GC-MS analysis of bioactive compounds in methanol extracts of three traditional rice varieties (*Oryza sativa* L.) in Kerala

Jahana K. P.[1], Najlath T.[2] and Deepa P.[2*]

[1]*Post Graduate Department of Botany, MES Kalladi College, Mannarkkad, Palakkad-678583, Kerala, India.*

[2] *Post Graduate Department of Botany, Korambayil Ahamed Haji Memorial Unity Women's College, Manjeri, Malappuram-676122, Kerala, India .*

*deepapsaj@gmail.com

Abstract

Background: *Oryza sativa* L. is a major cereal food crop and staple food in most of the developing countries which act as the rich source of nutrients and medicinal secondary metabolites. **Methods**: Present study revealed the diversity of phytochemical compounds in three traditional rice varieties, Sona Masuri, Thulasi and Supriya, of Kerala. Methanol extracts of seeds prepared using soxhlet apparatus and the compounds analyzed by GC-MS. **Findings**: The extract analysis showed the presence of the highest number of bioactive compounds in Supriya followed by Thulasi and Sona Masuri. Most important compound in Sona Masuri and Thulasi was 9,12-Octadecadienoic acid (z,z)-, while Oxacycloheptadec-8-en-2-one analyzed as the major notable compound in Supriya. These phytochemical compounds are with different biological activities, in turn leading the production of new pharmaceutical medicines in future.

Key words: *Oryza sativa* L., retention time, phytochemical compound, GC-MS analysis, Soxhlet apparatus.

1. Introduction

Rice, *Oryza sativa* L. is a perennial monocot plant in the family Poaceae and originated in India, Thailand and southern China. It is now cultivated on an estimated 3% of the world's agricultural land in wet tropical, semi-tropical and warm temperate areas around the world for the production of cereal grain; hence serves as a primary source of calories for over half the world's population [1]. There are two major ecotypes of *O. sativa,* namely *'indica'* adapted to the tropics, and '*japonica*', adapted to the temperate regions and tropical uplands and they show difference in seed shape. The seed of the ecotype, '*indica'*, is characteristically long and slim whereas *'japonica'* appears short and round [2].

Although there are no scientific reports available on the exact number of traditional varieties found in Kerala, it is documented that nearly 2000 traditional varieties with nutritional or medicinal values are predominantly cultivated [3]. Some of the important aromatic and medicinal rice varieties include Chennellu, Njavara, Gandhakasala, Jeerakasala, Chomala, Kayama, Kothampala etc.; while Thekkan, Chettadi, Kuttadan, Jyothi, Uma, Jaya etc. have high nutritional value [4,5]. All these varieties grow in various agro-climatic conditions of the state. But most of the varieties have disappeared from Kerala due to reduced cultivation and by various unfavourable abiotic conditions. It can be overcome by *ex-situ* and *in-situ* conservation methods, in turn confirming the germplasm conservation of rice diversity.

According to the American Diabetes Association, carbohydrates are the body's main source of energy. Rice is a good source of carbohydrate and each variety contains different quantities. In addition, the cereal provides proteins, dietary fibres, macronutrients, micronutrients etc. [6]. Together with nutrients, some of the traditional rice varieties are rich with different medicinal secondary compounds. Gas Chromatography-Mass Spectrometry (GC-MS) analysis is commonly used to detect and analyze the presence of the volatile secondary compounds. It combines the features of Gas Chromatography and Mass Spectrometry to identify different substances within the sample even if it is in minor quantity [7]. Previous study reports that Njavara, one of the major nutritional and medicinal rice varieties, included about 109 compounds having different biological activities including anti-microbial, anti-oxidant, anticancerous etc. [8]. Using advanced methods, the detection of medicinally important secondary metabolites in traditional rice varieties of Kerala is very significant in the pharmaceutical industry of the coming years.

The present study focused on three traditional rice varieties of Kerala, Supriya, Sona Masuri and Thulasi. Supriya (PTB 61) is a high yielding rice variety developed at Mannuthi Rice Research Centre, Thrissur, Kerala; which yields six and half to seven tons per hectare. A Premium Aromatic Rice Tulsi Amrit (Thulasi) is the non- Basmati variety having short to medium grain rice with intermediate amylose and gelatinization temperature. Moreover, Sona Masuri is also one of the fine varieties of non-basmati rice with lightweight and low starch content. Both Sona Masuri and Thulasi have less carbohydrate content than other traditional varieties in Kerala; so it plays a significant role in the diet of diabetic patients [9]. The knowledge on secondary metabolites of these three economically important rice varieties is very interesting and will contribute more in the future of the pharmaceutical industry.

2. Materials and methods

2.1. *Collection of plant material*: The fresh seeds of selected rice varieties, Sona Masuri and Thulasi were collected from Chandragiri Modern Rice Mill, Thirurangadi, Malappuram, Kerala, India. Supriya seeds were procured from Regional Agricultural Research Station (RARS) Pattambi, Kerala, India.

2.2. *Preparation of extract*: The seeds were dehusked carefully and powdered well using motor and pestle. About 30 gm of seed powder extracted with 250 ml methanol at a temperature between 60 and 65 ^{0}C using Soxhlet apparatus. The extract was concentrated by a rotary vacuum evaporator to obtain viscous semi-solid mass which was subjected to GC-MS.

2.3. *GC-MS analysis*: The detection of phytochemical compounds in seed extracts carried out using the GC-MS instrument (Shimadzu, Model Number - QP2010S). The Rxi-5Sil MS column showed 30 meter length, 0.25mm ID and 0.25µm thickness. In the gas chromatography part, the temperature programme, oven temperature, was 70°C raised to 260°C at 6°C/min and injection volume was 1 µl. The samples dissolved in methanol were run fully at a range of 50-650 m/z and the results were compared using NIST 11 & WILEY8 Spectral Library Programme.

3. Results

Different types of volatile secondary metabolites were detected in methanolic extracts of Sona Masuri, Thulasi and Supriya in which Supriya showed the highest number of phytochemical compounds followed by Thulasi and Sona Masuri. Most of the analyzed compounds have different biological activities including antibacterial, anticancerous, antiinflammatory, antioxidant, antifibrotic properties etc. In GC-MS analysis, 9, 12-Octadecadienoic acid (z,z)- showed the highest area % of 61.55 and 74.66 in Sona Masuri and Thulasi respectively (Table 1 & 2). The phytochemical compounds in Supriya had lower area % compared to other varieties; among the compounds, Oxacycloheptadec-8-en-2-one had the highest value, 18.82 (Table 3). In case of RT, it was lowest for dodecanoic acid (20.56) in Supriya; while methylpalmitate (28.40) showed lowest RT in Sona Masuri and Thulasi (Table 1, 2 & 3). The study revealed that Supriya is highly medicinal compared to other varieties due to the presence of diverse secondary metabolites (Figure 1, 2 & 3).

Table 1. Phytochemical compounds detected in methanol extract of dehusked Sona Masuri.

RT	Area %	Phytochemical compounds	Bioactivity
28.4	2.56	Methylpalmitate	Anti-inflammatory & antifibrotic [25]
31.6	4.96	9,12-Octadecadienoic acid, methyl ester	Anticancerous [21]
31.7	4.94	10-Octadecenoic acid, methyl ester	Antimicrobial [22]
33.4	61.55	9,12-Octadecadienoic acid (z,z)-	Anti-inflammatory & cancer preventive [14]
35.1	0.67	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	-
38.9	6.29	Dicapryl phthalate	Plasticizer, dye carrier, adhesive, hepatotoxin & mucous membrane irritant [24]
39.3	6.50	Oxalic acid, hexyl pentadecyl ester	-
39.8	4.47	Oxalic acid, 3,5-difluorophenyl nonyl ester	-
40.1	6.72	Oxalic acid, pentadecyl propyl ester	-
40.8	1.43	Adipic acid, decyl ester	-

Table 2. Analyzed phytochemical compounds in methanol extract of dehusked Thulasi.

RT	Area %	Phytochemical compound	Bioactivity
28.4	2.23	Methylpalmitate	Anti-inflammatory & antifibrotic [25]
31.6	4.99	Methyl octadeca-9,12-dienoate	Antitumor [26]
31.7	5.44	Elaidinsaeure methyl ester	-
32.2	0.29	Methyl isostearate	-
32.9	1.62	Hexadecanoic acid	Antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant & haemolytic [26]
33.5	74.66	9,12-Octadecadienoic acid (z,z)-	Anti-inflammatory & cancer preventive [14]
34.0	1.75	Solasonine	-
38.9	3.01	Dicapryl phthalate	Plasticizer, dye carrier, adhesive, hepatotoxin & mucous membrane irritant [24]
39.3	1.42	Oxalic acid, hexyl pentadecyl ester	-
39.4	0.38	1-heptanol, 2-propyl-	-
42.8	3.69	9-Octadecenamide	-
43.1	0.82	Squalene	Anti-inflammatory, anti-atherosclerotic, skin aging & adjuvant activities [26]

Table 3. Phytochemical compounds present in methanol extract of dehusked Supriya.

RT	Area %	Phytochemical compound	Bioactivity
20.56	05.82	Dodecanoic acid	Antimicrobial, antifungal, antiinflammatory & anticancerous [23]

23.97	00.37	Tetradecanoic acid, methyl ester	Antibacterial & antifungal [27]
24.94	03.25	Tetradecanoic acid	Antimicrobial, antioxidant, antiinflammatory, anticancerous, hypercholesterolemic, larvicidal & repellent [28, 29, 30]
28.16	04.51	Hexadecanoic acid, methyl ester	Antimicrobial, antipasmodic, antiinflammatory, antioxidant, hypocholesteromic, antiandrogenic & hemolytic 5-Alpha reductase inhibitor [30, 31]
28.71	00.42	Dibutyl phthalate	Antibacterial, antibacterial & antifungal [32, 33]
29.22	13.03	Hexadecanoic acid	Antimicrobial & anticancerous [28, 34]
31.36	07.69	9,12-octadecadienoic acid, methyl ester	Antimicrobial [35]
31.48	06.96	Elaidinsaeure methylester	Antiinflammatory, antiarthretic, anticarcinogenic & hepato protective [34]
32.52	18.82	Oxacycloheptadec-8-en-2-one	Antimicrobial & antiasthmatic [17]
32.62	13.67	Cis-vaccenic acid	Antimicrobial [36]
34.76	01.37	3-cyclopentylpropionic acid, 2-dimethylaminoethyl ester	Antibacterial [37]
38.48	11.63	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	Antioxidant, hypocholesterolemic, nematicide, anti-androgenic & hemolytic 5-alpha reductase inhibitor [38]
41.13	06.70	9,12-octadecadienoic acid(z,z)-, 2-hydroxymethyl) ethyl ester	Antiasthmatic [17]
41.19	05.78	Oleoyl chloride	Antibacterial, antimicrobial, antifungal & endotoxin neutralizing activity [39, 40, 41, 42]

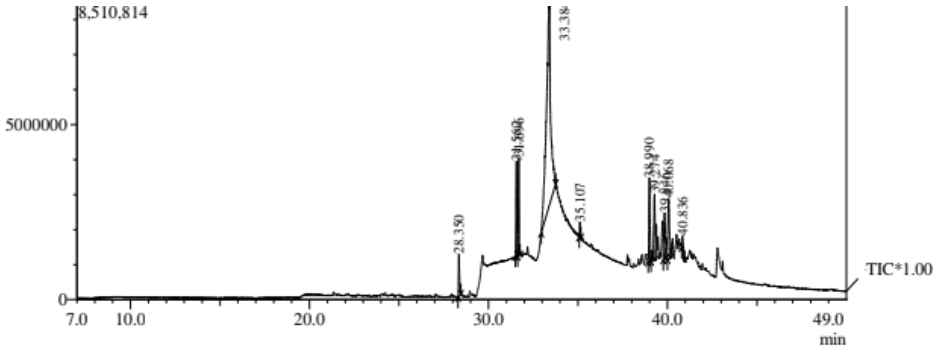

Figure 1. Chromatogram of phytochemical compounds present in hexane extract of seeds of *O. sativa* var. Sona Masuri.

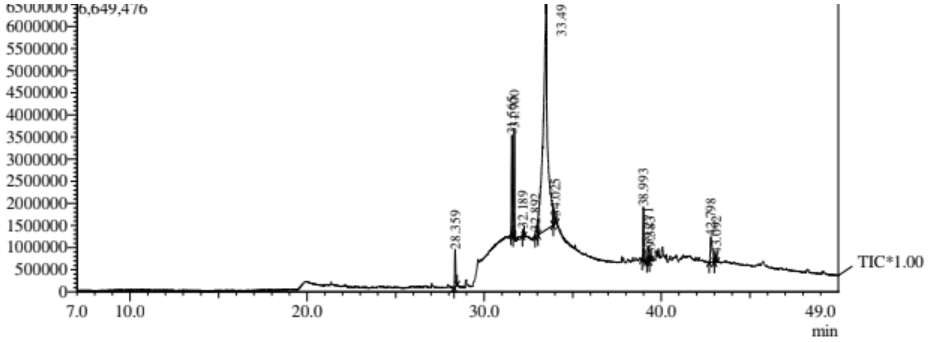

Figure 2. Chromatogram of phytochemical compounds present in hexane extract of seeds of *O. sativa* var. Thulasi.

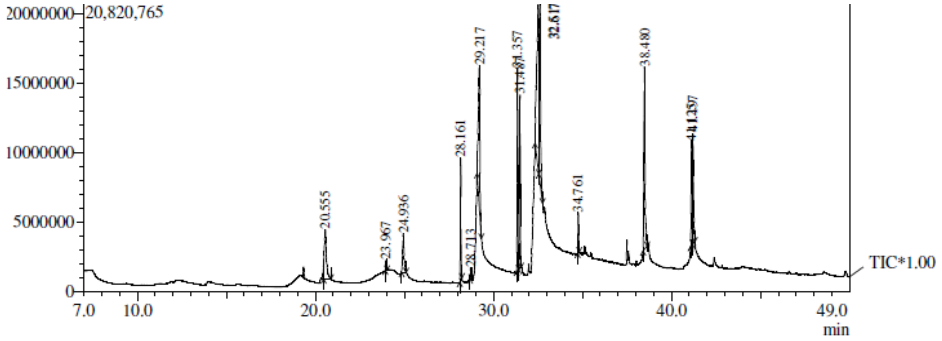

Figure 3. Chromatogram of phytochemical compounds present in hexane extract of seeds of *O. sativa* var. Supriya.

4. **Discussion**

In Kerala, many traditional rice varieties show medicinal values due to the presence of significant phytochemical compounds. GC-MS is an integrative technique for separation, identification and quantification of volatile compounds in plant extracts [10]. Quantification is an important step for data analysis and different softwares is being used for the calculation of retention time corresponding to specific peaks [11]. For the analysis of plant volatile compounds, the different solvents including ethanol, hexane, methanol, ethyl acetate etc. are commonly used [12]. GC-MS analysis of hexane extract of dehusked Njavara revealed the presence of 109 phytochemical compounds [8]. Similarly, Ashokkumar et al. detected volatile compounds including fatty acids, terpenes, alkanes, alkenes, alcohols, phenols, esters and amides in South Indian traditional rice varieties including Kichili samba, Seeraga samba, Kaiviral samba, Mappilai samba, Karuppu kavuni, Kattuyanam and Kuzhiyadichan [13].

In the present investigation, the methanol extracts of dehusked seeds of Supriya, Sona Masuri and Thulasi were used for analysis and detected 14, 12 and 10 compounds in Supriya, Thulasi and Sona

Masuri respectively. Many of above compounds are pharmaceutically important and their medicinal efficacies have been reported by many researchers including antioxidant, anticancerous, antibacterial, antiinflammatory and antiviral activities that will improve the drug designing in pharmaceutical industries (Table 1, 2 & 3); whereas some compounds show the industrial applications also. The compound, 9,12-Octadecadienoic acid (z,z)-, showed highest area percentage in Sona Masuri and Thulasi which has antiinflammatory and cancer preventive potential [14]; therefore, the rice varieties are useful to produce anticancerous medicines. The same compound is also reported from different plants like *Solena amplexicaulis*, *Adenophorae radix*, *Albizia adianthifolia, Pterocarpus angolensis* etc. [14,15,16]. The area percentage of Oxacycloheptadec-8-en-2-one is higher in Supriya compared to other compounds. The compound in the rice variety is with better potential for antimicrobial and antiasthmatic activities and can be used in pharmaceutical industry [17]. Similarly, Oxacycloheptadec-8-en-2-one is detected from hexane and dichloromethane (DCM) extracts of *Ageratum conyzoides* and essential oils of *Trollius europaeus* and *Arisaema amuremse* that makes the plant highly medicinal [18,19,20].

5. Conclusion

The rice varieties Supriya, Sona Masuri and Thulasi are the sources of different bioactive compounds that justify the pharmaceutical usage of the varieties. GC-MS analysis shows the presence of 14, 12 and 10 phytochemical compounds with different biological activities in methanol extract of Supriya, Sona Masuri and Thulasi respectively. In Sona Masuri and Thulasi, 9, 12-Octadecadienoic acid (z,z)- is the major compound; while Supriya consisted of Oxacycloheptadec-8-en-2-one as the significant phytoconstituent. Further investigations in this varieties will lead to the development of medicines in future.

6. Acknowledgement

The authors are graciously thankful to the Principal, Korambayil Ahammed Haji Memorial Unity Women's College, Malappuram, Kerala, India for providing the laboratory facilities.

7. References

1. Khush G. Productivity improvements in rice. Nutrition Reviews. 2003;61(6):S114-S116.
2. Sun CQ, Jiang TB, Fu YC, Wang XK. *Indica-Japonica* differentiation of paddy rice and its relationship with heterosis. Plant breeding. 20002;121(4):330-337.
3. Leena Kumari S. Status Paper on Rice in Kerala. Rice Knowledge Management Portal. 2012;1–32.

4. Devi PI, Solomon SS, Narayanan M. Conservation of traditional rice varieties for crop diversity in Kerala. Acharya N.G. Ranga Agricultural University. 2017;93.
5. Devraj L, Panoth A, Kashampur K, Kumar A, Natarajan V. Study on physicochemical, phytochemical and antioxidant properties of selected traditional and white rice varieties. Journal of Food Process Engineering. 2020;43(3):e13330.
6. M.C. Kik. Nutritive value of rice, nutrients in rice bran and rice polish and improvement of protein quality with amino acids. J. Agric. Food Chem. 4(2) (1956) 170-172.
7. Daferera DJ, Ziogas BN, Polissiou MG. GC-MS analysis of essential oils from some Greek aromatic plants and their fungitoxicity on *Penicillium digitatum*. Journal of Agricultural and Food Chemistry. 2000;48 (6):2576-2581.
8. Hima Rasheed, Deepa P. Nutrient evaluation, phytochemical analysis and *in vitro* propagation in Navara. International Journal of Current Research. 2018;10(5):68865-68869.
9. Deepa P, Najleth T. *In vitro* salt tolerance in *Oryza sativa* var. Sona Masuri & Thulasi. MESMAC International Conference, Applying Theory: Paradigms Practices Faultlines. 2020;ISBN-978-93-5396-247-0.
10. Leary J, Hiscox J, Eastwood DC, Savoury M, Langley A, McDowell SW, Müller CT. The whiff of decay: linking volatile production and extracellular enzymes to outcomes of fungal interactions at different temperatures. Fungal Ecology. 2019;39:336-348.
11. Johnsen LG, Skou PB, Khakimov B, Bro R. Gas chromatography–mass spectrometry data processing made easy. Journal of Chromatography A . 2017;1503:57-64.
12. Mamza UT, Sodips OA, Khan IZ. "Gas Chromatography-Mass Spectrometry (GCMS) Analysis of Bioactive Components of Phyllanthus amarus Leaves". International Research Journal of Plant Sciences. 2012;3(10):208-215.
13. Ashokkumar K, Govindaraj M, Sampathrajan Vellaikumar VG, Karthikeyan A, Akilan M, Sathishkumar J. Comparative Profiling of Volatile Compounds in Popular South Indian Traditional and Modern Rice Varieties by Gas Chromatography–Mass Spectrometry Analysis. Frontiers in Nutrition. 2020;7.
14. Krishnamoorthy K, Subramaniam P. Phytochemical profiling of leaf, stem, and tuber parts of Solena amplexicaulis (Lam.) Gandhi using GC-MS. International Scholarly Research Notices. 2014.

15. Mustafa GHULAM, Ahmed S, Ahmed NISAR, Jamil AMER. Phytochemical and antibacterial activity of some unexplored medicinal plants of Cholistan desert. Pakisthan Journal of Botany. 2016;48(5):2057-2062.
16. Lan H, Lai B, Zhao P, Dong X, Wei W, Ye Y, Wu Z. Cucumber mosaic virus infection modulated the phytochemical contents of *Passiflora edulis*. Microbial Pathogenesis. 2020;138:103828.
17. Soorya R, Dhamodaran P, Rajesh K, Duraisamy B. In Silico Studies of the Secondary Metabolites of *Solanum torvum* sw. For their Antiasthmatic Activity. International Journal of Current Pharmaceutical Research. 2017;9(4):38-41.
18. Witkowska-Banaszczak E. Identification of the components of the essential oil from *Trollius europaeus* flowers. Acta Physiologiae Plantarum. 2013;35(5):1421-1425.
19. Mihigo SO, Ndombele L, Masesane IB, Sichilongo K. Preliminary GC-MS Profiling and Anti-bacterial activity Investigation of *Ageratum conyzoides* Linn. (Asteraceae). 2013.
20. Li G, Jiang Y, Li Y, He T, Wang Y, Ji T, Zhou X. Analysis and biological evaluation of *Arisaema amuremse* Maxim essential oil. Open Chemistry Journal. 2019;17(1):647-654.
21. Sharma A, Rai PK, S. Prasad. GC–MS detection and determination of major volatile compounds in *Brassica juncea* L. leaves and seeds. Microchemistry Journal. 2018;138 :488-493.
22. Rahman MM, Ahmad SH, Mohamed MTM, Ab Rahman MZ. Antimicrobial compounds from leaf extracts of *Jatropha curcas*, *Psidium guajava*, and *Andrographis paniculata*. Scientific World Journal. 2014.
23. Bakdash A, Almohammadi OH, Taha NA, Abu-Rumman A, Kumar S. Chemical composition of propolis from the Baha region in Saudi Arabia. Czech Journal of Food Sciences. 2018;36 (2):00-10.
24. https://www.nlm.nih.gov/
25. El-Demerdash E. Anti-inflammatory and antifibrotic effects of methyl palmitate. Toxicology and Applied Pharmacology . 2011;254 (3):238-244.
26. George Hunter M, Paul Beckett R, Clements JM, Mark. Antibacterial agents.US Patent. 2005;6:908.
27. Agoramoorthy G, Chandrasekaran M, Venkatesalu MV, Hsu MJ. Antibacterial and antifungal activities of fatty acid methyl esters of blind-your-eye mangrove from India. Brazilian Journal of Microbiology. 2007;38(4):739-742.

28. Kahriman N, Albay CG, Dogan N, Ustat A, Alpay S, Yayli KN. Volatile Constituents and Antimicrobial activities from flower and fruit of *Arbutus unedo* L. Asian Journal of Chemistry. 2010;22(8):6437-6442.
29. Kavitha A, Mangamoori LN. Phytochemical compound identification and evaluation of antimicrobial activity of *Eugenia bracteata* Roxb. International Journal of Biotechnology & Biochemistry. 2016;12(1):73-78.
30. Lina Suliman AM, Ali Marouf AS. Gas chromatography analysis and photoluminescence of *Nigella sativa* oil. International Journal of Advanced Biochemistry Research. 2018;2(2):64-67.
31. Al-Shammari L, Wafaa Hassan HB, Hanan Al-Youssef M. Chemical composition and antimicrobial activity of the essential oil and lipid content of *Carduus pynocephalus* L. growing in Saudi Arabia. Pharmaceutical Chemistry Journal. 2012;4(2):1281-1287.
32. Khatiwora E, Vaishali Adsul B, Manic Kulkarni M, Deshpande NR, Kashalkar RV. Antibacterial activity of Dibutyl Phthalate: A secondary metabolite isolated from *Ipomoea carnea* stem. Journal of Pharmacy Research. 2012;5(1):150-152.
33. Ahsan T, Chen J, Zhao X, Irfan M, Wu Y. Extraction and identification of bioactive compounds (eicosane and dibutyl phthalate) produced by streptomyces strain KX852460 for the biological control of *Rhizoctonia solani*. *AMB* express. 2017;7(1).
34. Usharani, Chithra M. GC–MS analysis of methanol extract of leaf of *Wattakaka volublis*. International research journal of pharmaceutical and applied sciences. 2013;3(4):161-165.
35. Abubakar MN, Majinda RR. GC-MS analysis and preliminary antimicrobial activity of *Albizia adianthifolia* (Schumach) and *Pterocarpus angolensis* (DC). Med. 2016;3(1):3.
36. Hussein HM. Antimicrobial activity and spectal chemical analysis of methanolic leaves extract of *Adiantum capillus-veneries* using GC-MS and FT-IR spectroscopy. College of Science. Al-Qadisiya University, Iraq. 2012.
37. Hunter MG, Raymond Paul Beckett, John Martin Clements, Mark. Antibacterial agents.US Patent, 2005;6:908,91.
38. Kumar Suluvoy J, Berlin Grace VM. Phytochemical profile and free radical Nitric oxide (NO) Scavenging activity of *Averrhoa bilimbi* L. Fruit extract. Biotechnology. 2017;7(1):85.
39. Zorko M, Andreja Majerle, David Sarlah, Mateia Mancek keber, Barbara Mohar, Roman. Combination of antimicrobial and endotoxin-neutralizing activities of novel oleoylamines. Antimicrob. Agents Chemotherapy. 2005;49(6):2307-2313.

40. Xing K, Chen XG, Li YY, Liu CS, Liu CG, Cha DS. Antibacterial activity of oleoylchitosan nanoparticles: A novel antibacterial dispersion system. Carbohydrate Polymers. 2008;74(1):114-120.
41. Evran S, Yasa I, Telefoncu A. Modification of lysozome with oleoyl chloride for broadening the antimicrobial specificity. Preparative Biochemistry & Biotechnology. 2010;40(4):316-325.
42. Xing K, Shen X, Zhu X, Ju X, Mia X, Tian J, Fena J. Synthesis and *in vitro* antifungal efficacy of oleoyl-chitosan nanoparticles against plant pathogenic fungi. International Journal of Biological Macromolecules. 2016;82:830-836.