Open Access

International journal of Horticulture, Agriculture and Food science(IJHAF)
Vol-4, Issue-4, Jul-Aug, 2020
https://dx.doi.org/10.22161/ijhaf.4.4.4
ISSN: 2456-8635

In Vitro Assessment of Antioxidant Activity, Total Phenolic and Flavonoid Contents of Sweet Marjoram (*Origanum majorana* L.) Extract

Talal LAHRECHE*[1], Mohamed Lamine BRADAIE[2], Taha Mossadak HAMDI[3]

[1] Laboratory of Food Hygiene and Quality Insurance System, High National Veterinary School, Issad Abbes St., Oued Smar, Algiers, Algeria
Department of Biology, Faculty of Natural Sciences, Ziane Achour University, Djelfa, Algeria
[2] High National School of Agronomy, Hassan Badi Ave., El Harrach, Algiers, Algeria
[3] Laboratory of Food Hygiene and Quality Insurance System, High National Veterinary School, Issad Abbes St., Oued Smar, Algiers, Algeria
* Corresponding author

***Abstract*— *Sweet marjoram (Origanum majorana L.) is cultivated as a condiment for its aromatic leaves for culinary purposes and utilized as a medicinal plant for many diseases. The aim of this study was to evaluate in vitro antioxidant activity of marjoram extract by the 2, 2–diphenyl–1–picrylhydrazyl–hydrate (DPPH) free radical scavenging method while total phenolic and flavonoid contents were quantified by spectrophotometry using Folin−Ciocalteu and aluminum chloride colorimetric methods, respectively. The extraction yield of sweet marjoram obtained by maceration in absolute ethanol at a ratio of 1/5 (w/v) for 24h at room temperature was 8.41 ± 0.76 % (w/w). The obtained results showed that the investigated extract contained a higher amount of phenolics: 164.96 ± 4.61 mg GAE/g of dry plant, lower flavonoid contents: 44.61 ± 2.08 mg QE/g of dry plant, and exhibited a strong antioxidant activity (IC_{50} value: 40.09 µg/ml) almost like those of the used standard products, namely ascorbic acid and butylated hydroxytoluene (BHT). Based on the obtained results, marjoram (Origanum majorana L.) features a potential application as natural antioxidants that could be exploited by the pharmaceutical and food industries.*

Keywords*— *Antioxidant activity, DPPH, ethanolic extract, flavonoid contents, marjoram, phenolic contents.

I. INTRODUCTION

In recent decades, the employment of natural antioxidants from plant sources as green chemicals has attracted major interest and has provided a potential alternative to the commonly used synthetic antioxidant molecules that present potential human health risks especially butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Rodrigues et al., 2019; Suhaj, 2006).

Polyphenols and flavonoids are considered as natural compounds widely presents in plants (San-Feliciano et al., 2012). They are products of the secondary plant metabolism that are involved in hormone regulation of plant growth, in protection against UV rays and microbial infections, in the attraction of pollinators and contribute to the plant pigmentation (Naczk and Shahidi, 2004). Polyphenol and flavonoid compounds arouse important interest due to their potential beneficial effects on public's health as they have demonstrated a multiple biological activities, such as antioxidant, antibacterial, antifungal, anti-spasmodic, analgesic and anti-hyperglycemic activities (Chishti et al., 2013). Their natural antioxidant activities are attracting increasing interest widely exploited in food and pharmaceutical industries (Chen et al., 2004). In food industry, phenolic compounds are used as additives and contribute particularly to slowing the oxidative degradation of lipids, thus improving the microbial and nutritional quality of food (Jukic et al., 2015). In addition, their presence in human diet is associated with beneficial

International journal of Horticulture, Agriculture and Food science(IJHAF)
Vol-4, Issue-4, Jul-Aug, 2020
https://dx.doi.org/10.22161/ijhaf.4.4.4
ISSN: 2456-8635

pharmacological effects that reduce the risk of various chronicle diseases related to the oxidative stress (Zhou et al., 2006).

Origanum majorana L. also called sweet marjoram is a species of perennial plant in the Lamiaceae family, indigenous from Mediterranean and cultivated as a condiment for its aromatic leaves for culinary purposes (Sud and Kumar, 2004). Marjoram is also used as a medicinal plant for various diseases because it contains high amount of phenolic compounds such as carvacrol and thymol (Burt, 2004). Various authors have previously studied the effects of sweet marjoram extracts from different origin and demonstrated their antioxidant (Duletic-Lausevic et al., 2018; Guerra-Boone et al., 2015; Roby et al., 2013; Chrpova et al., 2010; Vagi et al., 2005), antimicrobial (Duletic-Lausevic et al., 2018; Guerra-Boone et al., 2015; Hussain et al., 2011; Kozlowska et al., 2010; Charai et al., 1996), anti-inflammatory (Arranz et al., 2015), anticancer (Rao et al., 2014; Erenler et al., 2016), hormonal and menstrual cycle regulatory (Haj-Husein et al., 2016), cardio-protective (Ramadan et al., 2013), hepato-protective (Mossa et al., 2013), gastric secretory (Rafsanjani et al., 2007), anticholinesterase (Chung et al., 2001; Mossa et al., 2011), antiulcer (Al-Howiriny et al., 2009) and antineurodegenerative activities (Duletic-Lausevic et al., 2018).

The present study is part of the context of exploiting and enhancing the biodiversity of aromatic plants for their natural properties. The aim consisted in assessing the total phenolic contents and the concentration of flavonoids, as well as to evaluate the antioxidant activity of the ethanolic sweet marjoram extract (*Origanum majorana* L.).

II. MATERIALS AND METHODS

Chemicals

Absolute ethanol and methanol were purchased from Honeywell Riedel-de Haën, Seelze, Germany. Standards of phenolic acid (Gallic acid), Folin−Ciocalteu's phenol reagent, 2, 2–diphenyl–1–picrylhydrazyl–hydrate (DPPH), 3, 5−di−tert−butyl−4−hydroxytoluene (BHT) and ascorbic acid were purchased from Sigma-Aldrich Chemical Co., St Louis, MO, USA. Flavonoid (Quercetin) was purchased from Merck Chemicals Ltd., Nottingham, United Kingdom. Aluminum chloride (AlCl3) was from Fluka Chemie AG, Buchs, Switzerland. Sodium hydroxide (NaOH), sodium nitrite (NaNO2), and sodium carbonate (Na2CO3) were purchased from Carlo Erba reagent, Milan, Italy. Ultra-pure water was obtained with a Milli-Q water purification system.

Plant material

Fresh marjoram was purchased from local market in April 2019. The plants were botanically identified as *Origanum majorana* L. Aerial parts of Marjoram were dried at room temperature (20 °C) in the dark to preserve their molecular integrity. The plant materials were individually ground into a fine powder (500 µm, ISO, 1999).

Preparation of plant extract

Extraction was made in line with the method reported by Bensid et al. (2014) with slight modifications. Sample was macerated at room temperature in absolute ethanol in a ratio of 1:5 for 24h. Extract was filtered over Whatman No. 1 filter paper. Activated carbon was appended to filtrate (20 g of activated carbon / 100 g of plant material) and was immediately removed by filtration. After that, all ethanol was evaporated under reduced pressure using a Büchi Rotavapor R-200 at 50 °C. Then, extract was stored in amber flasks at refrigerated conditions until use.

Determination of total phenolic compounds

Total phenolic contents (TPC) were evaluated using the spectrophotometric analysis with Folin−Ciocalteu's phenol reagent (Waterhouse, 2002). Briefly, 100 µL of appropriately diluted sample was added to 400 µL of a 1:10 diluted Folin−Ciocalteu's reagent. After 5 min, 500 µL of a saturated sodium carbonate (Na2CO3) solution and 1.5 mL of ultrapure water were added. The mixture was incubated in dark at room temperature for 2 h and the absorbance was determined at 765 nm against water blank on spectrophotometer. Calibration curve was made using gallic acid standard solution (100, 80, 60, 40 and 20 µg/mL) under the same procedure as above and results are expressed as milligram of gallic acid equivalents (GAE) per g of dry Plant.

Determination of flavonoid contents

Total flavonoids were determined using a colorimetric assay (Kim and lee, 2004). A 500 µL aliquot of appropriately diluted sample was put into a volumetric flask containing 2 mL of ultrapure water. At zero time, 150 µL of 5 % sodium nitrite (NaNO2) was added to the flask. At 5 min, 150 µL of 10 % aluminum chloride (AlCl3) was added. At 11 min, 1 mL of 1M sodium hydroxide (NaOH) was appended into the mixture. Forthwith, the contents of the reaction flask were diluted with 1.2 mL of ultrapure water and carefully blended. Mixture absorbance was read at 510 nm against water blank. Calibration curve for flavonoids was set using quercetin standard solution (100, 80, 60, 40 and 20 µg/mL) under the same procedure as above and results are expressed as milligram of quercetin equivalents (QE) per g of dry Plant.

International journal of Horticulture, Agriculture and Food science(IJHAF)
Vol-4, Issue-4, Jul-Aug, 2020
https://dx.doi.org/10.22161/ijhaf.4.4.4
ISSN: 2456-8635

Evaluation of antioxidant activity

The plant extract ability to scavenge DPPH free radicals was evaluated by the standard method as reported by Stankoviç (2011). Briefly, 1950 µL of methanolic solution of DPPH were mixed with 50 µL of diluted solutions obtained from each stock solution (100, 80, 60, 40 and 20 µg/ml). The mixture was loudly shaken and incubated in darkness at room temperature for 30 min. The absorbance was recorded at 517 nm against water control. The percentage of the radical scavenging activity was calculated using the following equation: DPPH scavenged (%) = [(A of control − A of sample) / A of control] × 100, while IC_{50} value which defined as the concentration of the sample leading to inhibition of 50 % of the DPPH concentration was determined by linear regression and was expressed as milligrams per g of sample. Ascorbic acid and butylated hydroxytoluene BHT was used for comparison.

Statistical analysis

Average values and standard deviations were obtained from triplicate data. One-way ANOVA followed by Duncan's post-hoc was performed to compare differences of the data at P value < 0.05.

III. RESULTS AND DISCUSSION

Ethanolic extracts were prepared to examine the total phenolic compounds, flavonoid contents and antioxidant activity. The extraction yield of phenolic compounds obtained from plant material was determined to be 8.41 ± 0.76 % (w/w). Similarly, Benchikha et al. (2013) reported a yield of extract of 8.16 %.

Total phenolic compounds present in the examined plant extract measured by Folin–Ciocalteu's reagent were calculated using the standard curve equation of gallic acid: $y = 0.051x - 0.071$, $r^2 = 0.979$ and expressed in gallic acid equivalents (GAE) per gram dry plant weight. The concentration of total phenols in our study was found to be 164.96 ± 4.61 mg of GAE/g. Pereira et al. (2012) and Fernandes et al. (2016) reported lower values of 72.87 and 48.66 mg GAE/g, respectively.

The concentration of flavonoids present in the Marjoram extract was determined using spectrophotometric method with aluminum chloride. The flavonoid contents were calculated using the equation: $y = 0.013x - 0.053$, $r^2 = 0.998$ extracted from standard curve of quercetin. The concentration of flavonoids was found to be 44.61 ± 2.08 mg QE/g of dry plant, which was very similar to the value reported by Gawlic-Dziki (2012).

The recorded differences in the extraction yield and in the contents of polyphenol compounds (total phenolics and flavonoids) contained in the studied plant extract might be due on one hand to extrinsic factors related to the plant such as; the origin, plant species and considered organ (Valnet, 1980). Indeed, Smallfield (2001) and Bruneton (1993) report that environmental and climatic conditions, the stage and harvest period, the vegetative cycle and/or the techniques and time of conservation can influence the extraction yields and the contents of compounds. On the other hand, due to factors related to the extraction procedures used such as; particle size, choice and concentration of solvent, exhaustion, temperature, pressure, time, ratio and / or extraction methods (maceration, decoction, infusion, hydro distillation, etc.) (Silva et al., 2007; Min and Chun-Zhao, 2005; Naczk and Shahidi, 2004; Zhou and Yu, 2004).

It should be noted that the determination of the content by those methods do not reflect the absolute value of the amount of phenolic compounds present in the extract (Sengul et al., 2009; Singleton et al., 1999). The interference of the sought compounds with other products used during the assay can also affects the final results. For example, the low specificity of the Folin−Ciocalteu reagent is the main inconvenient of the colorimetric assay (Roby et al., 2013; Kim and Lee, 2004). This reagent is extremely sensitive to the reduction of all hydroxyl groups, not only those of phenolic compounds but also of certain sugars, proteins, carotenoids, etc. (Gomez-Caravaca et al., 2006). Therefore, the assay carried out by this reagent represents a crude evaluation of all hydroxyl groups contained in the extract because it is not specific to phenolic, but many compounds can react with this reagent, resulting in high values (Tawaha et al., 2007). For this reason, High Performance Liquid Chromatography (HPLC) analysis is interesting for the determination of the individual phenolic constituents in the plant extract.

The antioxidant activity of ethanolic plant extract from marjoram was evaluated using a methanol solution of DPPH reagent and was compared to that of natural and synthetic antioxidants currently used in therapy and food industry, namely; ascorbic acid and BHT, respectively. The antioxidant activity of all samples: the studied plant extract, natural and synthetic antioxidants (ascorbic acid and BHT) was determined using a spectrophotometer by following the transition of color from purple to yellow once the reduction of DPPH free radical (Majhenic et al., 2007). All the samples showed concentration-dependent increases significantly ($P < 0.05$) in free radical scavenging capacity (Fig. 1). In all experiment, free radical scavenging

International journal of Horticulture, Agriculture and Food science(IJHAF)
Vol-4, Issue-4, Jul-Aug, 2020
https://dx.doi.org/10.22161/ijhaf.4.4.4
ISSN: 2456-8635

activity of ascorbic acid was significantly different ($P < 0.05$) from that of BHT and ME which they showed no significant difference ($P > 0.05$). Ascorbic acid had relatively high free radical scavenging activity with 93.34 % at 0.1 mg/mL while BHT and marjoram extract showed values of 76.32 % and 72.53 % respectively at the same concentration. Our extract has an important antioxidant power which can replace synthetic antioxidant because it presents a potential free radical scavenging activity similar to those observed for the used reference standard antioxidant products. This potential free radical scavenging ability can be attributed to the active hydrogen donor ability of hydroxyl substitution due probably to the high level of phenolic compounds containing in extract (Siddhuraju, 2007).

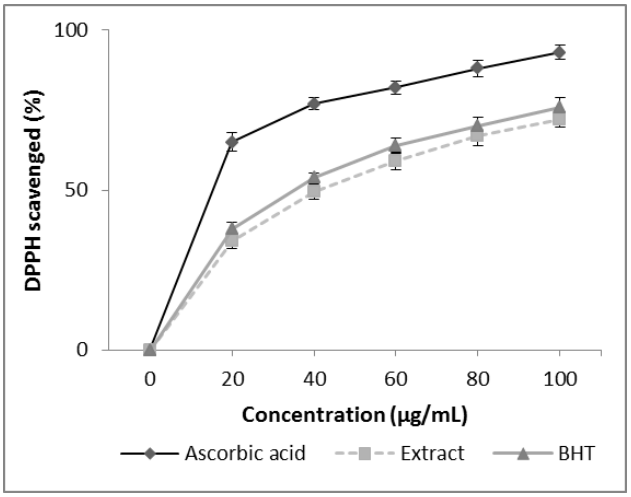

Fig. 1: Free radical (DPPH) scavenged percentages of marjoram extract compared to ascorbic acid and BHT. Means values of DPPH scavenged percentages (n=3). Standard deviations are indicated by bars.

The anti-radical activities of samples stated as IC_{50}, which was defined as the effective concentration (in µg/mL) of substrate required to scavenge the DPPH radical by 50 %, were determined by linear regression. Awa et al. (2018) and Spanou et al. (2008) reported that the lower the IC_{50} value the higher the antioxidant activity of a sample. IC_{50} values of ascorbic acid, BHT and the studied extract was determined as 15.34, 37.39 and 40.09 µg/mL, respectively. The results showed that ascorbic acid had the most powerful antioxidant activity ($P < 0.05$). However, natural extract obtained in our study (marjoram) and BHT exhibited similar anti-radical activities ($P > 0.05$), thus claiming that our extract had a significant antioxidant activity which can replace the synthetic antioxidant (BHT).

IV. CONCLUSION

Based upon the obtained results in the present study, we can conclude that ethanolic extract of marjoram (*Origanum majorana* L.) contains a considerable amount of phenols (phenolic and flavonoid contents), exhibits a strong antioxidant power and a potential free radical scavenging ability that can replace the synthetic antioxidant (BHT). These indicate that marjoram represents a significant source of natural antioxidants which might be helpful in the pharmaceutical and food industries. Nevertheless, further studies are required to determine and quantify the individual phenolic constituents containing in the extract.

ACKNOWLEDGEMENTS

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

[1] Al-Howiriny, T., Alsheikh, A., Alqasoumi, S., Al-Yahya, M., ElTahir, K. & Rafatullah, S. (2009). Protective effect of *Origanum majorana* L. 'marjoram' on various models of gastric mucosal injury in rats. The American Journal of Chinese Medicine, 37(03), 531-545.

[2] Arranz, E., Jaime, L., Lopez, M. C., Reglero, G. & Santoyo, S. (2015). Supercritical fluid extraction as an alternative process to obtain essential oils with anti-inflammatory properties from marjoram and sweet basil. Industrial Crops and Products, 67, 121 – 129.

[3] Awa, D., Konan, Y., Youssouf, S., Honora, T., Adama, B. & Witabouna, K. (2018). Pouvoir antioxydant et teneurs en composés phénoliques de deux espèces du genre *Albertisia*: *Albertisia cordifolia* (Mangenot & J. Miège) Forman et *Albertisia scandens* (Mangenot & J. Miège) Forman (Menispermaceae). European Scientific Journal, 14(30), 128-144.

[4] Benchikha, N. B., Menaceur, M. & Barhi, Z. (2013). Extraction and antioxidant activities of two species Origanum plant containing phenolic and flavonoid compounds. Journal of Fundamental and Applied Sciences, 5(1), 120-128.

[5] Bensid, A., Ucar, Y., Bendeddouche, B. & Özogul, F. (2014). Effect of the icing with thyme, oregano and clove extracts on quality parameters of gutted and beheaded anchovy (*Engraulis encrasicholus*) during chilled storage. Food Chemistry, 145, 681-686.

[6] Bruneton, J. (1993). Pharmacognosie, phytochimie, plantes médicinales. Technique et documentation. Lavoisier Ed., Paris, pp. 418-419.

[7] Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods—A review. International Journal of Food Microbiology, 94(3), 223-253.

[8] Charai, M., Mosaddak, M. & Faid, M. (1996). Chemical composition and antimicrobial activities of two aromatic plants: *Origanum majorana* L. and O. compactum Benth. Journal of Essential Oil Research, 8(6), 657-664.

[9] Chen, F. A., Wu, A. B. & Chen, C. Y. (2004). The influence of different treatments on the free radical scavenging activity of burdock and variations of its active components. Food Chemistry, 86, 479–484.

[10] Chishti, S., Kaloo, Z. A. & Sultan, P. (2013). Medical importance of genus *Origanum*: A review. Journal of Pharmacognosy and Phytotherapy, 5(10), 170-177. DOI: 10.5897/JPP2013.0285

[11] Chrpova, D., Kourimska, L., Gordon, M., Hermanova, V., Roubickova, I. & Panek, J. (2010). Antioxidant activity of selected phenols and herbs used in diets for medical conditions. Czech Journal of Food Sciences, 28(4), 317-325.

[12] Chung, Y. K., Heo, H. J., Kim, E. K., Kim, H. K., Huh, T. L., Lim, Y., Kim, S. K. & Shin, D. H. (2001). Inhibitory effect of ursolic acid purified from *Origanum majorana* L. on the acetylcholinesterase. Molecules and Cells, 11(2), 137-143.

[13] Duletic-Lausevic, S., Alimpic, A. A., Kolarevic, S., Vukovic-Gacic, B., Oalde, M., Zivkovic, J., Savikin, K. & Marin, P.D. (2018). Antineurodegenerative, antioxidant and antibacterial activities and phenolic components of *Origanum majorana* L. (Lamiaceae) extracts. Journal of Applied Botany and Food Quality, 91, 126–134.

[14] Erenler, R., Sen, O., Aksit, H., Demirtas, I., Yaglioglu, A. S., Elmastas, M. & Telci, İ. (2015). Isolation and identification of chemical constituents from *Origanum majorana* and investigation of antiproliferative and antioxidant activities. Journal of the Science of Food and Agriculture, 96(3), 822–836.

[15] Fernandes, R. P. P., Trindade, M. A., Tonin, F. G., Lima, C. G., Pugine, S. M. P., Munekata, P. E. S., Lorenzo, J. M. & De Melo, M. P. (2016). Evaluation of antioxidant capacity of 13 plant extracts by three different methods: cluster analyses applied for selection of the natural extracts with higher antioxidant capacity to replace synthetic antioxidant in lamb burgers. Journal of Food Sciences and Technology, 53(1), 451–460.

[16] Gawlic-Dziki, U. (2012). Dietary spices as a natural effectors of lipoxygenase, xanthine oxidase, peroxidase and antioxidant agents. LWT - Food Science Technology, 47, 138–146.

[17] Gomez-Caravaca, A., Gomez-Romero, M., Arraez-Roman, D., Segura-Carretero, A. & Fernandez-Gutiérrez, A. (2006). Advances in the analysis of phenolic compounds in products derived from bees. Journal of Pharmaceutical and Biomedical Analysis, 41(4), 1220-1234.

[18] Guerra-Boone, L., Alvarez-Román, R., Alvarez-Roman, R., Salazar-Aranda, R., Torres-Cirio, A., Rivas-Galindo, V. M., de-Torres, N. W., Gonzalez, G. & Perez-Lopez, L. A. (2015). Antimicrobial and antioxidant activities and chemical characterization of essential oils of *Thymus vulgaris*, *Rosmarinus officinalis*, and *Origanum majorana* from northeastern Mexico. Pakistan Journal of Pharmaceutical Sciences, 28(1), 363–369.

[19] Haj-Husein, I., Tukan, S., & Alkazaleh, F. (2015). The effect of marjoram (Origanum majorana) tea on the hormonal profile of women with polycystic ovary syndrome: a randomised controlled pilot study. Journal of Human Nutrition and Dietetics, 29(1), 105–111.

[20] Hussain, A. I., Anwar, F., Rasheed, S., Nigam, P. S., Janneh, O. & Sarker, S. D. (2011). Composition, antioxidant and chemotherapeutic properties of the essential oils from two *Origanum* species growing in Pakistan. Revista Brasileira De Farmacognosia, 21(6), 943–952.

[21] International Organization for Standardization. (1999). _ ISO 7925: 1999. Dried oregano — Whole or ground leaves specification. _ Geneva, Switzerland: ISO.5 p.

[22] Jukic. H., Habes, S., Aldzic, A., Durgo, K. & Kosalec, I. (2015). Antioxidant and prooxidant activities of phenolic compounds of the extracts of *Echinacea purpurea* (L.). Bulletin of the Chemists and Technologists of Bosnia and Herzegovina, 44, 43-52.

[23] Kim, D. O. & Lee, C. Y. (2004). Comprehensive study on vitamin C equivalent antioxidant capacity (VCEAC) of various polyphenolics in scavenging a free radical and its structural relationship. Critical Review in Food Sciences and Nutrition, 44(4), 253–273.

[24] Kozlowska, M., Laudy, A. E., Starosciak, B. J., Napiorkowski, A., Chomics, L. & Kazimierczuk, Z. (2010). Antimicrobial and antiprotozoal effect of sweet marjoram (*Origanum majorana* L.). Acta Scientiarum Polonorum Hortorum Cultus, 9(4), 133-141.

[25] Majhenic, L., kerget, M. S. & Knez, Z. (2007). Antioxidant and antimicrobial activity of guarana seed extracts. Food Chemistry, 104(3), 1258–1268.

[26] Min, G. & Chun-Zhao, L. (2005). Comparison of techniques for the extraction of flavonoids from cultured cells of *Saussurea medusa* Maxim. World Journal of Microbiology and Biotechnology, 21, 1461-1463.

[27] Mossa, A. T. & Nawwar, G. A. (2011). Free radical scavenging and antiacetylcholinesterase activities of *Origanum majorana* L. essential oil. Human and Experimental Toxicology, 30(10), 1501-1513.

[28] Mossa, A. T., Refaie, A. A., Ramadan, A. & Bouajila, J. (2013). Amelioration of prallethrin-induced oxidative stress and hepatotoxicity in rat by the administration of *Origanum majorana* essential oil. BioMed Research International, 2013, 859085, 11p.

[29] Naczk, M. & Shahidi, F. (2004).Extraction and analysis of phenolics in food. Journal Of Chromatography A, 1054, 95-111.

[30] Pereira, M. A, C. & Jorge, N. (2012). Antioxidant potential of oregano extract (*Origanum vulgare* L.). British Food Journal, 114(7), 954–965.

[31] Rafsanjani, F. N., Shahrani, M., Ardakani, Z. V. & Ardakani M. V. (2007). Marjoram increases basal gastric acid and pepsin secretions in rats. Phytotherapy Research, 21(11), 1036-1038.

[32] Ramadan, G., El-Beih, N. M., Arafa, N. M. & Zahra, M. M. (2013). Preventive effects of Egyptian sweet marjoram (*Origanum majorana* L.) leaves on hematological changes and cardiotoxicity in isoproterenol-treated albino rats. Cardiovasc Toxicology, 13(2), 100-109.

[33] Rao, S., Timsina, B. & Nadumane, V. K. (2014). Evaluation of the anticancer potentials of Origanum marjoranaon fibrosarcoma (HT-1080) cell line. Asian Pacific Journal of Tropical Disease, 4, S389-S394.

[34] Roby. M. H. H., Sarhana. M. A., Selima. K. A. & Khalel. K. I. (2013). Evaluation of Antioxidant activity, total phenols and phenolic compounds in thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), and marjoram (*Origanum majorana* L.) extracts. Industrial Crops and Products, 43, 827–831.

[35] Rodrigues, A. B., Almeida-Apolonio, A., Alfredo, T., Dantas, F., Campos, J., Cardoso, C., de PicoliSouza, K. & Oliveira, K., (2019). Chemical Composition, antimicrobial activity, and antioxidant activity of *Ocoteaminarum* (Nees & Mart.) Mez. Oxidative Medicine and Cellular Longevity, 2019, 1-14.

[36] San-Feliciano, A., Castro, M. A., Lopez-Perez, J. L. & Olmo, E. (2012). The importance of structural manipulation of natural compounds in drug discovery and development. In: Cechinel-Filho V. (ed), Plant bioactives and drug discovery. John Wiley & Sons Inc, Hoboken, NJ. pp. 127–160.

[37] Sengul, M., Yildiz, H., Gungor, N., Cetin, B., Eser, Z. & Ercisli, S. (2009). Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. Pakistan Journal of Pharmaceutical Sciences, 22, 102-106.

[38] Siddhuraju, P. (2007). Antioxidant activity of polyphenolic compounds extracted from defatted raw and dry heated *Tamarindus indica* seed coat. LWT -Food Science and Technology, 40, 982–990.

[39] Silva, E.M., Rogez, H. & Larondelle, Y. (2007). Optimization of extraction of phenolics from *Inga edulis* leaves using response surface methodology. Separation and Purification Technology, 55(3), 381-387.

[40] Singleton, V., Orthofer, R. & Lamuela-Raventós, R. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In: Packer, L. (Ed.). Oxidants and antioxidants, part A, methods in enzymology. Academic Press. New York. pp. 152-178.

[41] Smallfield, B. (2001). Introduction to growing herbs for essential oils, medicinal and culinary purposes. Crop and Food Research, 45, 1-4.

[42] Spanou, C., Bourou, G., Dervishi, A., Aligiannis, N., Angelis, A., Komiotis, D., Skaltsounis, A. & Kouretas, D. (2008). Antioxidant and chemopreventive properties of polyphenolic compounds derived from greek legume plant extracts. Journal of Agricultural and Food Chemistry, 56(16), 6967–6976.

[43] Stankovic, S. M. (2011). Total phenolic content, flavonoid concentration and antioxidant activity of *Marrubium peregrinum* L. Extracts. Kragujevac Journalof Science, 33, 63-72.

[44] Sud, R. K. & Kumar, S. (2004). Herbs: Culinary, Medicinal, Aromatic (Secrets and Human Happiness). Scientific Publishers Ed. Jodhpur, India. 283 p.

[45] Suhaj, M., 2006. Spice antioxidants isolation and their antiradical activity: a review. Journal of Food Composition and Analysis, 19, 531–537.

[46] Tawaha, K., Alali, F. Q., Gharaibeh, M., Mohammad, M. & Elimat T. (2007). Antioxidant activity and total phenolic content of selected Jordanian plant species. Food Chemistry, 104, 1372-1378.

[47] Vagi, E., Rapavi, E., Hadolin, M., Vasarhelyine Peredi, K., Balazs, A., Blazovics, A. & Simandi, B. (2005). Phenolic and triterpenoid antioxidants from *Origanum majorana* L. herb and extracts obtained with different solvents. Journal of Agricultural and Food Chemistry, 53(1), 17-21.

[48] Valnet, J., (1980). Aromathérapie : traitement des maladies par les essences des plantes. 9e Ed. Maloine. 510 p.

[49] Waterhouse, A. L. (2002). Determination of total phenolics. Current Protocols in Food Analytical Chemistry, 6(1), 111–118.

[50] Zhou, K. & Yu, L. (2004). Effects of extraction solvent on wheat bran antioxidant activity estimation. LWT - Food Science and Technology. 37, 717-721.

[51] Zhou, K. & Yu, L. (2006). Total phenolic contents and antioxidant properties of commonly consumed vegetables grown in Colorado. LWT - Food Science and Technology, 39, 1155-1162.