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ANTIOXIDANT AND A-AMYLASE INHIBITION ACTIVITY OF *RUTA CHALEPENSIS* L EXTRACTS

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ABSTRACT

In the present study total phenolic compounds (TPC), Total flavonoids content (TFC), α-amylase inhibitory activity, and antioxidant activity were measured by the DPPH test of methanol and ethyl acetate extracts of the leaves and flowers of *Ruta chalepensis* L were evaluated. The extraction yield using methanol for the flower and leaves were about 25%, while those for ethyl acetate were about 3.4%. TPC of the methanol extracts for the flowers and leaves of the Ruta was around 1150 mg GAE /100 g dried Ruta, while TPC of ethyl acetate extract of the Ruta leaves and flowers were 760 and 290 mg GAE /100 g dried Ruta. The methanolic extracts of Ruta leaves and flowers exhibited the strongest DPPH radical scavenging activity. The IC50 for both extracts were about 12 mg TPC/mL). However, the ethyl acetate extract of flowers showed the lowest DPPH radical scavenging activity (IC50 = 96.7 mg TPC/ ml) and it was significantly different than that of leaves (IC50 = 62 mg TPC/ml). The inhibitory effect of methanolic extracts of leaves on the α-amylase was the lowest (42.2%) followed by ethyl acetate of flowers (53.9%). Whereas, the ethyl acetate extract of leaves showed the highest inhibitory effect against α- amylase (63.7%) followed by methanolic extract of flowers (57.9%). The results obtained in this study clearly indicate that R. *chalepensis* L has a significant potential to use as a natural antioxidant as well as an antidiabetic agent.

1. Introduction

The use of different types of synthetic antioxidants such as Butylated Hydroxyanisole (BHA) and Butylated Hydroxy Toluene (BHT) in the food industry has raised many potential risks, and recent studies have shown the susceptibility to different types of cancer when using these antioxidants, thus recent research is moving towards the use of natural sources of antioxidants (Li et al., 2014). Moreover, there is a global interest in the use of herbs and aromatic plants in food preservation and in folk medicine (Christaki et al., 2012). Recent research has focused on the properties and characteristics of the extracts of the aromatic plants as well as their essential oils, as they resemble and acquire antimicrobial in addition to antioxidant activities (Chouhan et al., 2017). Antioxidant and antimicrobial activities of aromatic plants are attributed to many potent compounds, including flavonoids, eugenol, coumarins, carvacrol, and cinnamaldehyde (Khameneh et al., 2019). Active research has grown rapidly to look for more effective and safer plant-based hypoglycemic compounds (Saleh et al., 2013), despite the fact that these plants have been used since ancient times (Subbulakshmi and Naik, 2011). Many drugs used nowadays are from plant sources, as they

are considered as their primary sources, for example, the plant Galega officinalis is the primary source of glucophage (metformin), which is a hypoglycemic drug (Kumar and Nandi, 2017).

Ruta graveolens L, *Ruta chalepensis* L, and *Ruta montana* L are species from Ruta, which is a genus of the Rutaceae family (Pollio et al., 2008). *Ruta graveolens* L (also known as garden Rue)is a dicot herb grown mainly in many parts of the world, native to the Mediterranean region(including Jordan), it is also grown in southern Europe, northern Africa, India, and other tropical regions (Asgarpanah and Khoshkam, 2012), it's used by different populations (including the Jordanian population) for many beneficial purposes, especially in traditional medicine for its antirheumatic activity, analgesic and antispasmodic effects (Soare et al., 1997), it is also used as anticancer, anti colic, antiseptic, abortifacient, anthelmintic and antihypertensive (Ahmad et al., 2010).

Its aerial parts are mainly used in Jordan for locally produced ghee to enhance its color and flavor (Ahmad et al., 2010).

This study aimed at investigating the total phenolic content of methanol and ethyl acetate extracts of the leaves and flowers of *Ruta chalepensis* L., their antioxidant activity, and their effect on the activity of α-amylase used in the hydrolysis of starch. health has been determined. Yoghurt, which is suitable for lactose intolerant individuals, is also easy to digest (Dewit, 2010; Pochart and Desjeux, 1988).

2. Materials and methods

2.1. Materials

The plant material of the present study *Ruta chalepensis* L. was collected from the north region of Jordan (Bani Kenana district) and purchased in the spring season of 2018. Folin-Ciocalteu Reagent and sodium was from AppliChem, GmbH (Darmstadt, Germany). 2-chloro-p-nitrophenyl-α-D-maltotrioside, Quercetin, Gallic acid, α-Amylase, and Aluminum Trichloride were from Sigma-Aldrich (Steinheim, Germany), 2,2-Diphenyl-1-picrrylhydazyl was from (ICN, Biomedical INC, USA). Sodium carbonate was from Merck (Darmstadt, Germany). The used solvents were of HPLC grade.

2.2.1. Preparation of methanol and ethyl acetate extracts of leaves and flowers of Ruta chalepensis L.

The fresh leaves and flowers of *Ruta chalepensis* L were dried in an electrical oven at 40ºC. The dried samples were grinded using a domestic coffee grinder. Four extracts were prepared by boiling 10 g of the grinded leaves and flowers in 100 ml of ethanol or ethyl acetate. The methanol and ethyl acetate extracts were filtered in a 250 and 100 ml volumetric flask, respectively and the volume was made to the mark with the corresponding solvent.

2.2.2. Determination of the yield of extracts

To determine the yield of extraction, 20 ml (in duplicates) from each extract was placed in a previously weighed Petri dish, and the extract was evaporated at 80ºC in an oven for 3 hrs.

2.2.3 Determination of Antioxidants

2.2.3.1. Determination of total phenolic compounds (TPC)

The phenolic compounds in the 4 extracts of the ground flowers and leaves of *Ruta chalepensis* L were determined by the Folin-Ciocalteu reagent (FCR) according to the method of Al-Ismail *et al* (2006). Briefly, 0.1 ml of methanol extracts (10 mg/ml), ethyl acetate extracts (3.3 mg /ml), and standard solution (gallic acid) were mixed with 0.5 ml of Folin-Ciocalteau reagent. After 3 min, 2 ml of 10% (w/v) of sodium carbonate solution was added. The final mixture was shaken and then incubated for 1 h in dark at room temperature. The absorbance of all samples was measured at 650 nm using a spectrophotometer (Labomed spectrophotometer, model UVD-2900, Labomed, USA). and the results are expressed in mg gallic acid equivalents (GEA) per 100g dry weight of plant material

2.2.3.2. Determination of total flavonoid content (TFC)

The content of flavonoids in the 4 extracts of the ground flowers and leaves of *Ruta*

chalepensis L were determined according to the method reported by Miliuskas *et al*., (2004). 0.5 ml from each extract or standard (quercetin) was mixed with 1 ml of 2 % aluminum trichloride in ethanol solution; the mixture was diluted with water into a 25-mL volumetric flask and allowed to stand for 40 min at room temperature. The absorbance of the sample was then measured at 415 nm using a spectrophotometer (Labomed spectrophotometer, model UVD-2900, Labomed, USA). Flavonoids content was expressed in mg of quercetin equivalent (QE) per 100g dry weight of plant material

2.2.3.3. Determination of Diphenyl-1-picrryylhydazyl (DPPH) free radical scavenging activity of the extracts.

DPPH radical scavenging effect was determined according to the method of Al-Ismail *et al* (2006). A 0.2 ml of ethanol solution of DPPH (2,2-Diphenyl-1-picrylhydrazyl) (50 mg/ 100 ml) was mixed with different levels of each extract, and the mixture was brought to a total volume of 4.0 ml with the corresponding extracting solvent. The mixture was mixed thoroughly and was allowed to stand for 45 min in a dark place. The absorbance was then measured at 515 nm and the radical scavenging activity of the tested samples was expressed as %inhibition according to the following formula (Brand-Williams *et al*., 1995),

Inhibition (%) = [(Abs. control –Abs. sample)/ Abs. control] x 100

IC_{50} is the concentration of extract in mg/ml needed to scavenge 50% of the DPPH radical, which was calculated from their concentration-response curves.

2.2.3.4. Determination of α-amylase inhibitory activity by CNP-G3 Assay of the extracts.

According to the method of Suganuma, et al. (1997), the release of 2-chloro-4-nitrophenol (CNP) from CNP-G3 by porcine pancreas α-amylase was determined, where a 450 µl reaction mixture containing a solution of 0.2 M potassium thiocyanate and 0.15 mM CNP-G3 dissolved in 0.05 M phosphate buffer solution with a pH of 7.0 and an aqueous solution of the extract at a concentration of 3.3 mg/ml was pre-incubated for 5 minutes at 25ºC, which was followed by the addition of a volume of 20 µl of a freshly prepared α-amylase solution (1 mg/ml) in a phosphate buffer with a pH of 7.0.

During the reaction, the absorbance of the mixture was read at 405nm, and the increase in the absorbance determined the release of CNP from CNP-G3as follows,

The inhibitory activity% = [(A-B)/A] x 100

Where A is the increase in absorbance during the reaction when the extract is absent, and B is the increase in absorbance during the reaction when the extract is present.

2.3. Statistical Analysis

Statistical analysis was performed using (SPSS for Windows, Rel. 22.0, 2013, Chicago, SPrSS Inc.). Data were presented as mean ± SD, Significance was tested by ANOVA at *P*-value ≤0.05, and mean differences were determined by Duncan's multiple range test.

3. Results and discussions

3.1. Extraction yield

There are many steps to obtain phytochemicals from plants such as milling, grinding, homogenization, and extraction. Among these steps, extraction is the main step for recovering and isolating phytochemicals from plant materials (DO et al. 2014). In this study flowers and leaves of *Ruta graveolens* extracts were obtained by using methanol and ethyl acetate. Extraction yields of leaves and flowers using methanol (ca 25% w/w) were significantly greater than the corresponding extract using ethyl acetate (ca 3.4% w/w) (Table 1). It can be concluded that the more polar solvent will produce more extraction yield. The higher yield of methanol extract could be due to compounds other than phenolic, such as pigments, carbohydrates, and proteins, that may have been extracted by methanol (Zieli_nski and Kozłowska, 2000). The results of this study agree with the extraction yields of *Limnophila aromatic* (Do, 2014) and some medicinal plants (Sultana et. al. 2009).

3.2. *Total polyphenolic compounds (TPC) and flavonoids contents (TFC)*

Table 1 shows the TPC of the extracts measured using Folin Ciocalteu method. TPC of the methanol extracts of the flowers and leaves of the *Ruta* were 1175 and 1131 mg Gallic acid equivalent/100 g dried sample and they were not significantly different (P> 0.05). Whereas TPC of the ethyl acetate extract of *Ruta* leaves was significantly ($p \leq 0.05$) greater than that of flowers. The TPC of ethyl acetate extract of the leaves was 2.5 times greater than those of the ethyl acetate extract of flowers. The methanol extract of leaves and flowers contains more phenolic content as compared to the corresponding ethyl acetate extracts. The TPC of methanol extracts (Leaves or flowers) were about 4 and 1.6 times greater than those of ethyl extracts of leaves and flowers, respectively. It was observed that the effect of solvents on TFC is similar to that of TPC (Table 1). The highest TFC was obtained in the methanol extracts, while ethyl acetate showed lower efficiency in extracting flavonoids. Furthermore, the amount of TFC of the methanol extract of flower (409 mg/100g) was slightly but significantly greater than that of leaves (383 mg/100g. However, no significant difference in TFC between *Ruta* flowers or leaves was found. The TFC in the ethyl extracts was low when compared to TPC. The results indicated that solvent polarity plays a vital role in increasing phenol solubility (Sultan et al. 2009). These results indicate that the polarity of the solvent used in the extraction influences extracting of different phenolic compounds from the leaves and flowers of *Ruta.* The TPC of the methanolic extract of *Ruta chalepensis* leaves in this study was comparable to those reported by Ouerghemmi et al (2017) (1190.6 mg GAE/100 g), while those of the flowers were slightly greater (1688 GAE mg/ 100g. Mohammad et al. (2015) reported that there was no effect of methanol and ethyl acetate on the extraction level of polyphenols from *Ruta chalepensis* grown in Tunisia. On contrary to that, Athmouni *et al*. (2015) reported that the methanolic extract of *Scorzonera undulata* (*Asteraceae*) exhibited higher TPC than its ethyl acetate extract.

Table 1. The yield of extraction, total phenolic content (TPC), and total flavonoids (TFC) of the methanolic and ethyl acetate extracts of leaves and flowers of *Ruta chalepensis L*

Extract	Yield%	TPC (mg GAE /100 g dried Rutta)	(TFC) (mg /100 g dried Rutta)
Ethyl acetate flowers	3.2 ± 0.1^{b}	290 ± 5^{c}	22.6 ± 1.1^{c}
Ethyl acetate leaves	3.4 ± 0.2^{b}	726 ± 13^{b}	29.6 ± 1.4^{c}
Methanol flowers	25.8 ± 0.6^{a}	1175 ± 46^{a}	409 ± 12^{a}
Methanol leaves	25.4 ± 0.8^{a}	1131 ± 17^{a}	393 ± 13^{b}
• Different superscript within the same column are significantly different ($P \leq 0.05$)			

3.3. *Antioxidant activity*

Due to the presence of different antioxidant components in the crude extract and the complexity of the oxidation–antioxidation processes, no single testing method can provide a comprehensive picture of the antioxidant profile of a given sample. Several assay methods have been developed and applied to screen and evaluate the total antioxidant activity of plant extracts (Prabhakar et al., 2006). In this study, the antioxidant activity of *R. chalepensis* L. extracts have been determined by α-diphenyl-β-picrylhydrazyl (DPPH). DPPH radical is a stable organic free radical with an absorption band at 517 nm. It loses this absorption when accepting an electron or a free radical species, which results in a visually noticeable discoloration from purple to yellow. The DPPH activity was expressed by IC_{50}. The IC_{50} of a compound is inversely related to its antioxidant activity, as it expresses the amount of antioxidant required to decrease the DPPH concentration by 50%, which is obtained by 50%, which is obtained by interpolation from linear regression analysis (Liu et al. 2009). A lower IC_{50} indicates a higher antioxidant activity of a compound. Table 2 shows the IC_{50} values of the DPPH radical scavenging activity assay of the extracts. It was found that the methanolic extracts of *Ruta* leave and flowers possess the strongest DPPH radical activity (IC_{50} = around 0.0125 mg TPC/mL) and no significant difference between them. However, the ethyl acetate extract of flowers showed the lowest DPPH radical activity (IC50 = 0.242mg TPC/ ml) and it was significantly different than that of leaves (IC_{50} = 0.155 mg TPC/ml). Mohammad et al (2015) reported that methanolic extract *of R.* graveolens *L* showed higher DPPH activity than that of ethyl acetate extract which goes parallel with the results of this study. Generally, extracts that contain a high value of polyphenols exhibit high antioxidant activity (Srivastav *et al*., 2015).

Table 2. DPPH scavenging activity evaluated by IC50 and inhibition% of α-Amylase activity of the methanolic and ethyl acetate extracts of leaves and flowers of *Ruta chalepensis* L

Extract	IC_{50}		Inhibition% of α-Amylase activity
	mg TPC /ml	µl extract solution	
Ethyl acetate flowers	0.242 ±0.011	832 ± 6.4[a]	53.9 ± 1.3[c]
Ethyl acetate leaves	0.155 ± 0.013	213 ±4.9[b]	63.7± 1.2[a]
Methanol flowers	0.013± 0.006	27.1 ± 2.0[c]	57.9 ±0.6[b]
Methanol leaves	0.012± 0.006	25.7 ± 1.0[c]	42.2 ± 1.9[d]
*Different superscript within the same column is significantly different (P≤0 .05)			

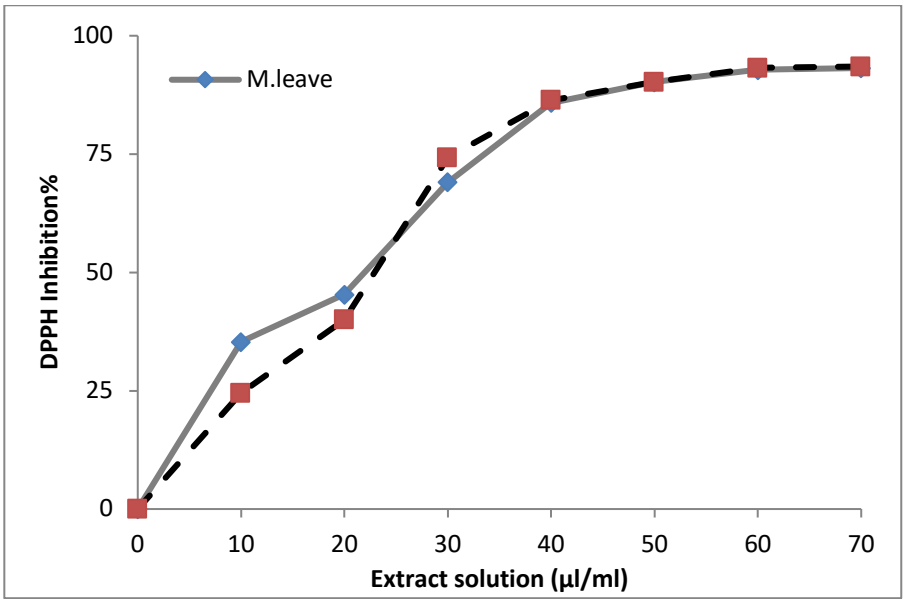

Figure 1. DPPH scavenging activity of methanol extracts of *Ruta* leaves and flowers

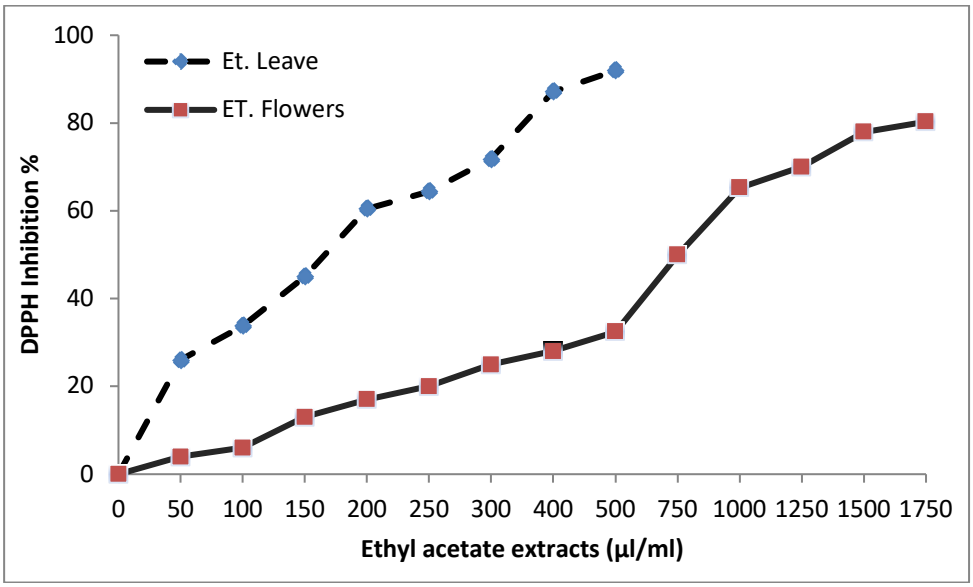

Figure 2. DPPH scavenging activity of Ethyl acetate extracts of *Ruta* leaves and flowers

3.4. *α- Amylase Inhibitory Activity of Rut chalepensis L. Extracts*

Alpha-amylase is a membrane-bound enzyme that is located on the brush border of the small intestine and is required for the breakdown of carbohydrates into monosaccharides (Lebovitz, 1997). Theα-amylase inhibitors act as an anti-nutrient that obstructs the digestion and absorption of carbohydrates and are potentially useful for the control of obesity and diabetes (Adesegun et al., 2013). The inhibitory effect of methanolic and ethyl acetate extracts on the α-amylase activity is reported in Table 2. The results indicated the inhibitory effect did not go parallel with those of antioxidant activity. The methanolic extracts of leaves with the highest

antioxidant and TPC showed the lowest inhibitory effect against α- amylase activity (42.2%) followed by ethyl acetate of flowers (53.9%) which had the lowest TPC and antioxidant activity. Whereas the ethyl acetate extract of leaves showed the highest inhibitory effect against α- amylase (63.7%) followed by the methanolic extract of flowers (57.9%). The results indicate that the inhibition of α- amylase activity might be due to other compounds rather than phenolic compounds.

4. Conclusions

The results of the present study revealed that the higher polarity of the solvent, the higher extraction yields, and the higher TPC and flavonoids. In the methanolic extracts, the distribution of TPC between flowers and leaves was equal. While it was not in the case of ethyl acetate extracts, since the TPC in leave extract was greater than that in flowers. Also, the results revealed that the higher TPC the higher antioxidant activity measured by the DPPH test. The inhibition effects the extract of α-amylase activity based on other factors than the amount of TPC, since the ethyl acetate extract of leaves showed the highest inhibitory effect followed by the methanolic extract of flowers. The positive results of this study may enhance the probability of using plant extracts to prolong the shelf life of many fat-based or oil-based products and the possible effect of such extracts as hypoglycemic agents and antidiabetic agents with consequent health benefits.

5. References

Adesegun, S. A., Fayemiwo, O., Odufuye, B. Coker, H.A.B. (2013). α-amylase inhibition and antioxidant activity of Pterocarpusosun Craib. *Journal of Natural Products,* 6, 90- 95.

Ahmad, N., Faisal, M., Anis, M. and Aref, I. M. (2010). In vitro callus induction and plant regeneration from leaf explants of Rutagraveolens L. *South African Journal of Botany*, 76(3), 597-600.

Al-Ismail, K., Hamdan, M. and Al-Delaimy, K. (2010). Antioxidant and ant Bacillus cereus activities of selected plant extracts. *Jordan Journal of Agricultural Sciences*, 2(2).

Al-Ismail, K., Aburjai, T. (2004). Antioxidant activity of water and alcohol extracts of chamomile flowers, anise seeds and dill seeds. *Journal of the Science of Food and Agriculture*, 84(2), 173-178.

AOAC, Association of Official Analytical Chemists (1990) Official methods of analysis. 15th ed. Washington DC, Association of Official Analytical Chemists.

Asgarpanah, J. and Khoshkam, R. (2012). Phytochemistry and pharmacological properties of Rutagraveolens L. *Journal of Medicinal Plants Research*, 6(23), 3942 - 3949

Athmouni, K., Belghith, T., Bellassouad, K., Feki, A.E., Ayadi, H. (2015). Effect of extraction solvents on the biomolecules and antioxidant properties of Scorzoneraundulata (Asteraceae), Application of factorial design optimization phenolic extraction. *Acta Scientiarum Polonorum Technologia Alimentaria*, 14(4), 313-330.

Chan, H.W. and Coxon, D.T. (1987). Lipid Hydroperoxides. In, Chan, H.W. (ed). Autoxidation of Unsaturated Lipids. Academic Press, London.

Chouhan, S., Sharma, K. and Guleria, S. (2017). Antimicrobial Activity of Some Essential Oils—*Present Status and Future Perspectives.Medicines*, 58,1-21

Christaki, E., Bonos, E., Giannenas, I. and Florou-Paneri, P. (2012). Aromatic Plants as a Source of Bioactive Compounds. *Agriculture*, 2, 228-243

Do, Q. D., Angkawijaya, Artik. E., Tran-Nguyen, P., Soetaredjo, F. E., Ismadji, S., Ju, Y. (2014). Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of Limnophilaaromatic. *Journal of food and drug analysis*, 22, 296-302.

Folin, O. and Ciocalteu, V. (1927). On tyrosine and tryptophane determinations in proteins. *Journal of biological chemistry*, 73(2), 627-650.

Khameneh, B., Iranshahy, M., Soheili, V. and Fazly Bazzaz, B. (2019). Review on plant antimicrobials, a mechanistic viewpoint. *Antimicrobial Resistance and Infection Control*, 8,118

Kumar, S. and Nandi, G. A. (2017). Present status of antidiabetic properties of carica papaya L. *International Journal of Advanced Research*, 5(7), 495-502

Lebovitz, H.E. (1997). α-Glucosidase inhibitors. *Endocrinology & Metabolism Clinics of North America*, 26, 539–551.

Li, S., Chen, G., Zhang, C., Wu, M., Wu, S. and Liu, Q. (2014). Research progress of natural antioxidants in foods for the treatment of diseases. *Food Science and Human Wellness*, 3,110–116.

Liu, S.C., Lin, J.T., Wang, C.K. (2009). Antioxidant properties of various solvent extracts from lychee (Litchi chinenesissonn.) flowers. *Food Chemistry*, 114, 577-581.

Mohammad, H.F., Mohammad, A., Roja, R., 2015. Role of dietary polyphenols in the management of peptic ulcer.World *Journal of Gastroenterology*. 21, 6499–6517.

Odoratissima, A. Banothua , V. Neelagiria, C, Adepallya, U., Lingamb, J. Bommareddya, K. (2017). Phytochemical screening and evaluation of in vitro antioxidant and antimicrobial activities of the indigenous medicinal plant. *Pharmaceutical Biology*, 55(1), 1155–1161.

Ouerghemmi, l., RebeyI. B., Rahali.Z., Bourgou, S. Pistelli,L., Ksouri, R., Marzouk, B., Tounsi, M. S. (2017). Antioxidant and antimicrobial phenolic compounds from extracts of cultivated and wild-grown Tunisian Rutachalepensis. *Journal of Food and Drug Analysis*, 25(2), 350-359

Pollio, A., De Natale, A., Appetiti, E., Aliotta, G. and Touwaide, A. (2008), Continuity and change in the Mediterranean medical tradition, Ruta spp. (Rutaceae) in Hippocratic medicine and present practices. *Journal of Ethnopharmacology*, 116 (3), 469-482.

Prabhakar, K. R., Veeresh, V. P., Vipan, K., Sudheer, M., Priyadarsini, K. I., Satish, R. B., Unnikrishnan, M. K. (2006). Bioactivity-guided fractionation of Coronopusdidymus, A Free radical scavenging perspective. *Phytomedicine*, 13, 591–595.

Soare, J. R., Dinis, T. C., Cunha, A. P., and Almeida, L. (1997). Antioxidant activities of some extracts of Thymus zygis. *Free radical research*, 26(5), 469-478.

Suganuma, T., Maeda, Y., Kitahara, K., and Nagahama, T. (1997). Study of the action of human salivary alpha-amylase on 2-chloro-4-nitrophenyl -maltotrioside in the presence of potassium thiocyanate. *Carbohydrate Research*, 303, 219–227.

Sultana, B., Anwar, F., Ashraf, M. (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinalplant extracts. *Molecules*,14, 2167-2180.

Zieli_nski, H., Kozłowska, H. (2000). Antioxidant activity and totalphenolics in selected cereal grains and their differentmorphological fractions. *Journal of Agriculture of Food Chemistry*, 48, 2008-2016.