GC-MS Analysis and Antioxidant Effect of Some Iraqi Plants

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Abstract

Aromatic oils found in many plants are called essential oils. Essential oils are extracted from plants and utilized in various fragrances, foods, medications, and cleaning items. Essential oils, thought to include molecules that a plant utilizes to fight illnesses and fight against bacteria and parasites, have been called the "lifeblood" of a plant by some scientists. The flowers were from Tikrit, Iraq, nurseries. The oil from the flowers was extracted using a Clevenger after the flowers were rinsed completely. Weigh out 10 grams of flower and put it into a 500-milliliter round-bottom flask. Combine the 150 mL of distilled water. Extract oil from the flower by starting a Clevenger-type distillation, turning on the heating mantle for 2 hours, collecting the oil in a tiny measuring cylinder, and then testing the oil for GC-MS and antioxidant action. Different chemical elements of the Jasmine flower were identified based on their limited peak area, retention time, and molecular formula, as shown in the chromatogram generated by the GC-MS. Flower oil was analyzed, and 10 chemical components were present, each with a unique peak area percentage. The antioxidant activity of gardenia was studied using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, the most commonly used method for testing plant extracts for antioxidant properties. The stable free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) is associated with an extra electron.

Keywords: Jasminum Sambac, Gardenia, Essential oils. GC-MS.DPPH

INTRODUCTION

Aromatic oils found in many plants are called essential oils. Essential oils are extracted from plants and utilized in various fragrances, foods, medications, and cleaning items. Essential oils, which are thought to be a plant's "blood," are thought to include components that the plant employs to fight infections and ward off parasites and other harmful organisms. Numerous essential oils have antifungal, antiparasitic, and antibacterial, properties, as demonstrated by scientific research, which has isolated hundreds of compounds in essential oils.^[1] The integration of gas chromatography and mass spectrometry into a single procedure, known as gas chromatographymass spectrometry (GCMS), allows for more precise qualitative and quantitative examination of test samples. Phase characteristics of the gas chromatograph's column (material, type, length, film thickness, diameter) determine how the instrument is used. For this purpose, the mass spectrometer ionizes each molecule and then detects the resulting fragments based on their mass-to-charge ratio. In addition to its use in detecting drugs and plasma, GC-MS is also useful in analyzing the environment, identifying explosives, and investigating fires. As a bonus, it can pick

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out minute amounts of substances long considered to be gone forever. The goal of this research was to identify and catalog the chemical components given out by Jasminum nitidum. In addition to being the biggest organ in the body, the liver plays a crucial function in the control of a wide range of physiological processes. Detoxification, secretion, storage, and metabolism are only a few of the many key processes that are linked to its action.^[2] It can detoxify external (toxic molecules) and endogenous (waste metabolites) toxins in organisms. Both nutrition and energy production are metabolic processes in which the liver plays a role. It also plays a role in bile production, vitamin storage, and fat and carbohydrate metabolism.^[3,4] Hepatic disorders have recently emerged as a major public health concern worldwide. Liver disease is responsible for over 20,000 fatalities annually.^[5] Damage to the liver's cells, tissues, and function is called hepatic disease. Oxidative stress, which may harm organs, results from an imbalance between antioxidants and pro-oxidants.^[6] The tropical Asian regions home to the jasmine species Jasminum

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How to cite this article: Ahmed O H. GCMS Analysis and Antioxidant Effect of Some Iraqi Plants. J Nat Sc Biol Med 2023;14:115-121 sambac (Arabian jasmine or Sambac jasmine) also include the Indian subcontinent and Southeast Asia. It is grown in various parts of the world, most notably in South, Southeast, and West Asia.[7-9] Jasminum sambac flowers have a rich history of being employed in distilling aromatic oil and infusing beverages with a distinct jasmine fragrance. These flowers possess exceptional medicinal properties and have traditionally been used to address ailments such as ear, mouth, and eye illnesses, skin disorders, ulcers, and even leprosy. They exhibit a combination of bitter, pungent, and cooling characteristics, making them valuable as a brain tonic, purgative, and effective in addressing tri dosha imbalances, biliousness, itchy sensations, fever, and vomiting. Furthermore, these flowers have demonstrated their efficacy in treating conditions such as diarrhea, stomach discomfort, asthma, conjunctivitis, wound healing, cancer, dermatitis, and toothache.

Historically, the wounds were treated with bandages infused with Jasminum sambac flowers, while the leaves of the plant were employed in preventing and treating breast cancer. By brewing the blossoms into a tonic, women could minimize the risk of developing breast cancer and regulate their menstrual bleeding. Additionally, herbal remedies incorporating this plant have been utilized to address mental illness and seizures, providing a holistic approach to healthcare.[10-18] Emmenagogue, diuretic, and Anthelmintic properties are attributed to the whole plant. ^[19] Women in Malaya traditionally used wet flowers as facial cleansing wipes. Women experiencing lactation problems would apply a poultice from the blossoms to their breasts.^[20] People have relied on the plant's leaves and roots for centuries as a natural remedy for anything from fever and discomfort to inflammation and pain.[21] Jasmine oil was traditionally utilized in the fields of perfumes, soaps, flavors, and cosmetics in addition to its many therapeutic uses. Medicinally, it was used as an antidepressant, antibacterial, uterine tonic, sedative, and antispasmodic, to treat dry, oily, irritated, and sensitive skin; to calm inflamed coughs; to soothe aching muscles; and to cure sprains and muscle soreness.[22-^{26]} proteins, Carbohydrates, coumarins, amino acids, tannins, glycosides, phenolic compounds, phenolics, flavonoids, steroids, saponins, lipids, essential oils, fixed oils, terpenes, resin, and salicylic acid were found in the early phytochemical investigation of Jasminum sambac. ^[27,28] The evergreen blooming plant Gardenia jasminoides, often known as gardenia and cape jasmine, belongs to the Rubiaceous family. Its original range includes most of Southeast Asia.^[29] Triterpenoids, Iridoids, organic acids, Iridoids glucosides, and volatile chemicals are only some of the identified and characterized chemical components of G. jasminoides. The primary bioactive chemicals in G. jasminoides include geniposide, genipin, garden side, crocin, and iridoid. Under optimal extraction conditions, geniposide yielded 10.9%.^[30] Botanical Chemistry The principal phytoconstituents of Gardenia jasminoides that have been employed in the phytochemical analysis

and pharmacological investigations include genipin, geniposide, crocin, geniposidic acid, and crocetin. Many newly isolated phytochemicals have been reported to have therapeutic effects against various diseases and conditions, including cancer, inflammation, oxidative stress, infection, depression, neuropathy, protozoa, and melanoma. In traditional medicine, Gardenia *jasminoides* was used to alleviate inflammation and as a folk remedy for various other medical conditions. fever, headaches, inflammation, jaundice, hepatic problems, hypertension, and other conditions are all treated with this ancient Chinese folk medication.^[30]

This research aims to examine the antioxidant impact of gardenia flowers and determine the bioactive elements of jasmine using a GC-MS analysis of the flowers' phytochemicals.

MATERIALS AND METHODS *Extraction method*

Weigh out 10 grams of flower and put it into a 500-mL round-bottom flask. Added 150 mL of distilled water. Oil collected from the flower was analyzed for GC-MS composition and antioxidant activity after being distilled using a Clevenger-type apparatus for two hours.

GC/MS of the flower of Jasminum

The SHIMADZU/Gas Chromatography GC 2010 Plus GC/MS was the instrument of choice.

The following are the experimental parameters for GC/ MS analysis:

- 70.0 C° in the column.
- The optimal injection temperature is 240.0 C°.
- Splitless injection mode.
- The sampling interval is one minute.
- Pressure-based flow regulation.
- Tension: 100.0 kPa.
- 1.53 mL/min column flow rate.
- Its linear speed is 45.40 cm/sec.
- 3.0mL/min at purge.
- Divisor value=10.0

Column and injector oven temperature schedules for petroleum ether and chloroform fractions

Tabl	e 1:	Hexan	fraction	column	and	injector	oven
tem	pera	ture pro	ograms				

Rate	Temperature (°C)	Hold time (min)		
0.0	70.0	3.00		
12.00	150.0	2.00		
12.00	240.0	8.00		
Total elution time	31 5min			

Total elution time 31.5min

Table 2: Leaves oil column and injector oven temperature settings

Rate	Temperature (°C)	Hold time (min)
0.0	60.0	2.00
10.00	300.0	10.00
Elution	n time: 38 minutes in total	

Antioxidant effect of gardenia DPPH Scavenging Assay

The ability of the gardenia to scavenge free radicals was measured using a standard DPPH method (Sigma-Aldrich, USA). The synthesized compounds were added to DPPH in a total volume of 500 μ L, and the final volume was brought up to 2 mL with 100% ethanol. Each compound's absorbance was evaluated at 517 nm.^[31,32]

Statistical Analysis

Data were analyzed statistically using GraphPad Prism 6^[33] and reported as the mean±SD of three replicates per experiment.^[34]

RESULTS AND DISCUSSION *Methods of Extraction*

Extraction is the initial process of extracting valuable substances from their source sources. The specific extraction method will vary from substance to drug. There has been a rise in oils pressed from various plants, fruits, and herbs. They have a wide range of uses, from aromatherapy to medicine. The method used to extract the oil from plants must be tailored to the specific plant or flower species. Each approach provides the optimum means to extract the plant's active ingredients, leaving you with the most effective final product possible.

Extraction method (hydrodistillation by Clevenger)

Clevenger apparatus is used to extract essential oils from plant materials such as leaves, stems, flowers, and fruits, and then GC/MS analysis is done on the oil to determine its antioxidant impact.

GC/MS analysis of Jasmine flower

Different chemical elements of the Jasmine flower were identified based on their limited retention time, peak area, and molecular formula, as shown in the chromatogram generated by the gas chromatograph–mass spectrometer. Figure 1 displays the findings, which indicated the existence of 10 natural chemical components in the oil extract of flowers, recognized with various peak area percentages.





Propanoic Acid, Ethyl Ester



Caryophyllene Terpene



Tetradecanoic Acid



Methyl Stearate

The analytical technique known as gas chromatographymass spectrometry (GC-MS) combines the strengths of gas chromatography and mass spectrometry to determine the presence of many compounds in a given sample. Drug detection, fire analysis, environmental analysis, explosives analysis, and the identification of unknown materials are just some of the many uses for GC-MS. Gas chromatography (GC) is a common analytical method used in a wide variety of fields and industries, particularly for ensuring food safety and conducting environmental analysis because it is the separation technique of choice for volatile and semivolatile organic molecules like hydrocarbons, alcohols, aromatics, pesticides, steroids, fatty acids, and hormones. Separating complicated mixtures, quantifying analytes, identifying unknown peaks, and determining minuscule amounts of contamination are all possible using GC-MS thanks to the detection capability of mass spectrometry (MS). The analysis of fig leaves revealed the presence of the 10 chemicals listed in Figure (1-11).

Compound 1: Amino-5-Methylbenzoic Acid Compound 2: Anthracenamine Compound 3: Arsenous Acid Compound 4: 1H-Trindene Compound 5: Urea Compound 6: Pyrrolidinethione Compound 6: Pyrrolidinethione Compound 7: Propanoic Acid, Ethyl Ester Compound 8: Caryophyllene Terpene Compound 9: Tetradecanoic Acid Compound 10: Methyl Stearate

Antioxidant effect of gardenia

A DPPH test was used to examine gardenia for its antioxidant properties. A free radical with a spare electron is present in DPPH (2,2-diphenyl-1-picrylhydrazyl). Figure 12 displays the percent scavenging activities for DPPH radicals.



Figure 12: Antioxidant activity of gardenia, The results are represented as the mean±SD.

Most plant extracts are tested using the 2,2-Diphenyl-1picrylhydrazyl (DPPH) assay, which measures the amount of antioxidants present. Consumption of polyphenolrich foods has been shown to raise plasma polyphenol levels, and this, in turn, has been shown to significantly boost plasma antioxidant capacity, as determined by the DPPH test in most investigations. Gardenia's numerous constituents include flavonoids and polyphenols that act as antioxidants by scavenging free radicals. Compounds with one or more unpaired electrons, known as free radicals, are often produced during cellular metabolism. Reactive free radicals, such as reactive oxygen molecules and reactive nitrogen molecules, are thought to play a role in the onset and progression of many diseases, including cancer, liver disease, and cardiovascular disease by disrupting cell membranes, damaging membrane proteins, and mutating DNA, and novelty in this study consider first done in Iraq, investigation of 10 compounds by GC/ MS analysis of Jasmine flower and biological activity of these compounds has anticancer, antioxidant and antimicrobial properties for example about β -caryophyllene.^[35-40] anticancer and antioxidant activity of Methyl Stearate.[41,42] Also, gardenia is a plant that has many health benefits, including antioxidant effects, gardenia extract can protect the liver, improve the immune system, and prevent lipid accumulation.

CONCLUSIONS

From the findings, several inferences can be drawn:

- 1. Phytochemical screening was conducted to isolate and identify a diverse range of phytochemicals by taking advantage of the chemical differences between the flowers of Jasminum and Gardenia. This suggests these two species possess unique chemical profiles, which can be explored for potential applications.
- 2. GC-MS analysis, using parameters such as molecular weight, retention time, and MS/MS fragmentation, was employed to identify and quantify the specific chemicals present in a combination. This indicates that GC-MS can be a valuable analytical tool for determining the composition of complex mixtures.
- 3. The findings of this study align with the results of previous research conducted by foreign scientists who have also studied this plant. This suggests that the outcomes of the current investigation support and validate the existing body of knowledge in the field.
- 4. In this early investigation, the extract of oil from Jasminum flowers demonstrated a noteworthy antioxidant effect, which was observed for the first time. This implies that the extract possesses strong antioxidant properties, which could be of significant interest in various applications related to health and wellness.

FUTURE STUDY

in future work can carry out several biological studies for the oil fraction of Jasmine flower for example antimicrobial, anticancer, and anti-inflammatory.

REFERENCES

- Borg-Karlson A-K, Valterová I, Nilsson LA. Volatile compounds from flowers of six species in the family Apiaceae: bouquets for different pollinators? Phytochemistry. 1993; 35(1): 111-19. doi: https://doi. org/10.1016/S0031-9422(00)90518-1.
- Robinson MW, Harmon C, O'Farrelly C. Liver immunology and its role in inflammation and homeostasis. Cell Mol Immunol. 2016; 13(3): 267-76. doi: https://doi.org/10.1038/cmi.2016.3.
- Zaefarian F, Abdollahi MR, Cowieson A, Ravindran V. Avian Liver: The Forgotten Organ. Animals (Basel). 2019; 9(2): 63. doi: https://doi.org/10.3390/ani9020063.
- Pandit A, Sachdeva T, Bafna P. Drug-induced hepatotoxicity: a review. J Appl Pharm Sci. 2012; 02(05): 233-43. doi: http://dx.doi.org/10.7324/ JAPS.2012.2541.
- Hurkadale PJ, Shelar PA, Palled SG, Mandavkar YD, Khedkar AS. Hepatoprotective activity of Amorphophallus paeoniifolius tubers against paracetamol-induced liver damage in rats. Asian Pac J Trop Biomed. 2012; 2(1): S238-S42. doi: https:// doi.org/10.1016/S2221-1691(12)60167-1.
- Kurutas EB. The importance of antioxidants which play the role in cellular response against oxidative/ nitrosative stress: current state. Nutr J. 2015; 15(1): 71. doi: https://doi.org/10.1186/s12937-016-0186-5.
- GRIN-Global. Jasminum sambac (L.) Aiton. Agricultural Research Service (ARS), United States Department of Agriculture (USDA). Updated 2 July, 2020. Available from: http://www.tn-grin.nat.tn/ gringlobal/taxon/taxonomydetail?id=20676.
- López González Gs. Árboles y arbustos de la Península Ibérica e Islas Baleares. 2nd ed. Ediciones Mundi-Prensa; 2006.
- PIER. Jasminum sambac (L.) Aiton, Oleaceae. Pacific Island Ecosystems at Risk. Updated 15 May 2013. Available from: http://www.hear.org/pier/species/ jasminum_sambac.htm.
- Joshi SG. Medicinal plants. Oxford and IBH publishing; 2000. Available from: https://www.abebooks. com/9788120414143/Medicinal-plants-Jan-2000-Joshi-8120414144/plp.
- Gowdhami T, Rajalakshmi A, Sugumar N. Phytochemical characterization using various solvent extracts and GC analysis of ethanolic extract of Jasminum sambac Linn. Int J Curr Res. 2015; 7(9): 19950-55. Available from: http://www.journalcra. com/sites/default/files/issue-pdf/10485.pdf.
- 12. Kiritikar KR, Basu BD. Indian medicinal plants with Illustrations. 2nd ed. vol 7. Dehradun: International Book Distributors; 2003:2093-96.
- Vidya D, Dhanashri M. RP-HPLC determination of rutin and isoquercitrin from leaves of Jasminum sambac Ait. IJRPC. 2014; 4(1): 141-47. Available from: http:// www.ijrpc.com/files/22-439.pdf.

- Zhang Y-J, Liu Y-Q, Pu X-Y, Yang C-R. Iridoidal glycosides from Jasminum sambac. Phytochemistry. 1995; 38(4): 899-903. doi: https://doi.org/10.1016/0031-9422(94)E0200-C.
- Biswas TK, Mukherjee B. Plant medicines of Indian origin for wound healing activity: a review. Int J Low Extrem Wounds. 2003; 2(1): 25-39. doi: https:// doi.org/10.1177/1534734603002001006.
- Khare CP. Indian Herbal Remedies: Rational Western Therapy, Ayurvedic and Other Traditional Usage, Botany. Springer Berlin, Heidelberg; 2004. doi: https://doi.org/10.1007/978-3-642-18659-2.
- 17. Kalaiselvi M, Narmadha R, Ragavendran P, et al. In vivo and in vitro antitumor activity of Jasminum sambac (Linn) Ait Oleaceae flower against Dalton's ascites lymphoma induced Swiss albino mice. Int J Pharm Pharm Sci. 2011; 4(1): 144-47. Available from: https://innovareacademics.in/journal/ijpps/ Vol4Issue1/2925.pdf.
- Rahman MA, Hasan MS, Hossain MA, Biswas NN. Analgesic and cytotoxic activities of Jasminum sambac (L.) Aiton. Pharmacologyonline. 2011; 1: 124-31. Available from: https://pharmacologyonline. silae.it/files/archives/2011/vol1/011.rahman.pdf.
- Kunhachan P, Banchonglikitkul C, Kajsongkram T, Khayungarnnawee A, Leelamanit W. Chemical composition, toxicity and vasodilatation effect of the flowers extract of Jasminum sambac (L.) Ait."G. Duke of Tuscany". Evid Based Complement Alternat Med. 2012; 2012: 471312. doi: https://doi. org/10.1155/2012/471312.
- 20. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants. CSIR, New Delhi, India; 1956.
- 21. Brown W. Useful Plants in the Philippines, Technical Bulletin 10. Bureau of a Printing, Manila; 1958.
- Shrivastav P, George K, Balasubramaniam N, Jasper MP, Thomas M, Kanagasabhapathy AS. Suppression of puerperal lactation using jasmine flowers (Jasminum sambac). Aust N Z J Obstet Gynaecol. 1988; 28(1): 68-71. doi: https://doi.org/10.1111/j.1479-828x.1988. tb01614.x.
- Younis A, Mehdi A, Riaz A. Supercritical carbon dioxide extraction and gas chromatography analysis of Jasminum sambac essential oil. Pak J Bot. 2011; 43: 163-68. Available from: https://www.pakbs.org/ pjbot/PDFs/43(SI)/24.pdf.
- 24. Kang J-Y, Kim K-S. Effect of aromatherapy on anxiety and fatigue in students nurses experiencing their first clinical practice. J Korean Acad Fundam Nurs. 2002; 9(2): 226-36. Available from: https://www.koreamed.org/SearchBasic. php?RID=0091JKAFN/2002.9.2.226&DT=1.
- 25. Maxia A, Marongiu B, Piras A, et al. Chemical characterization and biological activity of essential oils from Daucus carota L. subsp. carota growing wild on the Mediterranean coast and on the Atlantic coast. Fitoterapia. 2009; 80(1): 57-61. doi: https://doi. org/10.1016/j.fitote.2008.09.008.

- Lawless J. The Illustrated Encyclopedia of Essential Oils. Elements Books, Boston, USA; 1995. Available from: https://www.abebooks.com/9781852306618/Illustrated-Encyclopedia-Essential-Oils-Complete-1852306610/plp.
- 27. Sabharwal S, Vats M, Sardana S, Aggarwal S. Pharmacognostical, physico and phytochemical evaluation of the leaves of Jasminum sambac Linn. (Oleaceae). Int J Pharm Pharm Sci. 2011; 3(4): 237-41. Available from: https://innovareacademics.in/journal/ ijpps/Vol3Issue4/2597.pdf.
- Joy P, Raja DP. Anti-bacterial activity studies of Jasminum grandiflorum and Jasminum sambac. Ethnobotanical leaflets. 2008; 2008(1): 59. Available from: https://opensiuc.lib.siu.edu/ebl/vol2008/iss1/59.
- GRIN-Global. Gardenia jasminoides. Agricultural Research Service (ARS), United States Department of Agriculture (USDA). Updated 2 July, 2020. Available from: http://www.tn-grin.nat.tn/gringlobal/taxon/ taxonomydetail?id=70315.
- 30. Yang B, Liu X, Gao Y. Extraction optimization of bioactive compounds (crocin, geniposide and total phenolic compounds) from Gardenia (Gardenia jasminoides Ellis) fruits with response surface methodology. Innovative Food Science & Emerging Technologies. 2009; 10(4): 610-15. doi: https://doi. org/10.1016/j.ifset.2009.03.003.
- Jabir M, Sahib UI, Taqi Z, et al. Linalool-Loaded Glutathione-Modified Gold Nanoparticles Conjugated with CALNN Peptide as Apoptosis Inducer and NFκB Translocation Inhibitor in SKOV-3 Cell Line. Int J Nanomedicine. 2020; 15: 9025-47. doi: https://doi. org/10.2147/ijn.s276714.
- 32. Rashid TM, Nayef UM, Jabir MS, Mutlak FA. Study of optical and morphological properties for Au-ZnO nanocomposite prepared by Laser ablation in liquid. J Phys Conf Ser. 2021; 1795(1): 012041. doi: https:// doi.org/10.1088/1742-6596/1795/1/012041.
- 33. Khashan KS, Jabir MS, Abdulameer FA. Carbon Nanoparticles decorated with cupric oxide Nanoparticles prepared by laser ablation in liquid as an antibacterial therapeutic agent. Materials Research Express. 2018; 5(3): 035003. doi: https://doi.org/10.1088/2053-1591/aab0ed.
- 34. Jabir MS, Sulaiman GM, Taqi ZJ, Li D. Iraqi propolis increases degradation of IL-1β and NLRC4 by autophagy following Pseudomonas aeruginosa infection. Microbes Infect. 2018; 20(2): 89-100. doi: https://doi.org/10.1016/j.micinf.2017.10.007.
- 35. Sattayasai J, Bantadkit J, Aromdee C, Lattmann E, Airarat W. Antipyretic, analgesic and anti-oxidative activities of Aquilaria crassna leaves extract in rodents. J Ayurveda Integr Med. 2012; 3(4): 175-9. doi: https://doi.org/10.4103/0975-9476.104427.
- 36. Jermsri P, Jiraviriyakul A, Unajak S, Kumphune S. Effect of Aquilaria crassna crude extract on simulated ischemia induced cardiac cell death. Int J Pharm Bio Sci. 2012; 3(3): 604-13. Available from: https://kukr. lib.ku.ac.th/kukr_es/BKN/search_detail/result/337599.

- 37. Kamonwannasit S, Nantapong N, Kumkrai P, Luecha P, Kupittayanant S, Chudapongse N. Antibacterial activity of Aquilaria crassna leaf extract against Staphylococcus epidermidis by disruption of cell wall. Ann Clin Microbiol Antimicrob. 2013; 12: 20. doi: https://doi.org/10.1186/1476-0711-12-20.
- Novriyanti E, Santosa E. The role of phenolics in agarwood formation of Aquilaria crassna Pierre ex Lecomte AND Aquilaria microcarpa Baill Trees. Indonesian Journal of Forestry Research. 2011; 8(2): 101-13. doi: https://doi.org/10.20886/ijfr.2011.8.2.101-113.
- Dahham SS, Ahamed MBK, Saghir SM, Alsuede FS, Iqbal AM, Majid AMSA. Bioactive essential oils from Aquilaria crassna for cancer prevention and treatment. Glob J Adv Pure Appl Sci. 2014; 4: 26-31. doi: http://dx.doi.org/10.13140/2.1.3315.7125.
- 40. Kakino M, Sugiyama T, Kunieda H, et al. Agarwood (Aquilaria Crassna) Extracts Decrease High-protein High-fat Diet-induced Intestinal Putrefaction Toxins in Mice. Pharm Anal Acta. 2012; 3(3): 1000152. doi: https://doi.org/10.4172/2153-2435.1000152.
- Naidu JR, Ismail RB, Yeng C, Sasidharan S, Kumar P. Chemical composition and antioxidant activity of the crude methanolic extracts of Mentha spicata. Journal of Phytology. 2012; 4(1): 13-18s. Available from: https://updatepublishing.com/journal/index. php/jp/article/view/2753.
- 42. Hameed IH, Hussein HJ, Kareem MA, Hamad NS. Identification of five newly described bioactive chemical compounds in methanolic extract of Mentha viridis by using gas chromatography-mass spectrometry (GC-MS). J Pharmacognosy Phytother. 2015; 7(7): 107-25. doi: https://doi.org/10.5897/JPP2015.0349.