INVESTIGATION OF LIPOIDIAL CONTENTS AND THEIR ANTI MICROBIAL ACTIVITY OF FORSSKAOLEA VIRIDIS AND TRICHODESMA EHRENBERGII WILDLY DISTRIBUTED IN EGYPT

Taha A.I. El Bassossy, Fatma Ali Ahmed
Medicinal and Aromatic Plants Department, Desert Research Center, Cairo, Egypt. 1st Matariya Museum, Desert Research Center, Cairo, Egypt.

ABSTRACT
Objective: The aim of this work was to assess the antimicrobial activity and investigation of lipoidal contents of F. viridis and T. ehrenbergii wildly distributed in Gebel Elba, Southeast of Egypt for the first time.

Methods: The phytochemical investigation of the ether extracts of F. viridis and T. ehrenbergii carried out by saponification of two lipoidal extracts and using gas chromatography (GC) with reference standards. The antimicrobial activity of the ether extract was performed as in vitro studies by diffusion agar technique for selected +ve and –ve Gram bacterial and fungal strains with reference used drug as a control.

Results: The findings of this study revealed that the two lipoidal extracts have sufficient steroidal and fatty acid methyl ester compounds where F. viridis contain (22) hydrocarbons, (6) sterols and (14) fatty acid methyl esters while, T. ehrenbergii contain (20) hydrocarbons, (5) sterols and (17) fatty acids where β-amyrin, stigmasterol and palmitic and Tricyclic acid were the major concentration of steroidal and fatty acid methyl ester contents of F. viridis and T. ehrenbergii respectively. The lipoidal extract of F. viridis and T. ehrenbergii exhibited moderate antimicrobial activity against all tested strains as compared to reference used drug.

Conclusion: It can be elicited that the etheeral extracts of two plants have moderate antimicrobial activity against selected strains.

Keywords: Antimicrobial, F. viridis, Lipoidal extract, T. ehrenbergii.

Article Info: Received 20 November 2020; Revised 11 December; Accepted 3 January, Available online 15 January 2021

Cite this article- El Bassossy TAI, Ahmed FA. Investigation of lipoidal contents and their anti microbial activity of Forskkaolea viridis and Trichodesma ehrenbergii wildly distributed in Egypt. Universal Journal of Pharmaceutical Research 2020; 5(6):43-48. DOI: https://doi.org/10.22270/ujpr.v5i6.511

Address for Correspondence:
Taha A.I. El Bassossy, Medicinal and Aromatic Plants Department, Desert Research Center, Cairo, Egypt. Tel- 00201000028656; E-mail: tahachemist2008@gmail.com

INTRODUCTION

Herbal plants have been recognized and used in the human history. Plants make many chemical compounds that have many biological activity, such as protection against insects, fungi. The use of plants as medicine exist before written history of the human. Most of the herbs and spices used by man in food and useful therapeutic compounds. Forskkaolea is a small genus in the Urticaceae family, represented by 6 species, distributed in over the world. Trichodesma ehrenbergii is a small genus in Boraginaceae family where, is an annual erect herb, 15-45 cm high, densely short hairy. Lipid compounds (sterols, terrenes, free fatty acids, esters of fatty acids) have antimicrobial activity where, the efficacy of these lipids over microorganisms is related to their chemical structure. Where, saturated compounds are effective against microorganisms at lower chain lengths, while unsaturated compounds with longer chain lengths are more active. The position of double bonds is important for long chain fatty acids. The therapeutic use of lipoidal compounds, with particular regard to topical applications for the treatment of bacterial or fungal infections. The survey on the previous studies on the F. viridis and T. ehrenbergii plants showed no chemical and biological studies performed on it so, current study aimed to investigate the chemical constituents in addition to their biological activity in our previous studies. Because of isolation and identification of some of active chemical constituents and their biological activity of two plants as hepatoprotective, antimicrobial, antitumor and antioxidant activity of different solvents extracts. It was decided to complete this study chemical investigations and antimicrobial activity. In this study on two lipoidal fractions of the two plants to obtain a complete chemical and biological profile of two
important plant species of two different families from the same location of Gebel Elba, Haliab, Southeast of Egypt.

MATERIALS AND METHODS

Plant Material
The plant parts of *F. viridis* and *T. ehrenbergii* were collected from their wild habitat in wadi kanthesrob, sarmati, Gebel Elba region, southeast corner of Egypt. The plant specimens were identified by Dr. Omran Ghaly, a researcher of plant taxonomy, department of Plant Ecology and Ranges, authenticated and deposited in the herbarium of Desert Research Center.

Preparation of lipoidal matter
The dried powder of *F. viridis* and *T. ehrenbergii* aerial portions (250 g) were exhaustively extracted separately by petroleum ether: di ethyl ether (1:1) using Soxhlet continuous extraction until exhaustion. The solvent was evaporated at 40°C under reduced pressure to give 24 g and 26 g residue of lipoidal matter7,11.

Preparation of the Unsaponifiable Matter
Total 3 g of lipoidal matter of two plants were saponified by refluxing in soxhlet apparatus with 50 ml of 10% alcoholic KOH for 6 hr followed by evaporating the alcohol, diluting with distilled water and extracting with ether exhaustively. The all combined ethereal extracts were cleaned with distilled water till being get rid of alkalinity, then dried over Na₂SO₄ and then concentrated to give 1.5 g unsaponifiable matter (USM) residue7,11.

Preparation of saponifiable matter (fatty acids)
The remaining saponifiable basic (alkaline) aqueous layer left afterward withdrawal of unsaponifiable matter with ether was acidified with 2N HCl to release the free fatty acids, and then extracted more times with di ethyl ether solvent. Then the ether portions were washed away more times with dist. H₂O until neutralization, dried above anhydrous Na₂SO₄. The residual were kept for analysis the fatty acid contents12.

Preparation of fatty acid methyl esters
The preparation of methyl esters of free fatty acids (0.6 g) was carried out by refluxing with 100 ml 99.9% MeOH and 5 ml H₂SO₄ for 2 hr. The major part of alcohol was distilled off and the residue was solubilized with distilled water and then extracted more times with ether. The collected fractions were washed with dist. H₂O, till free from any acidity then drying the ethereal layer and the rest part was dehydrated over anhydrous Na₂SO₄ then evaporate the ether extract to give residue of the fatty acid methyl esters and kept for GC analysis12.

GC analysis of the lipoidal matter conditions:
The saponifiable and unsaponifiable matter of aerial parts of the plant was carried by method described in13. Using GC Hewlett Packard hp 6890 Series Agilent Gas Chromatograph. Authentic samples according to the apparatus library from C₁₀ to C₃₂. With Capillary column hp-5 (5% diphenyl-95% dimethyl polysiloxane, 150 mm x 4mm), 2 ml/min of chart speed 80/280°C for initial/Final time for 25 minutes.

Antimicrobial Activity
Antimicrobial activity of the two lipoidal extracts was determined by diffusion agar technique in Regional Center for Mycology and Biotechnology Al-Azhar university, Cairo, Egypt (RCMB) according to CLSI13,14. Bacterial and fungal strains were obtained from the bacteria stock existing at RCMB. Petri dishes comprising on 20 ml of Nutrient (for bacteria) or Malt extract (for fungi), Agar medium was seeded with 1-3 day cultures of microbial inoculums (standardized inoculums 1-2X10⁷ cfu/ml 0.5 Mcfarland standard). Wells (6 mm in diameter) were cut off into agar and 100μl of the two plant extracts were tested in a concentration of 5mg/ml and incubated at 37°C for 24 h (bacterial strains) and at 25°C for 7 days (fungal strains). The assessment of antimicrobial activity was built on account of the diameter of the inhibition zone formed around the well. Ketoconazole with MIC 100 mg/ml was used for fungi positive control while, Gentamycin with MIC 4 mg/ml was used for bacteria strains positive control.

RESULTS AND DISCUSSION

Investigation of saponifiable matter using GC
The data recorded in Table 1: revealed that, there were 22 hydrocarbons beside 6 sterols and 20 hydrocarbons beside five sterols compounds were detected where, β-amyrin followed by β-sitosterol and stigmasterol followed by cholesterol were represented the major concentration of the sterols for *F. Viridis* and *T. ehrenbergii* ethereal extract respectively, the high concentration of the phytosterols in the lipoidal extracts may be related to their lipid absorption inside the cell membrane of the plant through converting the lipoidal matters to constituents which have sterols chemical structures, where they acts a dynamic role in cell membrane structure and used as a precursor to steroid hormones and fat-soluble vitamins (A, D, E, K)15. The high relative percent of β-amyrin and stigmasterol earned *F. viridis* and *T. ehrenbergii* plants some medicinal importance, where previous studies showed activity of β-amyrin and stigmasterol as human bladder cancer, skin epidermoid, anticancer, anti microbial, anti-inflammatory, and breast cancer16,17. Also it can be a probable effective compound for drug development in diabetes and atherosclerosias β-amyrin and stigmasterol have prospective antihyperglycemic and hypolipidemic effects18. While, the relatively high percent of β-sitosterol and cholesterol in the lipoidal extract of *F. viridis* plays a vital role in therapeutic drugs used for improving sexual activity, relieving symptoms of menopause, lowering of high bad blood cholesterol level and treating benign prostatic hyperplasia by reducing the quantity of cholesterol absorbed by the body. Also, used for improving the immune system and for avoiding colon cancer and in synthesis of cortisone as well as for gallstones19,20.

Investigation of saponifiable matter using GC:
The fatty acids methyl esters results represented in Table 2: indicated that, there were 14 fatty acid methyl ester, 10 saturated beside 4 unsaturated and 16 fatty acid methyl ester, 13 saturated beside 4 unsaturated of
both plants *F. viridis* and *T. ehrenbergii* saponifiable extracts respectively, the investigation of saponifiable contents showed that the palmitic and oleic acid were major concentrations of saturated and unsaturated fatty acids methyl ethers of *F. viridis* respectively.

### Table 1: Hydrocarbons and sterols determined of *F. viridis* and *T. ehrenbergii* using GC.

| No. of C atom | RT  | Name                  | M. F.   | *F. viridis* Area (%) | *T. ehrenbergii* Area (%) |
|---------------|-----|-----------------------|---------|------------------------|---------------------------|
| C13           | 9.791  | n-Tridecane          | C₁₃H₂₈  | 0.421                  | 0.596                     |
| C14           | 10.755 | n-Tetradecane        | C₁₄H₂₉  | 0.793                  | 2.357                     |
| C15           | 12.060 | n-Pentadecane        | C₁₅H₃₀  | 1.665                  | 12.220                    |
| C15:1         | 12.879 | n-Pentadecene-1     | C₁₅H₃₀  | 1.048                  | 6.439                     |
| C16           | 13.457 | n-Hexadecane         | C₁₆H₃₂  | 7.370                  | 14.974                    |
| C17           | 13.884 | n-Heptadecane        | C₁₇H₃₃  | 4.146                  | 5.519                     |
| C17:1         | 14.386 | n-Heptadecene-1     | C₁₇H₃₈  | 4.513                  | 14.920                    |
| C18           | 14.869 | n-Octadecane         | C₁₈H₃₄  | 15.309                 | 3.351                     |
| C18:1         | 15.767 | n-Octadecene-1      | C₁₈H₃₈  | 4.580                  | 3.003                     |
| C19           | 16.129 | n-Nonadecane         | C₁₉H₄₀  | 12.599                 | 0.596                     |
| C19:1         | 16.524 | n-Nonadecene-1      | C₁₉H₄₈  | --                     | 0.829                     |
| C20           | 17.015 | n-Eicosane           | C₂₀H₴₂  | 2.811                  | 0.409                     |
| C21           | 17.832 | n-Heneicosane        | C₂₁H₴₄  | 2.959                  | --                        |
| C22           | 17.975 | n-Docosane           | C₂₂H₴₆  | 0.956                  | 0.506                     |
| C23           | 18.953 | n-Tricosane          | C₂₃H₴₸  | 0.707                  | 0.456                     |
| C24           | 21.090 | n-Tetracosane        | C₂₄H₅₀  | 0.541                  | 0.344                     |
| C24:1         | 21.738 | n-Tetracosene-1     | C₂₄H₅₈  | 0.562                  | --                        |
| C25           | 22.086 | n-Pentacosane        | C₂₅H₅₂  | 0.627                  | 0.563                     |
| C26           | 23.068 | n-Hexacosane         | C₂₆H₅₄  | 1.741                  | --                        |
| C27           | 23.616 | n-Heptacosane        | C₂₇H₅₆  | 1.354                  | 0.563                     |
| C28           | 24.913 | n-Octacosane         | C₂₈H₆₈  | 4.642                  | 0.174                     |
| C28:1         | 25.464 | n-Octacosene-1      | C₂₈H₆₈  | --                     | 0.303                     |
| C29           | 26.729 | n-Nonacosane         | C₂₉H₆₀  | 2.275                  | 1.004                     |
| C30           | 29.063 | n-Triacontane        | C₃₀H₆₂  | 4.714                  | 1.359                     |

| No. of C atom | RT  | Name                  | M. F.   | *F. viridis* Area (%) | *T. ehrenbergii* Area (%) |
|---------------|-----|-----------------------|---------|------------------------|---------------------------|
| C:27          | 30.239 | Cholesterol          | C₂₇H₅₈O | 2.750                  | 6.450                     |
| C:28          | 32.055 | Campesterol           | C₂₈H₅₀  | 3.211                  | 2.797                     |
| C:29          | 34.228 | Stigmasterol          | C₂₉H₅₂  | 2.612                  | 13.575                    |
| C:29:1        | 35.138 | β-Sitosterol          | C₃₀H₅₄  | 3.956                  | 4.890                     |
| C:30          | 37.168 | γ-Amyrin              | C₃₀H₅₀  | 3.652                  | 1.894                     |
| C:30:1        | 38.734 | β-Amyrin              | C₃₀H₅₀O | 4.978                  | ----                      |

*RT = Retention time, M.F. = Molecular formula*

### Table 2: Saponifiable matter (fatty acids) of *F. viridis* and *T. ehrenbergii* using GC.

| No. of C atom | Systemic name | Trivial name | RT  | *F. viridis* Area (%) | *T. ehrenbergii* Area (%) |
|---------------|---------------|--------------|-----|------------------------|---------------------------|
| C:10          | Decanoic acid | Capric acid  | 8.562 | 4.403                  | ---                        |
| C:11          | Undecanoic acid | Undecylcic acid | 8.673 | 7.680                  | 3.723                     |
| C:12          | Dodecanoic acid | Lauric acid  | 9.398 | 2.351                  | 15.102                    |
| C:13          | Tridecanoic acid | Tridecylcic acid | 11.018 | ---                  | 22.140                    |
| C:14          | Tetradecanoic acid | Myristic acid | 12.657 | 2.400                  | 6.084                     |
| C:15          | Pentadecanoic acid | Pentaenylcic acid | 14.094 | ---                  | 1.062                     |
| C:16          | Hexadecanoic acid | Palmitic acid | 15.605 | 29.482                 | 16.225                    |
| C:17          | Heptadecanoic acid | Margaric acid | 17.522 | 2.060                  | 2.540                     |
| C:18          | Octadecanoic acid | Stearic acid  | 18.685 | 7.190                  | 4.639                     |
| C:18:1        | Cis-9-Octadecanoic acid | Oleic acid | 19.258 | 21.073                 | 2.589                     |
| C:18:2        | Cis, cis-9, 12-Octadecanoic acid | α-Linoleic acid | 20.440 | 5.211                  | 3.160                     |
| C:18:2        | Trans, trans-9, 12-Octadecanoic acid | Linolealicyclic acid | 21.697 | 6.350                  | ---                        |
| C:18:3        | All Cis-9, 12, 15-Octadecatrienonic acid | γ-Linoleic acid | 22.523 | 6.701                  | 3.177                     |
| C:19          | Cis-10-Nonadecanoic acid | Nonadecanoic acid | 4.146 | ---                     | ---                        |
| C:20          | Eicosanoic acid | Arachidic acid | 23.346 | 0.512                  | 0.842                     |
| C:22          | Docosanoic acid | Behenic acid  | 24.316 | ---                     | 4.163                     |
| C:24          | Tetracosanoic acid | Lignoceric acid | 26.985 | 0.355                  | 4.885                     |
| C:26          | Hexacosanoic acid | Ceric acid  | 28.293 | ---                     | 0.655                     |
| C:27          | Heptacosanoic acid | Carboxeric acid | 29.605 | ---                     | 8.485                     |
Tridecyclic and γ-Linoleic revealed the major percent for saturated and unsaturated fatty acid of *T. ehrenbergii* respectively. The essential fatty acids have great value where, they give the body healthy value as contrary to what was previously believed where, converted in the body by enzymes into long chain polyunsaturated fatty acids (LCPUFAs). Where γ-linolenic acid (ω-6) which needed for the maintenance of hormonal balance and healthy skin structure. The presence of essential unsaturated fatty acids in both plants, linoleic acid (ω-6 trans fatty acid), (ω-9) oleic acid, (ω-3)α-linolenic and (ω-6) γ-linolenic acid refers to the importance of the two plants as a source of all ω-3, ω-6 and ω-9 fatty acids as nutritional fats where, each acid of them has a great value in health benefits in the body by right equilibrium between them, where the imbalance between them may cause a number of chronic diseases. Oleic acid (ω-9) represented as non-essential fats, subsequently; they can be manufactured by the body. The high relatively percent of (ω-9) can qualify the plant to use as reducing agent of plasma triglycerides by 19% and very-low-density-lipoprotein cholesterol by 22% in patients with diabetes, enhanced insulin sensitivity and reduced inflammation. The relatively high percent of (ω-3) and (ω-6) may give more value of the plants for decreasing, blood pressure, liver fats, a number of symptoms of rheumatoid arthritis, triglycerides and the formation of arterial plaques, promoting of the bone health, preventing asthma. Otherwise, the two plants consists of high percent of saturated fatty acid, palmitic acid which has a vital role in cellular membrane functionality by improving their flexibility and permeability and it forms reversible links to cell membrane proteins, thus being involved in regulating the traffic of molecules in and out of cells and inter cells communication. Palmitic acid is then the precursor of palmitoyl ethanol amide (PEA) compound which formed by the body with anti-inflammatory, analgesic and neuroprotective activities.

**Table 3: Antimicrobial activity of lipoidal extract of *F. viridis* and *T. ehrenbergii*.**

| Tested Organism | Inhibition Zone Diameter (mm) |
|-----------------|-----------------------------|
|                 | Control | *F. viridis* | *T. ehrenbergii* |
| **Gram (+ve) Bacteria** | | |
| Gentamycin (MIC) 4 mg/ml (reference- drug) | | |
| *Micrococcus* sp. (RCMB 028) | 22 | 13 | 11 |
| *Streptococcus* mutants (RCMB017) (ATCC 25175) | 21 | 12 | 12 |
| Methicillin-Resistant *Staphylococcus aureus* | 15 | 11 | 13 |
| **Gram (-ve) Bacteria** | | |
| *Salmonella typhimurium* (RCMB 006) (ATCC 14028) | 17 | 10 | 10 |
| *Escherichia coli* (RCMB 010052) (ATCC 25955) | 30 | 13 | 11 |
| *Klebsiella pneumonia* (RCMB 003) (ATCC 13883) | 21 | 12 | 9 |
| **Filamentous Fungi** | | |
| Ketoconazole (MIC) 100 mg/ml (reference- drug) | | |
| *Aspergillus fumigatus* (RCMB 002008) | 17 | 2 | 7 |
| *Penicillium expansum* (RCMB 001001) | 17 | NA | 8 |
| **Yeasts** | | |
| *Candida albicans* (RCMB 005003) (ATCC 10231) | 20 | 1 | NA |
| *Cryptococcus neoformans* (RCMB 0049001) | 25 | 16 | 14 |

MIC = Minimum inhibitory concentration, NA= No activity, The sample was tested at mg/ml concentration

**Antimicrobial activity**

The antimicrobial activity of the lipoidal extract of *F. viridis* and *T. ehrenbergii* showed potent antibacterial activity against Gram (+) ve (*Methicillin-Resistant Staphylococcus aureus*) with activity 73% and 86% respectively, moderate activity against *Streptococcus mutants* and *Micrococcus sp.*) with activity 57,3, 57,3 and 59, 50%, respectively when compared with gentamicin as reference used drug. Also, it exhibited weak activity against all tested Gram (-) ve bacteria and there is no activity against tested filamentous fungi while, it exhibited moderate activity against yeasts fungi (*Cryptococcus neoformans*) with activity 64 and 56 % respectively, as compared to ketoconazole as used reference drug. The moderate activity of the lipoidal extract may be due to its phytosterols contents which characterized with antimicrobial activity and fat-soluble vitamins which have ability to inhibit the activity of micro-organisms and acts in cell membrane and DNA of microbial strains.
Figure 1: Inhibition zones of microbial activity of lipoidal extract of F. viridis and T. ehrenbergii.

From the previous obtained data the F. viridis show little improvement more than T. ehrenbergii as antimicrobial activity this is may be due to little changes in steroidal contents between them where the presence of β-Amyrin in F. viridis and absence in T. ehrenbergii. Also the high percent of stigmasterol in T. ehrenbergii may be act more activity against Penicillium expansum more than F. viridis. so we can say as general the two plants extract have moderate activity against some of tested strains as shown in Table 3.

CONCLUSION

The investigation of lipoidal contents of F. viridis and T. ehrenbergii using (GC) revealed that, of F. viridis contain 22 hydrocarbons, 6 sterols and 14 fatty acid methyl ester while T. ehrenbergii contain 21 hydrocarbons, 5 sterols and 16 fatty acid methyl esters. The in vitro antimicrobial studies showed that moderate antimicrobial activity of two plants against most Gram (-ve and + ve) bacteria while, weak and no activity of fungal strains while, the F. viridis showed little improvement than T. ehrenbergii.

ACKNOWLEDGEMENT

The authors are thankful to medicinal and aromatic plants department, DRC, Egypt for giving consent and all sorts of supports to conduct the research.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

AUTHOR’S CONTRIBUTION

All authors have worked equally for this work.

REFERENCES

1. Tapsell LC, Hemphill I, Cobiac L. Health benefits of herbs and spices: the past, the present, the future. Med J August 2006; 185. https://doi.org/10.5694/j.1326-5377.2006.tb00548.x
2. Kitikar KR, Basu BD, Singh B, Singh M. An ICS Indian Medical Plants, New Delhi, Second edition, III 1975: 2291-2298.
3. Alfarhan AH, Al-Turky TA, Basahy AY. Flora of Jizan Region. Vol. 1, King Abdulaziz City for Science and Technology (KACST) 2005.
4. Alfarhan AH, Al-Turky TA, Basahy AY. In “Flora of Jizan Region”, Final Report Supported by King Abdulaziz City for Science and Technology 2005; 1: 545. https://dx.doi.org/10.1008/16585355.2019.165477
5. Alves E, Dias M, Lopes D, Almeida A, Domingues MR, Rey F. Antimicrobial lipids from plants and marine organisms: An overview of the current state of the art and future prospects. Antibiotics 2020; 9:441. https://doi.org/10.3390/antibiotics9080441
6. McGaw LJ, Jager AK, Staden JV. Antibacterial effects of fatty acids and related compounds from plants. South African J Botany 2002; 68: 417-423.
7. Ahmed FA, El-Mesallamy AMD, El-Bassossy TAI. Phytochemical analysis and biological evaluation of Forsskaolea viridis aerial parts. Acta Polonae Pharmacuetica- Drug Res 2019; 76(5): 815-823. https://doi.org/10.32383/appdr/1085519
8. Ahmed FA, El-Bassossy TAI. Active constituents and biological activity of methanolic extract of Forsskaolea viridis aerial parts. Asian J Pharm Clin Res 2020; 13 (3): 40-46.https://doi.org/10.22159/ajpcr.2020.v13i3.36503
9. Ahmed FA, El-Mesallamy AMD, El-Bassossy TAI. Hepatoprotective and antitumor activity of phenolic content of Trichodesma ehrenbergii Schiewienf. ex Boiss aerial parts. World J Pharm Med Res 2016; 2(4): 119-125.
10. El-Mesllamy AMD, Ahmed FA, El-Hawe MH, Ibrahim TA. Chemical investigation and evaluation of antimicrobial activity of Trichodesma ehrenbergii Schiewienf. ex Boiss. growing at Gebel Elba region. Indo American J Pharm Sci 2015; 2(5): 947-953.
11. Johnson AR, Davenport JB. Biochemistry and Methodology of Lipids. New York: John Wiley and Sons, Inc., 1971; 31-33.
12. El-Said FM, Amer MM. Oils, Fats, Waxes and Surfactants. Cairo, Anglo-Egyptian Bookshop 1965; 130-131. 
   https://doi.org/10.1017/S0007145909289082
13. Vogel AJ. A Text Book of Practical Organic Chemistry, 3rd ed. English Language Book Society and Longman Group Ltd., London 1975; 969-971. 
   https://doi.org/10.4236/ce.2019.104054
14. CLSI. Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved Standard, 7th ed., CLSI document M02-A11. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087, USA, 2012.
15. Kametani T, Furuyama H. Synthesis of vitamin D3 and related compounds. Med Res Rev 1987; 7 (2): 147-71. 
   https://doi.org/10.1002/med.2610070202
16. Wei H, Xinchi Y, Paula V. Sterol synthesis in diverse bacteria. Frontiers in Microbiology 2016; 7: 990-109. 
   https://doi.org/10.3389/fmicb.2016.00990
17. Csapi B, Zsuzsanna H, Zupkó I, Berényi A, Forgo P, Szabó P,Hohmann J. Bioactivity-guided isolation of antiproliferative compounds from Centaurea arenaria. Phytotherapy Research 2010; 24: 1664-69. 
   https://doi.org/10.1002/med.2610070202
18. Acevedo RH, Terrazas T, González ME., Guzmán Y, Hernández M. Anti-ulcer activity of Cyrtocarpa procera analogous to that of Amphipterygium adstringens, both assayed on the experimental gastric injury in rats. J Ethnopharmacol 2011; 134: 67-73. 
   https://doi.org/10.1016/j.jep.2010.11.057
19. Santos FA, Frota TJ, Arruda RB, et al. Antihyperglycemic and hypolipidemic effects of α, β-amyrin, a triterpenoid mixture from Protium heptaphyllum in mice. Lipids in Health and Disease 2012; 11: 98-106. 
   https://doi.org/10.1186/1476-511x-11-98
20. Soy Infocenter. History of Soybean and Soyfoods in Mexico and Central America. Extensively Annotated Bibliography and Sourcebook 2009; 1877.
21. Rakel DMD. Integrative Medicine, (4th ed.). Elsevier, 2018; 1096-1123.
22. Garg A. High-monounsaturated-fat diets for patients with diabetes mellitus: a meta-analysis. Am J Clin Nutr 1998; 67(3): 577S-582S. 
   https://doi.org/10.1093/ajcn/67.3.577s
23. Finucane OM, Lyons CL, Murphy AM, and Reynolds CM. Monounsaturated fatty acid-enriched high-fat diets impede adipose NLRP3 inflammasome-mediated IL-1β secretion and insulin resistance despite obesity. Diabetes 2015; 64(6): 2116-28. 
   https://doi.org/10.2337/db14-1098
24. Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother 2002; 56(8): 365-379. 
   https://doi.org/10.1016/s0753-3322(02)00253-6
25. Hesselink JM, Hekker TA. Therapeutic utility of palmitoyl ethanolamide in the treatment of neuropathic pain associated with various pathological conditions: a case series. J Pain Res 2012; 5: 437–442. 
   https://doi.org/10.2147/jpr.s32143
26. Hesselink JM, Hekker TA. Therapeutic utility of palmitoyl ethanolamide in the treatment of neuropathic pain associated with various pathological conditions: a case series. J Pain Res 2012; 5: 437–442. 
   https://doi.org/10.2147/jpr.s32143