International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 09-10-2020; Accepted: 24-10-2020: Published: 12-11-2020
www.botanyjournals.com
Volume 5; Issue 6; 2020; Page No. 178-182

Phytochemical screening and GC-MS analysis of *Syzygium Caryophyllatum* (L.) Alston

Amitha S Chandra[1*], Siril EA[2], Radhamany PM[3]
[1-3] Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram, Kerala, India

Abstract
Medicinal plants are the local heritage with global importance and have therapeutic properties because of the presence of different complex chemical substances of varying composition, which are found as secondary metabolites in one or more parts of the plants. These phytochemicals have the ability to produce a definite physiological action on the human body. Keeping this view in mind the current investigation was carried out in *Syzygium caryophyllatum* (L.) Alston belongs to the family Myrtaceae. It is a low altitude evergreen tree; where the stem bark is traditionally used in the treatment of diabetes mellitus. Qualitative phytochemical analysis of the bark confirms the presence of various phytochemicals like alkaloids, flavonoids, tannins, saponins, terpenoids and quinone. From the GC-MS analysis using methanol extract totally 35 compounds were identified. Among the identified compounds major bioactive compounds are 9,12,15-Octadecatrienoic acid, n-Hexadecanoic acid, β-Sitosterol, 9,12,15-octadecatrienoic acid, ethyl ester, Stigmasterol, Vitamin E, Phytol, acetate and Octadecanoic acid. These chemical compounds are considered biologically and pharmacologically important.

Keywords: GC-MS analysis, methanol, phytochemicals, *Syzygium caryophyllatum*

1. Introduction
The traditional systems of medicine in India such as Unani, Ayurveda, Homeopathy and Siddha prescribe plant-based drugs and therefore, the search for new herbal drugs and their sources became the need of the hour [1]. Determination of chemical constituents in plants which in turn are responsible for the medicinal activity of the plants also became necessary. Most of the natural compounds are unique. It is very difficult to synthesize them anew with the same constituents and cofigurations as they exist in nature. Also, the combination of secondary products in a particular plant is unique, which poffers the distinctive medicinal value to the plant concerned. Traditional therapy includes the use of plant extracts or their active principles [2]. These chemical constituents from plants may be therapeutically active or inactive also called inert chemicals, which are collectively called as phytochemicals or secondary metabolites [3]. Some of these phytochemicals are alkaloids, phenolics glycosides, flavonoids, steroids, terpenoids, aminoacids and glycopeptides which are present in vegetables, fruits, flowers, leaves, stem, bark and roots that work with and act as a defence system more accurately to protect against diseases [4]. *Syzygium caryophyllatum* (L.) Alston (Myrtaceae) is a low-altitude evergreen tree, popularly known as 'Wild black plum'. The tree is endemic to Sri Lanka and also distributed in the Western Ghats of India. Many species of Myrtaceae are used for several medicinal purposes. Among them, the most important species are *S. cumini*, *S. malaccense*, *S. aromaticum* and *S.trvancoricum.* The species of *Syzygium* are reported to possess antibacterial [5], antidiabetic [6], antifungal [7], antihyperglycemic and hypoglycemic activities [8], due to the presence of active metabolites such as alkaloids, phenolics, glycosides, flavonoids, steroids, terpenoids, aminoacids and glycopeptides. The bark of *S. caryophyllatum* is traditionally used for treating diabetes mellitus [9]. The extracts of leaf and bark have been reported to possess antibacterial and antioxidant efficacies [10, 11]. The bark extract was used in veterinary medicine for the treatment of tympanitis in cattles [12].
The screening of plant extracts for finding therapeutically important compounds is an innovative method, which will help to develop novel drugs. Gas Chromatography–Mass Spectrometry (GC-MS) technique is used for the analysis of bioactive compounds in traditional medicine and for separation and identification of multicomponent mixtures such as essential oils, hydrocarbons etc [13]. Till now, the investigation of phytocomponents by GC-MS has not been done on the species. Taking into consideration of its medicinal importance, the present study deals with phytochemicals and GC-MS analysis of methanolic extract of *S. Caryophyllatum*, to ascertain the medicinal aspects of the plant.

2. Materials and Methods
2.1. Plant material
Fresh bark of *S. Caryophyllatum* collected from Thiruvananthapuram district of Kerala, India was used. Voucher specimen (KUBH 10255) was prepared, identified and deposited at the Herbarium of Department of Botany, University of Kerala.

2.2. Preparation of plant extract
The collected bark was chopped and shade dried under room temperature. After shade drying, packed in brown cover and kept in an oven at 50ºC for an hour to make grinding easy. After an hour, the barks were milled into coarse powder by the mechanical grinder. About 12 gm of the powder was subjected to Soxhlet extraction using different organic solvents like hexane, ethylacetate, acetone, methanol and aqueous for 6 hours. Filtered extract was concentrated using rotary evaporator under reduced pressure. Dried extracts were stored in 4°C until further use.

2.3. Phytochemical screening

As per the standard methods [14, 15] the phytochemical screening of *S.caryophyllatum* bark was done with four different extracts *viz.*, hexane, ethyl acetate, acetone methanol and aqueous.

2.4 GC-MS analysis

The analysis of the methonolic extract of bark was performed using GC-MS HP 7890 GC instrument integrated with an Agilent 5975C MSD mass spectrometer (Aligent, Santa Clara, CA, USA).The capillary column was an Agilent HP-5MS with 30 m length, 0.25mm diameter and 0.25µm film thickness. The helium (Purity > 99.999%) was used as the carrier gas, and the flow rate was 1 ml/min. The injector temperature was 250^0C, and the injection mode was splitless. The G.C oven temperature was held at 50^0C for 5min,which was increased to 210^0C at a rate of 3^0C/min, maintained at 210^0C for 3 min, and finally increased to 230^0C at 15^0C/min.1µl of the sample was injected into the column For GC–MS detection, electron ionization energy of 70eV was used.

Interpretation on mass specrum of GC-MS was done using National Institute Standard and Technology (NIST) library database having more than 62,000 patterns. The mass spectrum of the separated unknown component was compared with the spectrum of the known components stored in the NIST library [16]. The name, retention time, abundance and molecular weight of the test materials were ascertained.

3. Result

S. caryophyllatum bark extracts (hexane, ethylacetate, acetone, methanol and aqueous) were prepared for the preliminary phytochemical analysis to identify the bioactive constituents such as alkaloids, coumarins, flavonoids, phenols, terpenoids, saponins, tannins, reducingsugars, carbohydrates, quinones, steroids, iridoids and anthocyanins. The results of various phytochemical analysis are given in the Table 1. The methanolic extract showed positive result for majority of the phytyochemicals except reducing sugar, carbohydrate, iridoids and anthocyanins. The ethylacetate and aqueous extract of bark showed the presence of glycosides, phenols, terpenoids, tannins, quinones and anthocyanins. In hexane extract absence of all phytochemicals. Phytochemical test using acetone extract showed positive result similar to that of methanolic extract except for alkaloids, coumarins and saponins.

The methanolic extract of *S.caryophyllatum* (bark) on GC-MS analysis showed thirty five peaks indicating the presence of thirty five compounds in the sample as shown in figure 1. The molecular formula, the molecular weight, the retention time and the percentage constituents of the compounds are shown in Table 2. The compounds such as 1,2,3-benzenetriol, Phytol, acetate, Cyclopentane, pentyl-,1-Hexadecyne, trans-2-Undecen-1- ol, n-Hexadecanoicacid, Phytol, 9,12,15-Octadecatrienoic acid, 9,12-Octadecadienoic acid (Z, Z)-, methyl ester, Octadecanoic acid, 1,19-Eicosadiene, Bicyclo [11.3.0] hexadecane-2,14dione, 2-Dodecylcyclobutanone, Estragol, Octadecanoicacid, 2,3-dihydroxy propylester, Dibenzo (2, 3:10,11) perylo (1,12-bcd) thiophene, VitaminE, 4-Ethenyl-2-methoxyphenol, Heneicosanoic acid, 9,12,15-octadecatrienoic acid, ethyl ester, 1-Octadecyne, Stigmasterol, Heptacosane, 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol, 17- (1,5-Dimethylhexyl) -10,13-dimethyl-4-vinylhexadecahydrocyclopenta [a] phenanthren-3-ol, Alpha amyrin, Stigmasteryltosylate, gamma Tocopherol, Stigmastan-3,5-diene, Campestrol, Squalene, β-Sitosterol, Eicosane, 7-Hexyl, Urs -12-en-28-oic acid,3-hydroxy-,methyl ester, (3β)- were identified.

4. Discussion

Phytochemical analysis conducted on the bark extract has revealed the presence of phytochemicals considered as active medicinal chemical constituents. Myrtaceae plants, especially genus *Syzygium* have been used for several disorders traditionally as well as scientifically [17, 18, 19, 20, 21, 22]. It is due to the presence of non-nutritive plant phytochemicals such as alkaloids, phenolics, glycosides, flavonoids, steroids, terpenoids, tannins and saponins. These phytochemicals have been found to possess a wide range of activities in the biological system. Several workers have reported the analgesic [23], antispasmodic and antibacterial properties of alkaloids [24, 25]. The phenolics such as flavonoids, tannins are the group of compounds that acts as primary antioxidant or free radical scavengers due to their electron donating properties [26].Tannins have exhibited antiviral, anti-microbial and anti-tumour, antidiabetic and antilipogenic activities [27]. Flavonoids are referred as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies, anti-inflammatory, anti-microbial, anti-cancer activities [28]. In addition, flavonoids exhibited potent antihyperglycemic activity in animal studies [29, 30]. GC MS profile is firstly repored from this. The first active compound 1,2,3-benzenetriol was identified in less RT (10.751)with peak area1.33 %, and the last compound Urs-12-en-28-oic acid, 3-hydroxy-, methyl ester, (3β)- was identified in much longest RT (31.461) with peak area 0.86%. Major compounds detected were 9, 12, 15-Octadecatrienoic acid (20.74%), n-Hexadecanoic acid (18.41%), β-Sitosterol (6.38%), 9, 12, 15-octadecatrienoic acid, ethyl ester (4.40%) etc. The identified compounds possess many biological properties such as n-hexadecanoic acid possesses following properties: Anti-inflammatory, antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic, 5-alpha-reductase inhibitor, and potent mosquito larvicide [31]. Diterpene alcohol, phytol that have strong positive effects on insulin level in the body [32, 33]. The presence of stigmasterol and campesterol impart antioxidant and antimicrobial activities and prevent the fatty liver syndrome by lowering the cholesterol level. Stigmasterol is also a precursor of anabolic steroid boldenone and Vitamin D3 [34]. Oliveira *et al*. (2005) reported the hepatoprotective potential of alpha- and beta-amyrin against toxic liver injury [35]. Vitamin E, plays an important role in various stages of carcinogenesis, DNA repair and decreasing oxidative DNA damage [36].

Table 1: Preliminary phytochemical screening of *Syzygium caryophyllatum* bark

Phytochemical Constituents	Hexane	Ethylacetate	Acetone	Methanol	Aqueous
Alkaloids	-	-	-	+	+
Coumarins	-	-	-	+	-
Flavonoids	-	-	+	+	-
Glycosides	-	+	+	+	+
Phenols	-	+	+	+	+
Terpenoids	-	+	+	+	+
Saponins	-	-	-	+	-
Tannins	-	+	+	+	+
Reducing sugars	-	-	-	-	-
Carbohydrates	-	-	-	-	-
Quinones;	-	+	+	+	+
Iridoids	-	-	-	-	-
Steroids	-	+	+	+	+
Anthocyanins	-	+	+	-	-

+ indicates presence; - indicates absence

Table 2: GC-MS analysis of methanolic bark extract of *Syzygium caryophyllatum*

Peak No	R. Time	Name of Chemical Compounds	Molecular formula	Molecular Weight (g/mol)	Area %
1	10.751	1,2,3- benzenetriol	$C_{26}H_{18}ClN_3O_3$	455.9	1.33
2	15.223	Phytol, acetate	$C_{22}H_{42}O_2$	338.6	3.14
3	15.336	Cyclopentane, pentyl-	$C_{10}H_{20}$	140.27	0.52
4	15.621	1-Hexadecyne	$C_{16}H_{30}$	222.41	0.53
5	15.889	trans-2-Undecen-1-ol	$C_{11}H_{22}O$	170.29	0.92
6	17.363	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	18.41
7	18.636	Phytol	$C_{20}H_{40}O$	296.5	2.20
8	19.111	9,12,15-Octadecatrienoic acid	$C_{18}H_{30}O_2$	278.4	20.74
9	19.183	9,12-octadecadienoic acid (Z, Z)-, methyl ester	$C_{19}H_{34}O_5$	342.5	2.43
10	19.284	Octadecanoic acid	$C_{18}H_{36}O_2$	284.5	3.06
11	20.544	1,19-Eicosadiene	$C_{20}H_{38}$	278.5	0.59
12	20.747	Bicyclo[11.3.0]hexadecane-2,14-dione	$C_{16}H_{26}O_2$	250.38	0.69
13	21.323	2-Dodecylcyclobutanone	$C_{16}H_{30}O$	238.41	2.29
14	22.013	Estragol	$C_{10}H_{12}O$	148.2	1.33
15	22.120	octadecanoic acid,2,3-dihydroxypropyl ester	$C_{21}H_{45}BO_7$	420.4	1.09
16	22.334	Dibenzo(2,3:10,11)perylo(1,12-bcd)thiophene	$C_{28}H_{14}S$	382.5	1.81
17	22.536	Vitamin E	$C_{29}H_{50}O_2$	430.7	3.22
18	23.119	4-Ethenyl-2-methoxyphenol	$C_9H_{10}O_2$	150.17	3.45
19	23.393	Heneicosanoic acid	$C_{21}H_{42}O_2$	326.6	1.34
20	23.529	9,12,15-octadecatrienoic acid, ethyl ester	$C_{20}H_{34}O_2$	306.5	4.40
21	24.082	1-Octadecyne	$C_{18}H_{34}$	250.5	1.18
22	24.475	Stigmasterol	$C_{29}H_{48}O$	412.7	3.21
23	24.671	Heptacosane	$C_{27}H_{56}$	380.7	0.82
24	25.260	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180.2	3.65
25	25.337	17-(1,5-Dimethylhexyl)-10,13-dimethyl-4-vinylhexadecahydrocyclopenta[a]phenanthren-3-ol	$C_{29}H_{50}O$	414.7	0.49
26	25.676	Alpha amyrin	$C_{30}H_{50}O$	426.7	0.99
27	25.813	Stigmasteryl tosylate	$C_{36}H_{54}O_3S$	566.9	0.53
28	25.926	gamma-Tocopherol	$C_{28}H_{48}O_2$	416.7	0.59
29	26.211	Stigmastan-3,5-diene	$C_{29}H_{48}$	396.7	2.30
30	26.449	Vitamin E	$C_{18}H_{35}NO$	281.4	0.73
31	27.222	Campestrol	$C_{28}H_{48}O$	400.7	1.55
32	27.436	Squalene	$C_{30}H_{50}$	410.7	2.50
33	27.989	β-Sitosterol	$C_{29}H_{50}O$	414.7	6.38
34	28.096	Eicosane,7- Hexyl	$C_{26}H_{54}$	366.7	0.74
35	31.461	Urs-12-en-28-oic acid, 3-hydroxy-, methyl ester, (3β)-	C31H50O3	470.7	0.86

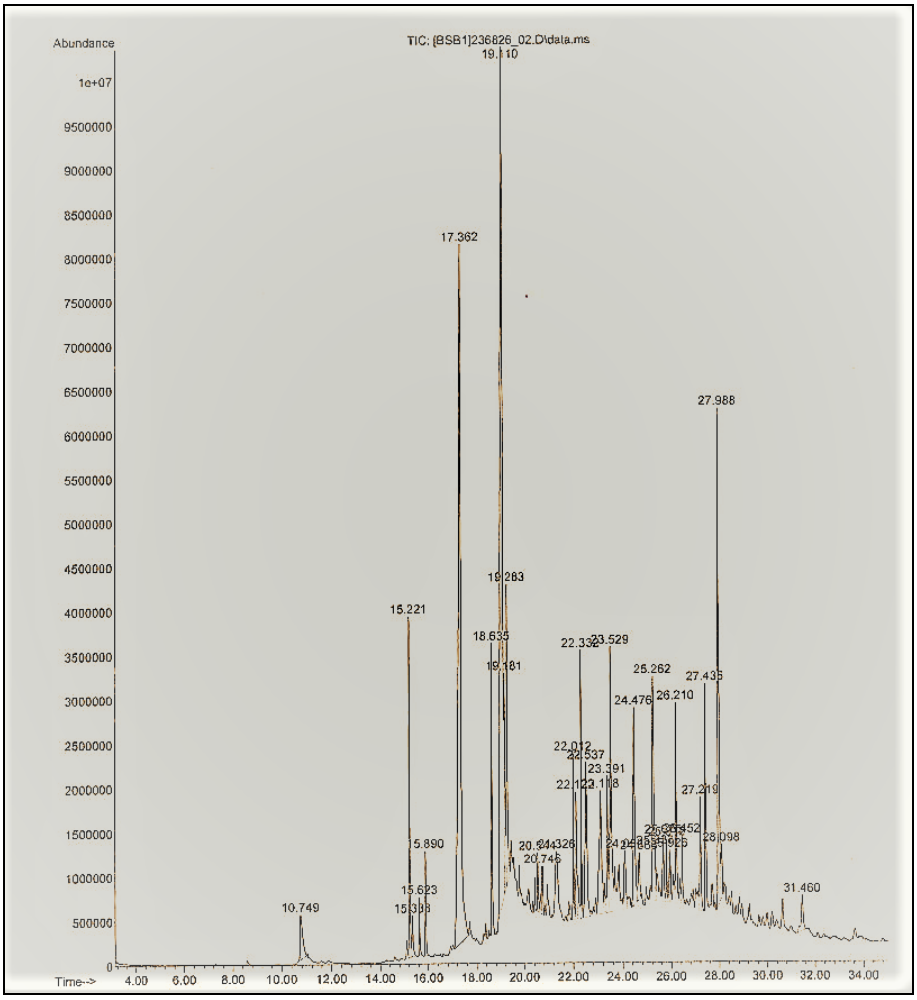

Fig 1: GC-MS Analysis of Methanolic bark extract of Syzygium *caryophyllatum*

5. Conclusion

This is the first report on the analysis of bioactive components present in *S.caryophyllatum* (bark). The phytochemical and GC-MS analysis revealed that the bark contains various bioactive phytoconstituents. The presence of these phytochemicals justifies the use of this plant in traditional medicine and one of the reason for considering this plant as a boon of nature by the tribal people. In view of the medicinal importance associated with the phytocompounds, further investigation should be carried out in order to purify, characterize the structure of these bioactive compounds and enhance their potentials as drugs.

6. Acknowledgement

We gratefully acknowledge the help offered by Bureau Veritas (India) Pvt. Ltd. for providing GC-MS analysis.The Head, Department of Botany, University of Kerala provided required facilities and the Council of Scientific & Industrial Research (CSIR) India, provided a Junior Research Fellowship (JRF) to carry out this research work.

7. References

1. Satyavati GV, Gupta AK, Tandon N. Medicinal Plants of India. Vol II CSIR publication, Indian Council of Medical Research, Cambridge printing worker, New Delhi, 1987, 557.
2. Elujoba AA, Odeleye OM, Ogunyemi CM. Traditional medical development for medical and dental primary health care delivery system in Africa. Afr J Trad CAM, 2005; 2(1):46-61.
3. Iyengar MA. Study of Crude Drugs. 8th Ed, Manipal Power Press, Manipal, India, 1995, 2.
4. Warjeet LS. Traditional medicinal plants of Manipur as anti-diabetics. J Medicinal plants Res. 2011; 5(5):677-687.
5. Murugan S, Uma Devi P, Kannika Parameswari N, Mani KR. Antimicrobial activity of *Syzygium jambos* against selected human pathogens.Int J Pharm Pharm Sci. 2011; 3(2):44-47.
6. Musabayme CT, Mahablele, Shode FO, Ojewole Jao. Effects of *Syzygium cordatum* (Hochst,) (Myrtaceae) leaf extract on plasma glucose and hepatic glycogen in streptozoctin-induced diabetic rats. J Ethnopharmacol. 2005; 97(3):485-490.
7. Chandrasekaran M, Venkatesalu V. Antibacterial and antifungal activity of *Syzygium jambolanum* seeds. Journal of Ethnopharmacology. 2004; 91:105-108.
8. Rao BK, Rao CH. Hypoglycemic and antihyperglycemic activity of *Syzygium alternifolium* (Wt.) Walp. Seed extracts in normal and diabetic rats. Phytomedicine. 2001; 8:88-93
9. Ediriweera ERHSS, Ratnasooriya WDA. Review on herbs used in treatment of diabetes mellitus by Sri Lankan and traditional physicians. Ayu. 2009; 30:373-391.
10. Shilpa KJ, Krishnakumar G. Nutritional, fermentation and pharmacological studies of *Syzygium*

caryophyllatum (L.) Alston and *Syzygium zeylanicum* (L.) DC fruits. Cogent Food & Agriculture. 2015; 1(1):1018694.
11. Stalin N. Screening of phytochemical and pharmacological activities of *Syzygium caryophyllatum* (L.) Alston. Clinical Phytoscience. 2018; 4(1):3.
12. Harsha VH, Sripathy V, Hedge GK. Ethnoveterinary practices in Uttara Kannada district of Karnataka, Indian J Tadit Knowl. 2005; 4:253-258.
13. Gamble JS. Flora of Presidency of Madras. Vol II, Bishen Singh Mahendra Pal Singh, Dehradun, 1921, 650-652.
14. Harborne JB. Methods of plant analysis. In: phytochemical methods. Chapman and Hall London, 1993, 74-79.
15. Trease GE, Evans WC. Pharmacognosy 2nd Edn. Braille Tiridel and Macmillan Publishers, 1989, 242-245.
16. Stein SE. National Institute of Standards and Technology (NIST), Mass Spectral Database and Software. Version 3.02. Gaithersburg, USA, 1990.
17. Teixeira CC, Pinto LP, Kessler FHP, Knijnik J, Pinto CP, Gastaldo G, *et al*. The effect of *Syzygium cumini* (L) skeels on postprantial blood glucose levels in non-diabetic rats and rats with streptozotocin-induced diabetes mellitus. J Ethnopharmacol. 1997; 56:209-213.
18. Jayasinghe UL, Kumarihamy BM, Bandara AG, Waiblinger J, Kraus W. Antifeedant activity of some Sri Lankan plants. Nat Prod Res. 2003; 17(1):5-8.
19. Ruan ZP, Zhang LL, Lin YM. Evaluation of the antioxidant activity of *Syzygium cumini* Leaves. Molecules. 2008; 13:2545-2556.
20. Savitha RC, Padmavathy S, Sundhararajan A. In-vitro antioxidant studies on leaf extracts of *Syzygium malaccense* (L.) Merr & Perry. Ancient Sci Life. 2011; 30(4):110-113.
21. Tanwar RS, Sharma SB, Singh UR, Prabhu KM. Antiatherosclerotic potential of active principle isolated from *Eugenia jambolana* in Streptozotocin-induced diabetic rats. Evid Based Compliment Alternat Med, 2011, 1-9.
22. Singh A, Marar T. Inhibitory effect of extracts of *Syzygium cumini* and *Psidium guajava* on glycosidases. J Cell Tissue Res. 2011; 11(1):2535-2539.
23. Antherden LM. Textbook of Pharmaceutical Chemistry, 8th edition, Oxford University Press, London, 1969, 813-814.
24. Stray F. The Natural Guide to Medicinal Herbs and Plants.Tiger Books International London, 1998, 12-16.
25. Okwu DE. Phytochemicals and vitamin content of indigenous species of southeastern Nigeria. Journal of Sustainable Agriculture & Environment. 2004; 6(1):30-37.
26. Hada M, Hino K, Takeuchi Y. Development of UV defense mechanisms during growth of spinach seedlings. Plant Cell Physiol. 2001; 42(7):784-787.
27. Chempakam B. Hypoglycemic activity of arecoline in betel nut *Areca catechu* L. Ind J Exp Biol. 1993; 31:474-475.
28. Aiyelaagbe OO, Osamudiamen PM. Phytochemical screening for active compounds in *Mangifera indica*. Plant Sci Res. 2009; 2(1):11-13.
29. Chakravarth BKS, Gupta SS, Gambir S, Gode KD. Pancreatic beta cell regeneration. A novel antidiabetic mechanism of *Pterocarpus marsupium* Roxb. Indian J Pharmacol. 1980; 12:123-127.
30. Geetha BS, Mathew BC, Augusti KT. Hypoglycemic effects of leucodelphinidin derivative isolated from *Ficus bengalensis* Linn. Indian. J Physiol Pharmacol. 1994; 38:220-222.
31. Abubakar MN, Majinda RR. GC-MS analysis and preliminary antimicrobial activity of *Albizia adianthifolia* (Schumach) and *Pterocarpus angolensis* (DC). Medicines (Basel), 2016, 3:E3.
32. Mohammad TG, Mohammed HE, Ali J, Seyedhossein H, Mohammad M. Antimicrobial activity, toxicity and stability of phytol as a novel surface disinfectant. Environ Health Eng. Manage J. 2015; 2:13-6.
33. Peter O, Malin H, Lars I Hellgren, Rikard H. Phytol: achlorophyll component with anti-inflammatory and metabolic properties. Recent Advances in Redox-Active Plant and Microbial Products, 2014, 345-359.
34. Kametani T, Furuyama H. Synthesis of Vitamin D3 and related compounds. Medicinal Research Reviews. 1987; 7(2):147-171.
35. Oliveira FA, Chaves MH, Almeida FR, Lima RC, Silva RM, Maia JL, *et al*. Protective effect of alpha- and beta-amyrin, a triterpene mixture from *Protium heptaphyllum* (Aubl.) March. Trunk wood resin, against acetaminophen-induced liver injury in mice. Journal of Ethnopharmacology. 2005; 98(1-2):103-108.
36. Machlin LJ, Bendich A. Free radical tissue damage: protective role of antioxidant nutrients. FASEB. J. 1987; 6:441-444.