | 6.05 | 0.6222 | 27.93 |
| **0.3** | 0.6416 | 24.92 | 0.4210 | 37.91 | 0.6570 | 20.72 | 0.4831 | 31.44 | 0.6986 | 12.63 | 0.4287 | 50.34 |
| **0.3** | 0.6413 | 24.96 | 0.4110 | 39.38 | 0.6517 | 21.36 | 0.4729 | 32.89 | 0.6958 | 12.98 | 0.4248 | 50.79 |
| **AVG** | 0.6415 | 24.94 | 0.4161 | 38.65 | 0.6544 | 21.03 | 0.478 | 32.17 | 0.6972 | 12.8 | 0.4268 | 50.57 |
| **0.6** | 0.4058 | 40.81 | 0.2850 | 57.9 | 0.5815 | 29.83 | 0.3931 | 44.21 | 0.5099 | 36.23 | 0.2925 | 66.11 |
| **0.6** | 0.4008 | 52.51 | 0.2901 | 57.21 | 0.5818 | 29.79 | 0.3927 | 44.27 | 0.5023 | 37.18 | 0.2705 | 68.67 |
| **AVG.** | 0.4033 | 40.66 | 0.3466 | 57.56 | 0.5817 | 29.81 | 0.3929 | 44.25 | 0.5061 | 36.71 | 0.2815 | 67.39 |
| **0.9** | 0.3273 | 61.70 | 0.1035 | 84.73 | 0.4560 | 44.97 | 0.3773 | 46.45 | 0.4742 | 40.69 | 0.1258 | 85.42 |
| **0.9** | 0.3153 | 63.11 | 0.1089 | 83.93 | 0.4529 | 45.35 | 0.3673 | 47.87 | 0.3979 | 50.24 | 0.1042 | 87.93 |
| **AVG.** | 0.3213 | 62.41 | 0.1062 | 84.34 | 0.4545 | 45.15 | 0.3723 | 47.17 | 0.4361 | 45.46 | 0.1150 | 87.93 |
| **1.2** | 0.1816 | 78.75 | 0.0764 | 88.73 | 0.3603 | 56.52 | 0.3154 | 55.24 | 0.2244 | 71.90 | 0.0481 | 94.42 |
| **1.2** | 0.1596 | 81.32 | 0.0757 | 88.83 | 0.3605 | 56.49 | 0.3200 | 54.59 | 0.2256 | 71.85 | 0.0486 | 94.37 |
| **AVG.** | 0.1706 | 80.04 | 0.0761 | 88.78 | 0.3604 | 56.51 | 0.3177 | 54.91 | 0.225 | 71.86 | 0.0484 | 94.5 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1.5** | 0.1197 | 85.99 | 0.1335 | 80.31 | 0.3616 | 56.37 | 0.2735 | 61.18 | 0.2026 | 74.66 | 0.0345 | 96.01 |
| **1.5** | 0.1061 | 87.58 | 0.1405 | 79.28 | 0.3028 | 63.46 | 0.2723 | 61.36 | 0.2136 | 73.28 | 0.0342 | 96.04 |
| **AVG.** | 0.1132 | 86.79 | 0.137 | 79.79 | 0.3322 | 59.91 | 0.2729 | 61.27 | 0.2081 | 73.98 | 0.1212 | 96.65 |
| **1.8** | 0.1021 | 88.05 | 0.143 | 78.90 | 0.1589 | 80.82 | 0.2199 | 68.79 | 0.2360 | 70.4 | 0.0353 | 95.91 |
| **1.8** | 0.1033 | 87.91 | 0.1400 | 79.35 | 0.1592 | 80.78 | 0.2177 | 69.11 | 0.2101 | 73.73 | 0.0358 | 95.85 |
| **AVG.** | 0.1027 | 87.98 | 0.1415 | 79.13 | 0.1591 | 80.80 | 0.2188 | 68.95 | 0.221 | 72.36 | 0.0356 | 95.88 |
| **2** | 0.1132 | 86.75 | 0.1486 | 78.08 | 0.1886 | 77.25 | 0.2728 | 61.28 | 0.2436 | 69.53 | 0.0398 | 95.38 |
| **2** | 0.1102 | 87.11 | 0.1422 | 79.01 | 0.1896 | 77.12 | 0.2812 | 60.09 | 0.2387 | 70.15 | 0.0409 | 95.26 |
| **AVG.** | 0.117 | 86.31 | 0.1454 | 78.55 | 0.1891 | 77.19 | 0.277 | 60.69 | 0.2412 | 69.83 | 0.0405 | 95.32 |

From Table 5, we understand that polar solvent extract of methanol and ethanol has strong antioxidant since both of them have maximum inhibition effect and $IC_{50}$ concentration than the other solvents. Phenolic compounds contributed significantly to antioxidant activity. Because previous study of shows linear relation between total phenolic content and antioxidant activities i.e. a plant with high-antioxidant activity, for which phenolic content versus antioxidant activity falls above the regression line is a type of plant that should be investigated for novel antioxidants (Middleton *et al.*, 1994). Even if phenolic compounds contribute for antioxidant activity, flavonoids and tannins also contribute directly to the antioxidant activity (Rice-Evans *et al.*, 1996). Water is strong polar solvent than the other solvents and it has greater total phenolic compound than the others, but shows maximum inhibition effect at large concentration when compared with hexane extract and have almost equal $IC_{50}$ with that of diethyl ether. Previous study reports that ethanol, aqueous and methanol extracts of *Ruta chalepensis* have 347.33 mg QE/g,87.12 mg QE/g and323.12 mg QE/g of total flovonoid respectively when Quercetin was taken as standard. This may be the reason why aqueous extract of *Ruta Chalepensis* have less strong antioxidant since flavonoids compounds have antioxidant activity (Nahed Fakhfakh et al., 2012). There were no previous study for Diethyl ether and Hexane extract and justification of their antioxidant activity were based on experimental data of this study. From Table 5 we can see that hexane extract has antioxidant activity than that of Diethyl ether and aqueous extract based on their $IC_{50}$ value. Methanol extract has the strongest antioxidant activity than the others solvents being followed by ethanol extract based their maximum IE and their IC50.

**Conclusion**

*Ruta chalepensis*is one of the medicinal and aromatic plants diversified in most countries and known by having essential oils and phenolic compounds that contributed for its antioxidant capacity. Essential oils of *Ruta chalepensi e*xtracted using Clevenger hydro steam distillation and its chemical composition identified using Gas chromatography- Mass spectroscopy (GC-MS) contains 2-undecanone (methyl nonyl ketone) (31.74), 1-dodecene or α-Dodecene (13.591), Tridecene (13.189),2-Nonanone (methyl heptyl ketone) (9.573),2-tetradecanol (4.856) as major components .These compounds possess dominantly the functional group of ketones, alkenes, and alcohol. *Ruta chalepensis* crude extract of solvents ethanol, methanol, distilled water, diethyl ether and hexane were compared with one another showed different amount of total phenolic compound. The Total phenolic compounds is 15.674 MEGA/ GDW for aqueous extract, 13.74 MEGA/ GDW for methanol extract, 10.81 MEGA/ GDW for ethanol extract, 9.686 MEGA/ GDW for diethyl extract and 4.127 MEGA/ GDW for hexane extract. Methanol crude extract has maximum antioxidant activity than all others followed by ethanoic crude extract in terms of both maximum inhibition effect and $IC_{50}$. Hexane crude extract has greater antioxidant activity than that of aqueous extract, diethyl ether extract in terms of $IC_{50}$.

**Recommendations**

The following recommendations are made based on a holistic review of the subject area of *Ruta chalepensis:*

- Studies on evaluation of *Ruta chalepensis* extract for its preservative effect on various food products.
- Studies on evaluation of *Ruta chalepensis* essential oil extracted by using steam distillation for the production of perfume, cosmetics formulation, drug formulation and other relevant products.
- Studies on optimization of processing variables to maximize the antioxidant potential of *Rutachalepensis* crude extracts.
- Studies on Techno – Economic evaluation of *Ruta chalepensis* essential oil production

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