International Journal of Aquatic Science
ISSN: 2008-8019
Vol 12, Issue 02, 2021

Gas Chromatography - Mass Spectrometry (Gc - Ms) Analysis Of Methanol, Hexane And Ethyl Acetate Extract Of Ziziphus Jujuba Fruit

Ponmani.S[1], Selvankumar T[2], Gopinath L. R[3]

[1] Department of Biotechnology, Mahendra Arts and Science College (Autonomous), Namakkal (Dt), Tamil Nadu, India- 637 501, Department of Biotechnology, Padmavani Arts and Science College, Salem, Tamil Nadu, India- 636 011.
[2]Department of Biotechnology, Mahendra Arts and Science College (Autonomous), Namakkal (Dt), Tamil Nadu, India- 637 501
[3] Department of Biotechnology, Vivekanandha College of Arts and Sciences for Women (Autonomous), Namakkal, Tamil Nadu, India-637205.

Abstract: Since from the past the phytochemicals identified from different plants plays a key role in treating human disorders.In this context, reviving the volatile compounds present in different solvents like methanol, hexane and ethyl acetate extract of Ziziphus jujuba fruit has been performed. The Hexane, Methanol and Ethyl acetate extracts of Ziziphus jujuba fruit was filtered and the compounds present in the above extracts were identified using GC – MS. The derivatives of fatty acids like 9,12-octadecadienoic acid, 9-octadecenoic acid, Eicosanoic acid, Octadecanoic acid and Tetradecanoic acid and some volatile compounds like 7,9-di-tert-butyl-1-oxaspiro(4,5), Eicosane, Hexatriacontane, Methyl stearate and phenol were found to be common in all the solvents. This serves as pool proof for its pharmacological activities from the ancient research work carried out by different researchers. Thus the present study was carried out to identify the biologically active compoundsfrom different solvents of Ziziphus jujuba fruit.

Key Words – GC – MS, Phytochemical compounds, fruits of Ziziphus jujuba, Hexane, Methanol and Ethyl Acetate.

1. INTRODUCTION

Medicinal plants were considered as a superior remedial agent in treating human disorders and the phytochemicals present in it also acts as an excellent source of drugs in many pharma industries due to its medicinal importance (Atanasov, *et. al*., 2015). The screening of biologically active substances from natural resources inculcates a great knowledge among the scientist in finding out new drugs for many ailments (Encarnacion and Keer, 1999). Among many natural sources, fruits of *Ziziphusjujuba*were found to contain enormous phytoconstitues with various pharmacological activities. *Ziziphus jujuba* belongs to the family of Rhamnaceae with 40 different spices. This plant was found to have its native in the tropical and subtropical parts in worldwide (Mukhtar, *et. al.,* 2004).
The fruits of *Ziziphus jujuba* were composed with calcium, phosphorous and also packed with vitamins like A, C and B (Pareek, 2002). Betulinic acid identified from the stem bark of *Ziziphus* family were found to have antiviral activity and also involved in the growth retardation of HIV infection (Mukharjee, *et. al*., 2003). A recent report states that compound

quantified from methanol extract of fruits of *Ziziphus jujuba* was proved to have antiulcer, anti-inflammatory and antispasmodic activity (Ganachari and Shiv, 2004). The compounds identified from the aqueous extract of *Ziziphus jujuba* were reported to have high and efficient anti allergic activity in In vitro condition (Su, *et. al*., 2004).It was also stated that the compounds identified from the Ethyl acetate extract of stem bark of *jujuba* species was found to have antisteroidogenic and antifertility activity in experimental mice (Gupta, *et. al*., 2004). Although the functions of different compounds from different parts of *Ziziphus* was examined the compounds present in the fruit with various biological properties was not entirely established to the world.

Thus the present research work was carried out to explore the biologically active phytoconstituents present in different solvents like Hexane, Ethyl Acetate and Methanol extracts of *Ziziphus jujuba* fruit using Gas Chromatography and Mass Spectrometry (GC – MS) technique. Hence, this work leads a way for the emergence of new and effective drug in the recent modern world for treating many disorders.

2. METHODOLOGY

Sample preparation

The healthy plant parts like leaves, seeds and fruits of *Ziziphus jujuba* were collected and washed thoroughly in tap water and distilled water also shade dried at room temperature for 21 days. The dried parts were powdered in a blender and extracts were prepared different solvents like chloroform, methanol, ethanol, petroleum ether, acetone and water with Soxhlet apparatus for 18 hrs. The prepared crude extracts were used for further process.

GC-MS analysis

The hexane, ethyl acetate and methanol extracts of *Ziziphus jujuba* was filtered and concentrated. The extracted material was taken for GC MS. The extracted samples were analyzed using gas-chromatography mass spectrometer (GC-MS). Gas chromatography mass spectrometry analysis was carried out at Advanced Instrumentation Research Facility (AIRF), Jawaharlal Nehru University (JNU), New Delhi on a Shimadzu GCMS- QP2010 PLUS GC-MS equipped with an AOC-20i auto injector and AOC-20s auto- sampler units on a GC clarus 500 Perkin Elmer system comprising a AOC20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument.

Identification of components

Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

3. RESULTS

The GC-MS chromatogram analysis for hexane extract of *Zizhipusjujuba* fruit showed the chromatogram peaks from 5 minutes to 24 minutes. The highest peaks were obtained from 15 minutes followed with 18 minutes to 20 minutes and 23 to 24 minutes. The moderate peaks were obtained at 9 minutes followed with 14 to 15 minutes and 21 to 23 minutes. The lowest peaks were obtained from 7 minutes to 9 minutes followed with 11 to 13 minutes and 16 to 17 minutes (Figure 1.1).Phytochemical compounds present in hexane extract of

Zizhipusjujuba fruit along with Retention Time (RT), Starting Retention Time (STR) and End Retention Time were tabulated In this extract nearly 107 compounds have been quantified with different retention times. The compounds like octadeconoic acid, stigmast-5-en-3-ol, (3.beta.,24s) and dotriacontane showed high Retention Time (24.07,24.195 and 24.82), Starting Retention Time (24, 24.1 and 24.78) and End Retention Time (24.1, 24.37 and 24.865) respectively.

The GC-MS chromatogram analysis for ethyl acetate extract of *Zizhipus jujuba* fruit showed the chromatogram peaks from 5 minutes to 24 minutes. The highest peaks were obtained from 5 minutes followed with 8 minutes to 10 minutes, 17 minutes and 18 to 16 minutes. The moderate peaks were obtained at 6 minutes to 9 minutes followed with 13 to 15 minutes and 21 to 24 minutes. The lowest peaks were obtained at 12 minutes to 14 minutes followed with 16 to 17 minutes and also at 20 minutes (Figure 1.2).Phytochemical compounds present in ethyl acetate extract of *Zizhipus jujuba* fruit along with Retention Time (RT), Starting Retention Time (STR) and End Retention Time were determined. In this extract nearly 105 compounds have been quantified with different retention times. The five compounds like 1-hexacosanol, Stigmast-5-en-3-ol,2(3h)-furanone, 1-hexacosanol,Ethyl tetracosanoate and Hexatriacontane showed high Retention Time (24.045, 24.155, 24.27, 24.37, 24.76 and 24.82), Starting Retention Time (24.005, 24.085, 24.24, 24.3, 24.715 and 24.795) and End Retention Time (24.085, 24.235, 24.3, 24.44, 24.795 and 24.865) respectively.

The GC-MS chromatogram analysis for Methanol extract of *Zizhipus jujuba* fruit showed the chromatogram peaks from 5 minutes to 24 minutes. The highest peaks were obtained from 5 minutes followed with 8 minutes to 10 minutes, 18 minutes and 24 minutes. The moderate peaks were obtained at 6 minutes to 7 minutes followed with 13 minutes and 21 to 24 minutes. The lowest peaks were obtained at 9 minutes followed with 11 minutes to 13 minutes and also followed with 16 to 17 minutes and also at 19 minutes to 23 minutes respectively (Figure 1.3).Phytochemical compounds present in Methanol extract of *Zizhipus jujuba* fruit along with Retention Time (RT), Starting Retention Time (STR) and End Retention Time were tabulated. In this extract nearly 115 compounds have been quantified with different retention times. The three compounds like 2-furancarboxaldehyde, stigmast-5-en-3-ol, (3.beta.,24s)- and Ethyl triacontyl ether showed high Retention Time (24.065, 24.325 and 24.66), Starting Retention Time (23.96, 24.11 and 24.59) and End Retention Time (24.11, 24.51 and 24.735) respectively.

The common phytochemical compounds identified from the different solvents like Hexane, Ethyl acetate and methanol of *Zizhipus jujuba* fruit along with its biological functions were discussed. In all the three solvents, 12 compounds like 7,9-di-tert-butyl-1-oxaspiro(4,5),9,12-octadecadienoic acid, 9-octadecenoic acid, eicosane, Eicosanoic acid, hexadecanoic acid, Hexatriacontane, Methyl stearate, N-Hexadecanoic acid, Octadecanoic acid, Phenol and Teradecanoic acid were found to be common. In this 9-di-tert-butyl-1-oxaspiro(4,5) was used as a surface treatment whereas 9,12-octadecadienoic acid, Hexatriacontane and Tetradecanoic acid were used as a flavouring and fragrance agent. Eicosane was used I petrochemical industry. Eicosanoic acid was used as a non pesticidal agricultural agent and also acts as lubricants. Hexadecanoic acid and its deraivatives like N-Hexadecanoic acid and octadecanoic acid was used in the preparation of soaps, detergents and cosmetics.

10 compounds like 1-docosanol, 2(3h)-furanone, 2-pyrrolidinone, 4-(1-hydroxy-ethyl), Docosane, Dodecanoic acid, Heptadecanoic acid, Palmitoleic acid, Pentadecanoic acid and tetracosane were found to be common in Hexane and Ethyl acetate extracts. In the above compounds, 1-docosanol was found to have antiviral activity and 2(3h)-furanone acts as a immunosuppressive agents. Docosane was involved in chlorination reactions. The compound

Dodecanoic acid was used as coating agents on fresh citrus fruits and also acts as defoaming agents.Palmitoleic acid had antithrombotic effects which help to prevent strokes. 5 compounds like 1-tetradecanol, benzene, Campesterol, olesterol and stigmast-5-en-3-ol, (3.beta.,24s)- were found to be common in both Hexane and Methanol, extract of *Zizhipusjujuba* fruit. In this, benzene was used as disinfectant and Campesterol was possessed to have anticancer and cholesterol lowering properties whereas olesterol was used as a emulsifying and solubilizing agents. In the solvents like Ethyl acetate and Methanol, 10 compounds like 1-hexacosanol, 2,5-furandione, 2-butenedioic acid (e), 2-furancarboxaldehyde, 2-hydroxypropane, 4-methyl itaconate, 5-acetoxymethyl-2-furaldehyde, azelaic acid and behenic alcohol were found to be common. From these the compounds like 1-hexacosanol and 2,5-furandione were acts as a Flavouring agents. The compound 2-furancarboxaldehyde was used in the manufacture of insecticides whereas azelaic acid and behenic alcohol was used as antibacterial and antiviral agents.

Figure 1.1 GC- MS Chromatogram for hexane extracts of *Z. jujuba* fruit

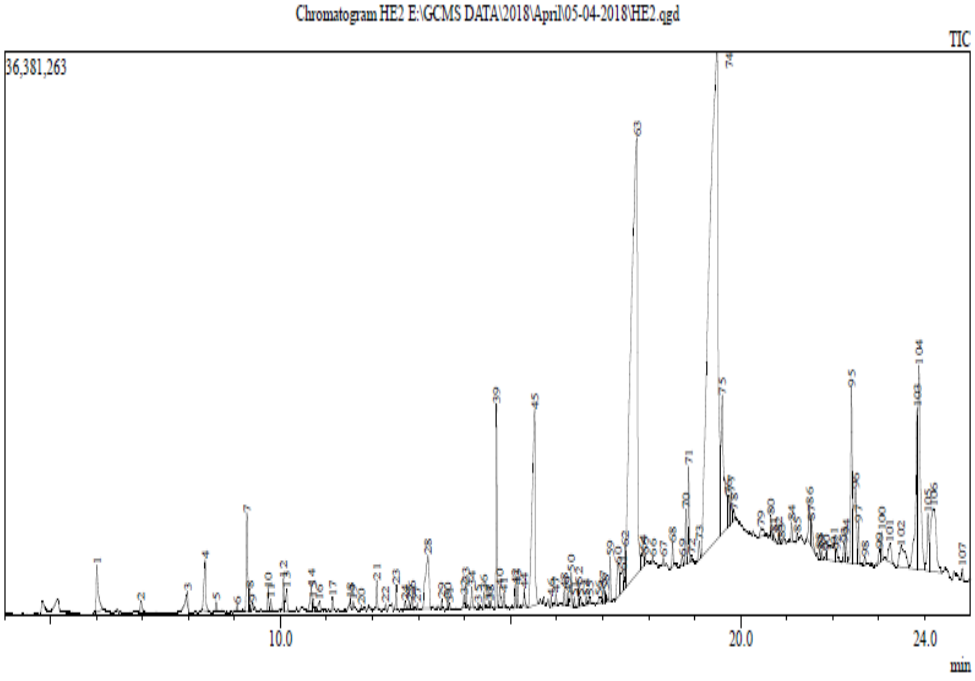


Figure 1.2 GC- MS chromatogram for ethyl acetate extract of *Z. jujuba* fruit

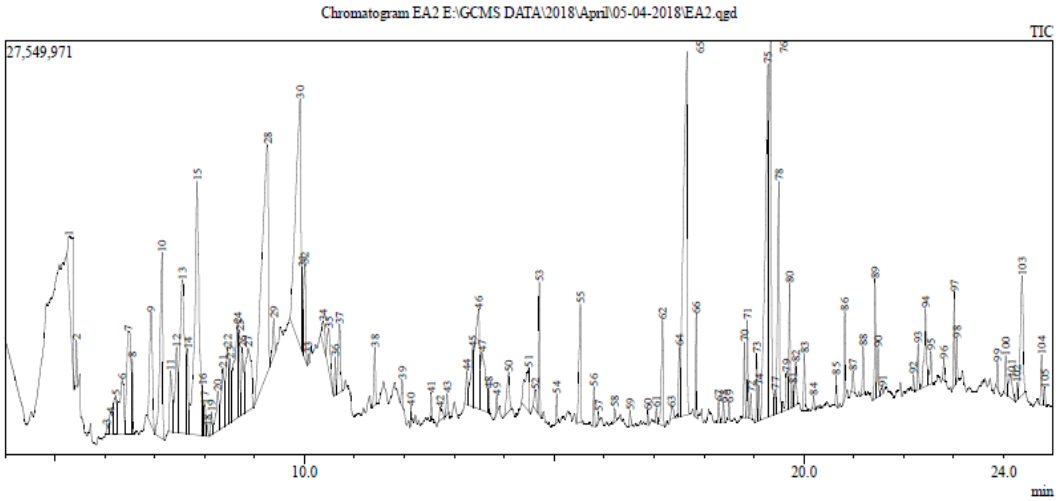
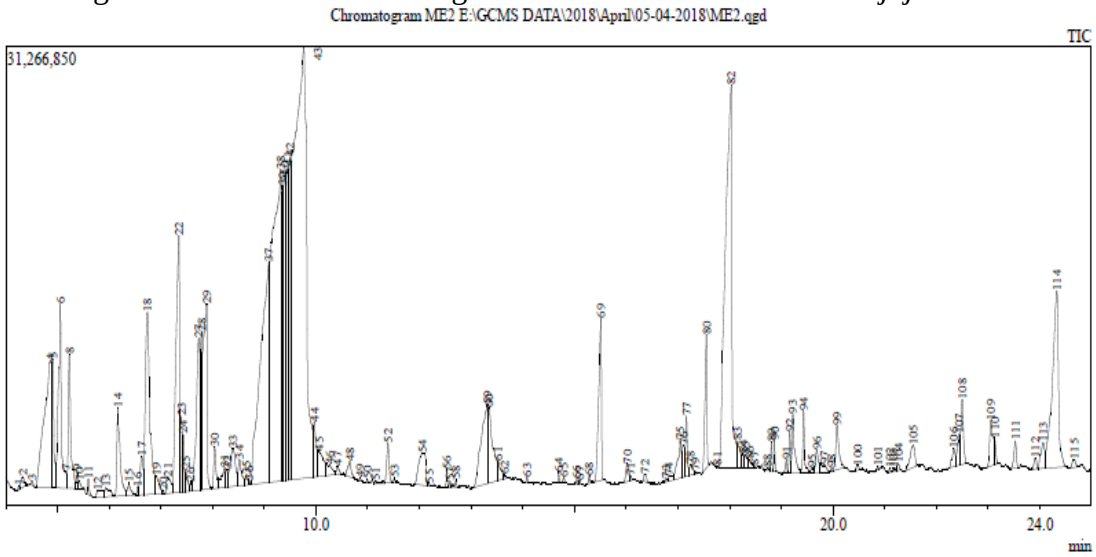


Figure 1.3 GC- MS Chromatogram for methanol extracts in *Z. jujuba* fruit.



4. DISCUSSION

Nowadays plants and its biological constituents play an important role in preventing and treating diseases which helps to reduce the side effects of other treatment methods (Bachrach, 2012). The herbal plants were rich in phytochemical constituents were highly involved in treating various diseases and such biologically active compounds were identified with the help of GC-MS (Farid, 2015, Wojdylo, 2015). A recent technique, Mass spectrometry combined with Gas Chromatography was proved to be very effective in identifying the biological components from many medicinal plants (Sermakkani and Thangapandian, 2012).
A recent report states that, vast amount of phytochemicals like alkaloids, polysaccharide, Saponins and polyphenols were extensively scattered in *Zizhipus* species (Li, 2014 and Guo, 2015). Hence, the present study was performed to identify the Phytochemical compounds present in different solvents like Hexane, Methanol and Ethyl acetate of *Zizhipus jujuba* fruit. In this, nearly 12 compounds were found to be common in all the three solvents with various properties and functions. A researcher proved that, GCMS analysis of ethyl acetate and

methanol extract of *Archidendron bubalinum* had been identified with hexadeconoic acids which were also reported to have antioxidant and antimicrobial properties (Candra, *et. al*., 2018). Hence from the present research work it was proved that the derivatives of such type of compounds were found to be common in all the three solvents of *Zizhipus jujuba* fruit.

The compound named 9-octadecenoic acid, methyl ester was proved to have various pharmacological functions like antifungal, antioxidant, antimicrobial activities and also acts as a good nematicide and pesticide (Chandrasekaran, *et.al*., 2011). An another compound called hexadeconoic acid was also possessed to have a potent antibacterial, antifungal and antimicrobial activity (Mangunwardoyo and Deasywaty, 2012). Hence the above research work also showed that the hexane and ethyl acetate extracts of *Zizhipus jujuba* fruit. Thus, it served as a pool proof that these two compounds identified from the hexane and ethyl acetate extracts of *Z. jujuba* fruit would be used against bacterial and fungal infections.

A recent research states that the jujube fruit was in bitter taste and also proved that the bitterness of the fruit was due to the presence of combined group of compound distribution like ketone, aldehyde and isoamylol (Li, *et.al*., 2013). Heptadecanolide, a derivative of fatty acid identified from aqueous extract of *Daniellao liveri* acts as a flavouring agent and also used as perfumes (Sistla, *et. al*., 2005). The other two compounds like Dodecanoicacid, 1-(hydroxymethyl)-1 and 2-ethanedidyl ester identified from the same species was proved to be used as emulsifiers in creams, lotions and also in hair conditioner (Elizabeth, et. al., 2013). The derivatives of fatty acids like Lauric anhydride was involved in drug formulation and volatile organic compounds like Tetrahydrofuran-2-carboxylic acid, dibenzofuran-3-ylamide and Heptadecanolide were used in the treatment of chemotheraphy for cancer patients and also used as food additive (Yakubu, *et. al*., 2017).

A researcher states that the sterol compounds like beta-sitosterol, lupeol identified from the leaf extract of Rhododendron arboretum was proved have anti-inflammatory, anti-diabetic, antibacterial and cardio protective properties (Prakash, *et. al*., 2008; Sonar, *et. al*., 2013 and Rezk, *et.al*., 2015). Hence, from the above research study it was proved that these types of compounds like campesterol, olestrol and stimasterol were found to be present in all the two solvents like Hexane and Methanol of *Zizhipus jujuba* fruit.

The phenolic compounds have the ability to degrade the formation of protein peptides on the cell surface of the microbes which leads to the death of the bacterial species. Thus, it was proved that the presence of phenolic compounds may exhibit the antibacterial activity against pathogens (Mangunwardoyo and Deasywaty, 2012). A researcher named Ikram, *et. al*., identified a compound called alpha-D-furopyranosyl was mainly used in the treatment of cancer and also extend its activity by lowering the cholesterol level in humans (Ogihara, *et. al.,* 1976). In this present study the similar compound and its derivatives found to be present in the Hexane and Ethyl acetate extract of *jujuba* fruit.

Many of the research says that the stem bark and seeds of *jujuba* species was present with cyclopeptide and peptide alkaloids like cyclopentanidione which were mainly involved in treating diabetes and also acts as a good flavouring agent from the past (Han and park,1986). It was proved from the above study that the same compound has been present in the methanol extract of *jujuba* fruit. The fatty acid derivatives present in different natural sources were proved to have many medicinal properties like anti-thrombotic and anti-inflammatory properties which also involved in lowering the lipids in human (Katalin and Ioana, 2017). Thus the present study also reveals that the fatty acids like Dodecanoic acid, Heptadecanoic acid, Palmitoleic acid, Eicosanoic acid and Pentadecanoic acid were found to be present in all the three extacts like Hexane, Methanol and Ethyl acetate of *Zizhipus jujuba* fruit.

Recent research proved that one of the hydrocarbon derivatives called Eicosane identified from the bark of *Ficusre ligiosa* has possessed the antifungal and antimicrobial activity against fungal and bacterial pathogens (Sundaramoorthy, *et. al.*, 2018). Thus from the present study it was clear that the same compound was identified from all the three solvents of *Zizhipus jujuba* fruit. Hence this may also proved to exhibit the same properties in further investigations.The glycoside derivatives like Mesifurane and 2, 5-dimethyl-4methoxy -3(2H)-furanone identified from berries were used as a flavoring agents in foods, beverages and also cosmetics (Heikki, 2018). Thus the same derivative of glycosides named 2(3h)-furanone was found to be identified from the Hexane and Ethyl acetate extracts as an immune suppressive agents.

5. CONCLUSION

The above discussion states that the plant Phytochemical compounds as secondary metabolites in plants play a major biological role in treating human disorders and also in industries as food additives and flavouring agents. Hence the present research work also proved that the compounds with various activities were found to be present in all three solvents like Hexane, Methyl acetate and Methanol of *Zizhipus jujuba* fruit with various properties. Thus this serves as a pool proof for many researchers in finding out the medicinal compounds for treating many human ailments in future.

6. REFERENCES

[1] Atanasov, A. G., Waltenberger, B., Pferschy-Wenzig, E. M., Linder, T., Wawrosch, C. and Uhrin, P. 2015. Discovery and resupply of pharmacologically active plant-derived natural products: a review. *Biotechnol Adv*. 3(8): 1582 - 614.

[2] Bachrach, Z. Y. 2012. Contribution of selected medicinal plants for cancer prevention and therapy. *ActaFacMedicaeNaissensis*. 29(3): 11 -23.

[3] CandraIrawan, Foliatini, Hanafi, LilisSulistiawaty and MamanSukiman. 2018. Volatile Compound Analysis using GC-MS, Phytochemical Screening and Antioxidant Activities of the Husk of "Julang-Jaling" (Archidendronbubalinum (Jack) I.C Nielsen) from Lampung, Indonesia. *Pharmacogn J*. 10(1): 92-98.

[4] Chandrasekaran M., Senthilkumar A. and Venkatesalu V. 2011. Antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of *Sesuviumportulacastrum L*. *Med. Pharmcol. Sci.* 15(7): 775 - 80

[5] Elizabeth, T., Aneesh, T. P., Della, G. T. and Anandam, R. 2013. GC-MS analysis of phytochemical compounds present in the rhizomes of *Nerviliaaragoana* GAUD. *Asian J Pharm Clin Res.* 6: 68 - 7.

[6] EncarnacionDimayuga, R. and Keer Garcia S. 1991. Antimicrobial screening of medicinal plants from Baja California Sur, Mexico. *J Ethnopharmacol*. 31(2): 181 - 92.

[7] Farid, M. M., Hussein, S. R., Ibrahim, L. F., Desouky, M. A., Elsayed, A. M. and Oqlah, A. A. 2015. Cytotoxic activity and phytochemical analysis of Arum palaestinumBoiss. *Asian Pac J Trop Biomed.* 5(11): 944 - 7.

[8] Ganachari, M. S. and Shiv, K. 2004. Anti-ulcer properties of *Ziziphus jujube* Lam leaves extract in rats. *Journal of Natural Remedies*. 4: 103 - 108.

[9] Guo, S., Duan, J. A., Zhang, Y., Qian, D., Tang, Y., Zhu, Z. and Wang, H. 2015. Contents Changes of Triterpenic Acids, Nucleosides, Nucleobases, and Saccharides in

Jujube (*Ziziphusjujuba*) Fruit during the Drying and Steaming Process. *Molecules*. 20: 22329 - 22340.

[10] Gupta, R. B. Sharma, S. Sharma, J. R. and Goyal, R. 2004. Study on the physicochemical characters of fruits of some wild and cultivated forms/spp. (*Ziziphus spp*.). *Haryana Journal of Horticultural Sciences*. 33 (3/4): 167 - 169.

[11] Han, B.H. and Park. M.H. 1986. Studies on the sedative alkaloids from *Ziziphusspinosasemen* (seed). *SaengyakHakhoechi*. 16(4): 233 –238.

[12] Heikki, P. Kallio. 2018. Historical review on identification of Mesifurane, 2,5-Dimethyl-4-methoxy-3(2H)-furanone and its occurance in berries and fruits. J. Agri Food Chem. 66: 2553 – 2560.

[13] Katalin Nagy and Ioana-DariaTiuca. 2017. Importance of fatty acids in physiopathology of human body.

[14] Li, A. P., Ding, Y. P., Chen, J. H. and Zeng, J. X. 2013. The Bitter Substances Origin and Composition Analysis in Jujube Wine. *Journal of Chinese Institute of Food Science and Technology*. 7: 236 - 241.

[15] Li, J., Hang, F., Ding, S. and Liu, Y. 2014. Composition and Antioxidant Activity of Polysaccharides from *Jujuba* by Classical and Ultrasound Extraction. *International Journal of Biological Macromolecules*. 63: 150 - 153.

[16] Mangunwardoyo, W. and Deasywaty, T. 2012. Antimicrobial and Identification of Active Compound *Curcuma xanthoriza*Roxb. *International Journal Basic and Applied Sciences* IJBAS-IJENS.12(01): 69 - 78.

[17] Mukharjee, P.K., Mukharjee, K., Rajesh Kumar, M., Pal, M. and Saha, B. P. 2003. Evaluation of the wound healing Activity of Some Herbal Formulations. *Phytother. Res*. 17: 265 - 268.

[18] Mukhtar, H. M., Ansari, S. H., Ali, M. and Naved, T. 2004. New compounds from *Ziziphus vulgaris*. *Pharm. Biol*. 42(7): 508 - 511.

[19] Ogihara, Y., Inoue, O., Otsuka, H., Kawai, K. I., Tanimura T. and. Shibata. S. 1976. Droplet counter current chromatography for the separation of plant products. *Journal of Chromatography*. 128(1): 218 – 223.

[20] Pareek, O. P. 2002. Ber-ziziphus Mauritania. Available by International centre for underutilized crops (online). Available: http://www. civil.soton.ac.uk/icuc/cd-icuc-ber-tamarind/content/ber/ber-book-html via the INTERNET. Accesed 2004 Apr. 6.

[21] Prakash, T., Fadadu, S. D., Sharma, U. R., Surendra, V., Stamina, P. and Kotresha, D. 2008. Hepatoprotective activity of leaves of *Rhododendron arboreum* in CCl4 induced hepatotoxicity in rats. *J Med Plants Res*. 2(11): 315 - 20.

[22] Rezk, A., Nolzen, J., Schepker, H., Albach, D. C., Brix, K. and Ullrich, M. S. 2015. Phylogenetic spectrum and analysis of antibacterial activities of leaf extracts from plants of the genus *Rhododendron*. *Complement Altern Med.* 15 (67): 11 – 12.

[23] Sermakkani, M. and Thangapandian, V. 2012. GC-MS analysis of *Cassia italica* leaf methanol extract. *Asian Journal of Pharmaceutical and Clinical Research*. 5(2): 90-4.

[24] Sistla, R., Tata, V. S. S. K., Kashyap, Y. V., Chandrasekar, D. and Diwan, P.V. 2005. Development and validation of a reversed-phase HPLC method for the determination of ezetimibe in pharmaceutical dosage forms. *J Pharm Biomed Anal*. 39: 517 - 522.

[25] Sonar, P. K., Singh, R., Verma, A. and Saraf S. K. 2013. *Rhododendron arboreum* (Ericaceae): Immunomodulatory and related toxicity studies. *OPEM*. 13(2): 127 - 31.

[26] Su, B. N., Farnsworth, N. R., Fong, H. H., Pezzuto J. M. and Kinghorn. A. D. 2002. Activity-guided fractionation of the seeds of Ziziphusjujuba using a cyclooxygenase-2 inhibitory assay. *Planta Med.* 68: 1125 - 1128.

[27] SundaramoorthyMaheshwari, KalyanaramanRajagopal, Kumar Lokesh, Meenambika, S. S. and ArulmathiRamalingam. 2018. 1-Eicosane, A hydrocarbon from curvularialutana an endophyticfungai isolated from the bark tissue of *Ficusreligiosa*. Research Journal of Pharm and Tech. 11(12): 5297 – 5303.

[28] Wojdylo, A., Carbonell-Barrachina, A. A., Legua, P., Hernandez, F. 2016. Phenolic Composition, Ascorbic Acid Content, and Antioxidant Capacity of Spanish Jujube (*Ziziphus jujube* Mill.) Fruits. *Food Chemistry*. 201: 307 - 314.

[29] Yakubu, O. E., OlawaleOtitoju and Joshua Onwuka. 2017. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of Aqueous Extract of *Danielliaoliveri* Stem Bark. *Pharm Anal Acta*. 8(11): 1 - 8.