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Extraction, Isolation and Identification of Useful Phyto Constituents from Dichloromethane Leave Extract of *Abutilon Pannosum* and *Grewia Tenax* Using Q-TOF LC/MS

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Abstract: *Plant extracts usually occur as a combination of various types of bioactive compounds or phytochemicals with different polarities. The present study investigates the qualitative and quantitative analysis of the major bioactive constituents of medicinally important plant Abutilon pannosum (A.P) and Grewia tenax (G.T) in its dichloromethane (DCM) extract of leaves by use of Liquid Chromatography Quadrupole Time of Fight Mass Spectrometry (Q-TOF LC/MS). Soxhlet extraction of sample was undertaken by continuous hot percolation method using DCM as a solvent. After extraction it was concentrated by use of distillation method. Crude DCM extracts were inserted in Quadrupole–time-of-flight (Q-TOF) LC/MS instrument for isolation and identification of valuable phytochemicals. The result of phytochemical investigates exhibited that there are very significant phytochemicals found in DCM extracts of A. pannosum leaves like alkaloids, sterol lipids, glycerophospholipids, fatty acid, steroid glycoside, antioxidant, and heterocyclics compounds and G. tenax leaves have alkaloids, sterol lipids, glycerophospholipids, fatty ester, fatty acid, glycoside, carotene, steroidal alkaloid, triterpene glycosides, glycerophosphates, sesquiterpenes, phosphatidylglycerol, antioxidant, antibacterial, antifungal, antiviralactivity and other biological function. Thus, DCM extract of G. tenax leaves gives decent medicinal activity compared to the DCM extract of A. pannosum leaves part. In this study, the G. tenax DCM extract have greatest number of bioactive compounds. This paper mainly deals with the extraction of active compounds from the leaves part of two plants by used of DCM solvent and investigation of the phytochemicals in DCM extract by used of Q-TOF LC/MS.*

Keywords: *Abutilon pannosum, Grewia tenax, Dichloromethane (DCM) extract, Q-TOF LC/MS, Phytochemicals*

1. INTRODUCTION

Plants are natural reservoir of medicinal agents. These are almost free from the side effects. [1] .Thus, since ancient times, people have been discovering the nature particularly plants in search of new drugs. This has resulted in the use of huge number of medicinal plants with curative properties to treat various diseases. [2] Approximately 80% of the world's population trusts on traditional medicines for primary health care, most of which contain the use of plant extracts. [3] Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The ingredients present in the plant play a significant role in the identification of crude drugs. [4]

Khapat is the native name of (*Abutilon pannosum*) is one of the valuable medicinal plant burn of Malvaceae family are reported here for the first time as a part of our studies to locate new oilseed resources *A pannosum* is a tomentose undershrub widely distributed in India, North Africa, S W Asia and Australia, and bears spherical fruits having about 25 carpels, each of which covers hairy plant widely distributed from tropical Africa to Australia through Asia. It grows to a height of 2 m and bears small, ovoid fruits which contain tasteless seeds. [5] It leaves have good medicinal activity for example antibacterial, antioxidant, antifungal etc. [6]

Guddaim is the native name of (*Grewia tenax*) is one of the valuable plant species in kachchh. It is basically spread in arid area such as sand and near mountains. [7] *Grewia tenax* is a tree spread in

Africa and Southeast Asiatic continents. It belongs to the *Tileacea* family. In fact, *Grewia tenax* is a plant that has been used in popular medicine in various ways in different countries. Roots are used to treat jaundice, pulmonary infections and asthma. Leaves are used against trachoma. Decoction and fruit juice are used for their tonic and anti-anemic properties. Fruits are small berries, round, orange sweetened and it may be consumed either fresh or dried. [8] There is commercial potential in using the fruits in beverages, ice cream, yogurt, and baby food. [9]

Quadrupole–time-of-flight (TOF) mass spectrometers have rapidly been incorporated by the analytical community as powerful and robust instruments with unique capabilities. [10] In particular, they combine the high performance of time of-flight analysis in both the mass spectrometry (MS) and tandem MS (MS/MS) modes, with the well accepted and widely used techniques of electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). [11]

The objectives of this study are to evaluate and study the potential bioactive compound of both plant leaves by used of Q-TOF LC/MS which can be considered an important for human health and the knowledge the nutritional value of both plant leaves and to take advantage of them in various nutritional applications.

2. Materials And Method

Abutilon pannosum and *Grewia tenax* leaves powder, dichloromethane, RBF (round bottom flask), condenser, heating mantle, measuring flask and thimble.

2.1. Preparation of Extracts

15 gm of leaves powder was extracted with 2-3 litre of dichloromethane (39.6°c) for 12 hour using soxhlet apparatus by continuous hot percolation method. After extraction, it was filtered and the exclusion of solvent was done under pressure by distillation process to afforded extract. Extract were collected in air tight glass tube.

2.2. Procedure

The prepared extract dissolve in 0.9 ml methanol and 0.1ml 0.1% formic acid in glass tubes, for recognition of the investigated compounds was achieved using a quadrupole coupled to time-of-flight analyzer (Q-TOF-MS 6540, Agilent Technologies, UHD). The mass spectrometry was equipped with an ESI Jet Stream source; identification and determination of the investigated drug was carried out in the SCAN mode.

2.3. LC-Q-TOF-MS Method

The separation of the analysts was carried out using an Agilent LC-Q-TOF-MS 6540, UHD.

2.3.1. LC-Parameter

The injected sample volume was 10µL; Mobile phases A and B were water and acetonitrile with 0.1% formic acid, respectively. The flow rate was 0.6mL/min. A 16 min run time was used after each analysis. The optimized chromatographic method held the initial mobile phase composition (10% B) constant for 0 min, followed by a linear gradient to 100% B after 14 min. and return back (10% B) at 14 min. the system featured a binary pump and vacuum degasser, well-plate auto sampler with a six-port micro-switching valve, a thermo stated column compartment. Samples were loaded onto a Reprosil C18 column (2.0mm×150mm, 2.5 µm – Dr Maisch, Germany) for metabolite separation.

2.3.2. Q-TOF Parameter

The LC system was connected to an Agilent 6450 ultrahigh definition quadrupole time-of-flight mass spectrometer equipped with dual electro spray Jet Stream Technology operating in positive ion mode. The operating parameters were as follows: capillary voltage: 4000V; nebulizer pressure: 45 psi(N2); drying gas: 8 L/min; gas temperature:325°C; nozzle voltage: 1000V; fragment or voltage: 150V; skimmer voltage: 65V, m/z; 100 to 1700, sheath gas temp350 °C and sheath gas flow 11 L min–1. The data recorded was processed with Agilent Mass Hunter software. Accurate mass measurements of each peak from the total ion chromatograms were obtained by means of an automated calibrant delivery system using a low flow of a calibrating solution (Calibrant solution A, Agilent Technologies, Santa Clara, CA, USA).

3. RESULTS

The compounds present in the DCM extract of leaves of *A. pannosum* and *G. tenax* were identified by LC-MS-Q-TOF analysis. The LC-MS-Q-TOF chromatogram (Figure 1) showed 10 peaks in *A. pannosum* and 34 peak in *G. tenax* indicating the presence of 10 and 34 phytochemical constituents. It is characterized and identified, which are listed with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) in Table 1 & 2. The major phytochemical constituents and their biological activities obtained through the LC-MS-Q-TOF study of *A. pannosum* and *G. tenax* are listed in Table 1 & 2 respectively.

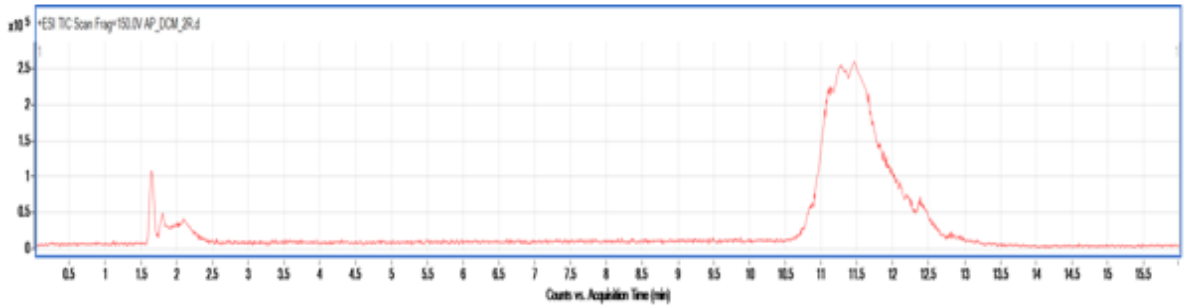


Figure1. *Chromatogram of A. pannosum DCM extract*

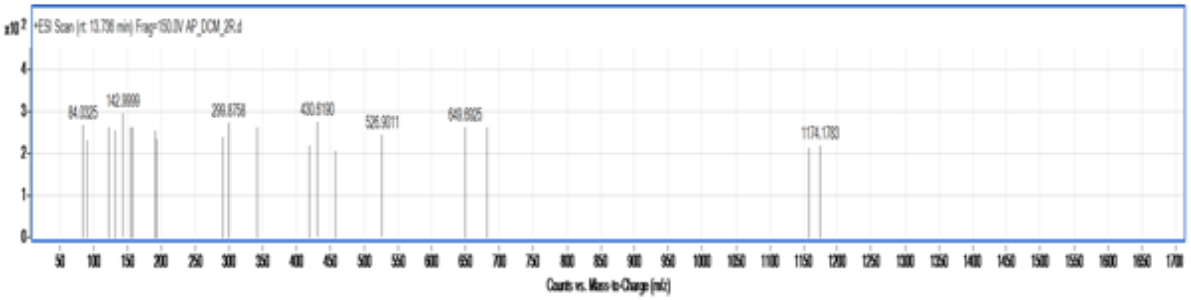


Figure2. *Mass spectrum of A. pannosum DCM extract*

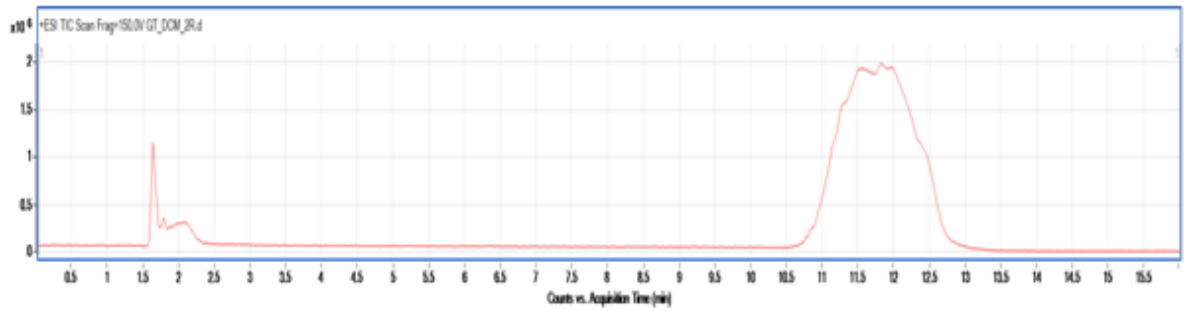


Figure3. *Chromatogram of G. tenax DCM extract*

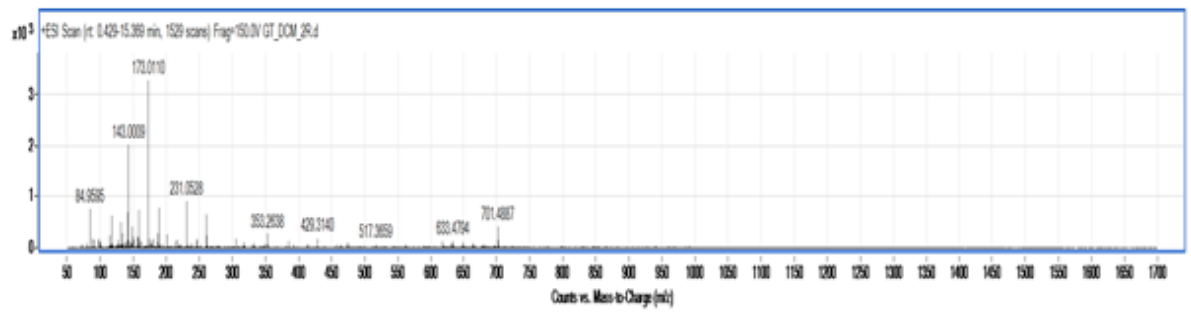


Figure4. *Mass spectrum of G. tenax DCM extract*

Table1. *DCM extraction of A. pannosum leaves sample*

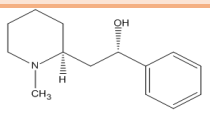
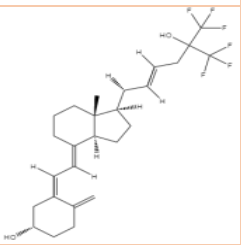
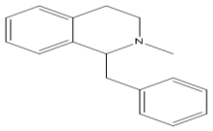
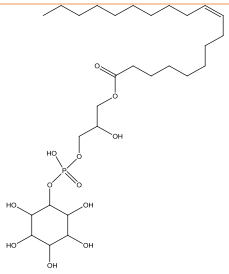
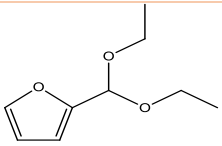
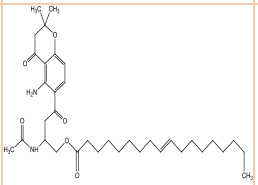
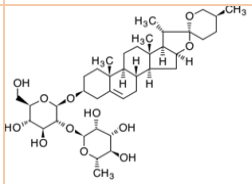
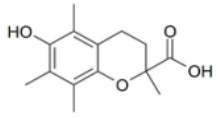
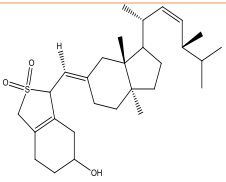
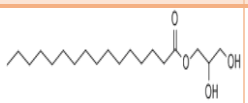
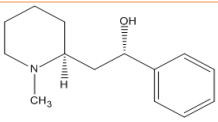
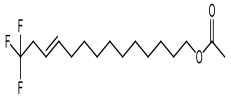
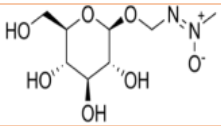
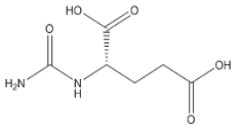
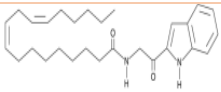
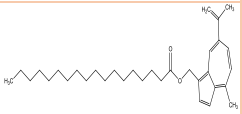
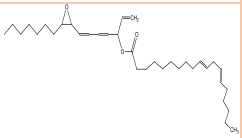
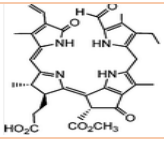
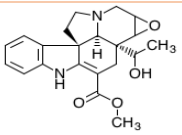
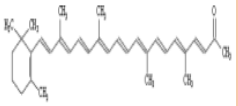
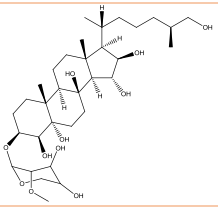
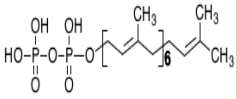
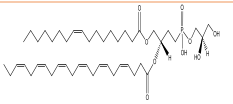
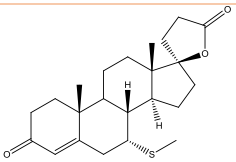
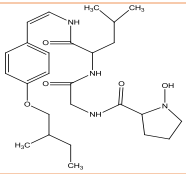
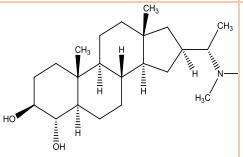
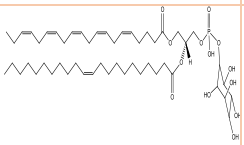
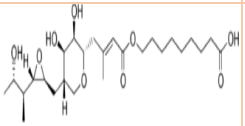
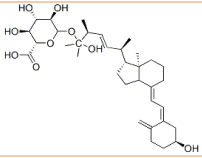
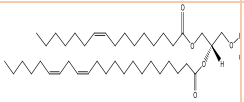
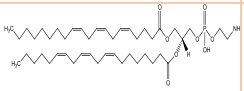
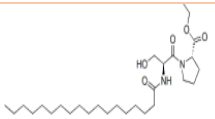
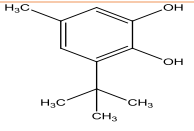
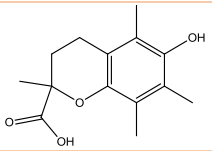
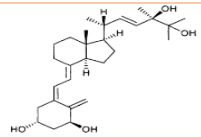
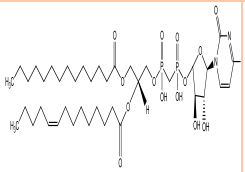
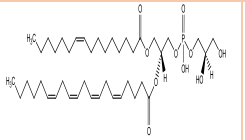
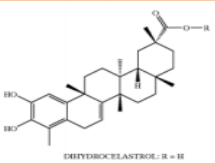
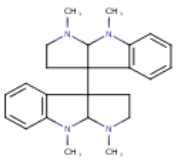
Sr	Name	M.F	RT	% of Conc	Mtln ID	Note	Structure	Ref.
1	(-)-Sedamine	$C_{14}H_{21}NO$	11.081	14.78	64453	Alkaloids		12, 22
2	(22E)-26,26,26,27,27,27-hexafluoro-25-hydroxy-22,23-didehydrovitamin D3	$C_{27}H_{36}F_6O_2$	12.109	20.41	42021	Sterol Lipids		13
3	N-Methyl-(R,S)-tetrahydrobenzylisoquinoline	$C_{17}H_{19}N$	11.148	12.27	66304	Alkaloids		14
4	8E-Tetradecenyl acetate	$C_{16}H_{30}O_2$	11.84	9.68	46290	Not reported		15
5	1-(9Z-nonadecenoyl)-glycero-3-phospho-(1'-myo-inositol)	$C_{28}H_{53}O_{12}P$	11.101	5.67	81183	Glycerophospholipids		16
6	Furfural diethyl acetyl	$C_9H_{14}O_3$	10.882	4.11	69937	Heterocyclics compounds (Uses as flavor additive and biofuel liquid)		17
7	3'-N-Acetyl-4'-O-(9-octadecenoyl)fusarochromanone	$C_{35}H_{54}N_2O_6$	11.664	13.13	93238	Fatty Acid		18
8	Yamogenin 3-O-neohesperidoside	$C_{39}H_{62}O_{12}$	12.218	2.16	67269	Steroid Glycoside (sapogenins)		19
9	Trolox	$C_{14}H_{18}O_4$	11.174	2.62	45333	Antioxidant		20, 44
10	(6R)-vitamin D2 6,19-sulfur dioxide adduct	$C_{28}H_{44}O_3S$	11.218	15.16	41911	Sterol Lipids		19

Table2. *DCM extraction of G. tenax leaves sample*

Sr.	Name	M.F	RT	% of Conc	Mtln ID	Note	Structure	Ref
1	1-Monopalmitin	$C_{19}H_{38}O_4$	12.908	4.09	24076	Intermediates and microwave reactor		21
2	(-)-Sedamine	$C_{14}H_{21}NO$	11.081	2.26	64453	Alkaloids		12, 22
3	14,14,14-Trifluoro-11E-tetradecenyl acetate	$C_{16}H_{27}F_3O_2$	11.51	12.71	46326	Fatty Ester		23
4	Cycasin	$C_8H_{16}N_2O_7$	10.902	1.54	65593	Carcinogenic, Toxic glycoside		24
5	8E-Tetradecenyl acetate	$C_{16}H_{30}O_2$	11.836	6.67	46290	No activity reported		15
6	N-Carbamylglutamate	$C_6H_{10}N_2O_5$	10.825	0.77	44787	Enzyme, protein synthase, enhanced growth performance and improved intestinal function in weaned piglets		25
7	Termitomycamide B	$C_{28}H_{40}N_2O_2$	11.583	1.32	96488	Fatty acid, Reduce oxidative stress in Endoplasmic reticulum (ER)		26, 27
8	Deterrol stearate	$C_{33}H_{50}O_2$	11.826	4.83	90042	Sesquiterpenes		28
9	Panaxydol linoleate	$C_{35}H_{54}O_3$	11.821	5.06	95626	Nutrient, Stabilizers, Surfactants and Emulsifiers, used in cosmetics		29
10	Red chlorophyll catabolite	$C_{35}H_{38}N_4O_7$	11.826	0.87	63971	Protein		30

11	**16Z-octadecenoic acid**	**$C_{18}H_{34}O_2$**	**12.069**	**1.07**	**34956**	**Trans fatty acid, Selectively inhibit eukaryotic DNA polymerase activities in vitro**		**31**
12	**Horhammericine**	**$C_{21}H_{24}N_2O_4$**	**11.745**	**2.61**	**64315**	**Alkaloid**		**32**
13	**Citranaxanthin**	**$C_{33}H_{44}O$**	**11.907**	**5.34**	**91883**	**Carotenoid**		**33**
14	**Kurilensoside F**	**$C_{33}H_{58}O_{11}$**	**12.084**	**2.03**	**84935**	**Sterol lipids**		**34**
15	**All-trans-heptaprenyl diphosphate**	**$C_{35}H_{60}O_7P_2$**	**12.214**	**1.16**	**53854**	**Terpenoids**		**35**
16	**1-(9Z-heptadecenoyl)-2-(4Z,7Z,10Z,13Z,16Z,19Z-docosahexaenoyl)-glycero-3-phospho-(1'-sn-glycerol)**	**$C_{35}H_{60}O_7P_2$**	**12.243**	**4.90**	**79102**	**Monoacylglycerophosphoglycerols**		**23**
17	**7α-(Thiomethyl)spironolactone**	**$C_{23}H_{32}O_3S$**	**11.026**	**1.10**	**2483**	**Diuretic and treatment of hypertension, metabolite of Spironolactone**		**36**
18	**Ceanothine D**	**$C_{27}H_{40}N_4O_4$**	**11.502**	**1.77**	**86202**	**Alkaloid**		**37**
19	**Terminaline**	**$C_{23}H_{41}NO_2$**	**11.86**	**2.89**	**68610**	**Steroidal alkaloid, cardiovascular, cancer therapy, hepatoprotectiv,**		**38**

						cholesterol-reducing, and antioxidant		
20	1-(5Z,8Z,11Z,14Z,17Z-eicosapentaenoyl)-2-(11Z-docosenoyl)-glycero-3-phospho-(1'-myo-inositol)	$C_{51}H_{87}O_{13}P$	12.069	4.13	80665	Diacylglycerophosphoinositols		23
21	1-(1Z-hexadecenyl)-2-dodecanoyl-glycero-3-phosphate	$C_{31}H_{61}O_7P$	11.664	7.11	82241	Glycerophosphates		39
22	Mupirocin	$C_{26}H_{44}O_9$	11.664	2.37	43266	Antibacteri, antibiotic, skin disorders, nasal infections, and wound healing		40
23	25 hydroxyergocalciferol 25-glucuronide	$C_{34}H_{52}O_8$	11.583	0.94	43182	Triterpene glycosides		19
24	1-(9Z-hexadecenoyl)-2-(13Z,16Z-docosadienoyl)-glycero-3-phosphate	$C_{41}H_{75}O_8P$	11.148	0.35	81388	Diacylglycerophosphates		39
25	1-Meadoyl-2-homo-gamma-linolenoyl-sn-glycero-3-phosphoethanolamine	$C_{45}H_{78}NO_8P$	12.028	1.60	60728	Phosphatidylethanolamine		23
26	1-eicosyl-glycero-3-phospho-(1'-sn-glycerol)	$C_{26}H_{55}O_8P$	12.218	1.95	80025	Monoacylglycerophosphoglycerols		23
27	N-stearoyl proline	$C_{23}H_{43}NO_3$	11.583	0.47	75501	Antimicrobial activivty		41
28	3-tert-Butyl-5-methylcatechol	$C_{11}H_{16}O_2$	11.416	2.95	66083	Polymerization inhibitor for hot styrene		42, 43

29	**Trolox**	**$C_{14}H_{18}O_4$**	**11.174**	**3.33**	**45333**	**Antioxidant activity**		**20, 44**
30	**(6R)-vitamin D2 6,19-sulfur dioxide adduct**	**$C_{28}H_{44}O_3S$**	**11.218**	**4.20**	**41911**	**Vitamin D**		**23**
31	**(2R,3R,5R)-5-(4-amino-2-oxopyrimidin-1-yl)-3,4-dihydroxyoxolan-2-yl**	**$C_{31}H_{59}O_8P$**	**11.354**	**2.31**	**81258**	**Glycerophospholipid**		**39**
32	**1-Palmitoleoyl-2-arachidonoyl-sn-glycero-3-phosphoglycerol**	**$C_{42}H_{73}O_{10}P$**	**11.988**	**1.02**	**61870**	**Phosphatidylglycerol**		**45**
33	**Dihydrocelastrol**	**$C_{29}H_{40}O_4$**	**11.907**	**2.08**	**44189**	**Derivative**		**19**
34	**(-)-Folicanthine**	**$C_{24}H_{30}N_4$**	**12.226**	**2.21**	**86790**	**Alkaloid, it is give antifungal activities, weak antiviral activities**		**46**

3.1. *pannosum* DCM extract

Table 1 represents DCM extract of *A. pannosum* gives alkaloids, sterol lipids, glycerophospholipids, fatty acid, steroid glycoside, antioxidant, and heterocyclic compounds.

According to above results showed that total ten type bioactive compound present in DCM extract of *A. pannosum*. It was mainly found to be in order of (22E)-26,26,26,27,27,27-hexafluoro-25-hydroxy-22,23-didehydrovitamin D3 (20.41%), (6R)-vitamin D2 6,19-sulfur dioxide adduct (15.16%), (-)-Sedamine (14.78%), 3'-N-Acetyl-4'-O-(9-octadecenoyl) fusarochromanone (13.13%), N-Methyl-(R,S)-tetrahydrobenzylisoquinoline(12.27%),1-(9Z-nonadecenoyl)-glycero-3-phospho-(1'-myo-inositol) (5.67%), Furfural diethyl acetal (4.11%), Trolox (2.62%) and Yamogenin 3-O-neohes-peridoside (2.16%).

3.2. *tenax* DCM extract

Table2 represents DCM extract of *G. tenax* leaves gives alkaloids, sterol lipids, glycerophospholipids, fatty ester, fatty acid, glycoside, carotene, steroidal alkaloid, triterpene glycosides, glycerol-phosphates, sesquiterpenes, phosphatidylglycerol, antioxidant, antibacterial, antifungal, antiviral activity and other biological function.

According to above results showed that total 34 type bioactive compound present in DCM extract of *G. tenax*. It was mainly found to be in order of 14,14,14-Trifluoro-11E-tetradecenyl acetate (12.71%), Citranaxanthin (5.34%), Panaxydol linoleate (5.06%), Deterrol stearate (4.83%), 1-Monopalmitin (4.09%), Trolox (3.33%), 3-tert-Butyl-5-methylcatechol (2.95%), Terminaline (2.89%), Horham-mericine (2.61%), Mupirocin (2.37%), (-)-Sedamine (2.26%), (-)-Folicanthine (2.21%), Kurilensoside F (2.03%), Ceanothine D (1.77%), Termitomycamide B (1.32%), All-trans-heptaprenyl diphosphate

(1.16%),7α-(Thiomethyl)spironolactone (1.10%), 16Z-octadecenoic acid (1.07%), 25 hydroxyergocalciferol 25-glucuronide (0.94%), Red chlorophyll catabolite (0.87%), N-Carbamylglutamate (0.77%) and N-stearoyl proline (0.47%).

4. DISCUSSION

A variety of herbs and herbal extracts contain different phytochemicals with biological activity that can be of valuable therapeutic index. Much of the protective effect of herbal plants has been attributed by phytochemicals, which are the non-nutrient compounds. [47] Different phytochemicals have been found to possess a wide range of activities, which may help in safety against sustained diseases. [48] The phytochemical investigation of the leaves of *A. pannosum* (A.P) and *G. tenax* (G.T) showed that the leaves contain most of the secondary metabolites analysed. They were revealed to possess sterol lipids, alkaloids, phenolic compounds, sterol glycosides, triterpene glycoside, steroids, carotenoid, tannins, fatty acids and triterpenoids. Phytosterols are the sterols of plant origin, which have been shown to possess cholesterol lowering [49] as well as anticancer property. [50]

According to the result two sterol lipids i.e.,(22E)-26, 26, 26, 27, 27, 27-hexafluoro-25-hydroxy-22, 23-didehydrovitamin D3 and (6R)-vitamin D2 6,19-sulfur dioxide adduct were shown in A.P and nine sterol lipids i.e.,(2R,3R,5R)-5-(4-amino-2-oxopyrimidin-1-yl)-3,4-dihydroxyoxolan-2-yl,1-Palmitoleoyl-2-arachidonoyl-sn-glycero-3-phosphoglycerol,1-eicosyl-glycero-3-phospho-(1'-sn-glycerol),1-(1Z-hexadecenyl)-2-dodecanoyl-glycero-3-phosphate,1-Meadoyl-2-homo-gammalinol-enoylsnglycero3 phosphoethanolamine,1-(9Z-hexadecenoyl)-2-(13Z,16Zdocosadienoyl)-glycero-3phos-phate,1(5Z,8Z,11Z,14Z,17Z-eicosapentaenoyl)-2-(11Z-docosenoyl)-glycero-3-phospho-(1'-myo-inositol),1-(9Zhepta decenoyl)-2-(4Z,7Z,10Z,13Z,16Z,19Z-docosahexaenoyl)-glycero-3-pho-sphor-(1'-snglycerol)and Kurilensoside F were shown in G.T.Sterol lipids are major components of biological membranes.It is present in smaller amounts act as enzyme cofactors, electron carriers and light absorbing pigments, hydrophobic anchors for proteins, emulsifying agents, hormones and intracellular messengers.[51] Here,1-Palmitoleoyl-2-arachidonoyl-sn-glycero-3-phosphoglycerol(i.e.,phosphatidylglycerols) exhibit important role as a precursor for the synthesis of cardiolipin.[52]

Yamogenin 3-O-neohesperidoside was shown only in A.P. It is sterol glycoside. Sterol glycosides have also been added to dietary supplements designed to improve lipid metabolism and immune function. [53] New findings within the past five years have discovered these compounds to be involved in complex cell-signal transduction mechanisms, resulting in selective control of human tumors but not normal cellular proliferation and they represent a promising form of targeted cancer chemotherapy. [54]

14,14,14-Trifluoro-11E-tetradecenyl acetate (12.71%) and Citranaxanthin are carotenoid, which are existing in G.T. Carotenoids may act as antioxidants and may exhibit chemo preventive anti atherosclerotic effects and anticancer effects[55] carotenoid pigments primarily plays role in the production of vitamin-A. It is support the maintenance of healthy epithelial cell differentiation, normal reproductive performance, and visual functions. [56-57]additionally, carotenoids also play an important role in human health as protecting cells and tissues from the oxidative damaging effects of free radicals and singlet oxygen. [58-59]Many studies show strong correlations between carotenoids intake and a reduced risk of some diseases, such as cancer, [60-61]atherogenesis, [62-63]bone calcification,[64] eye degeneration,[65-66] immune function[67-68] and neuronal damage.[69]

The human body needs essential fatty acids to construct and repair cell membranes enabling the cells to obtain optimum nutrition and expel harmful waste products. [70] A primary function of essential fatty acids, which support the cardiovascular, reproductive, immune and nervous systems, is the production of prostaglandins. [71] These regulate body functions such as heart rate, blood pressure, blood clotting, fertility and play a role in immune system by regulating inflammation. [72-74] In A.P present one fatty acid component which is 3'-N-Acetyl-4'-O-(9-octadecenoyl) fusarochromanone and G.T showed two fatty acid component i.e., termitomycamide B, and 16Z-octadecenoic acid are fatty acid.

Alkaloids that are responsible for the therapeutic effect of many medicinal plants. Earlier studies have indicated that alkaloids possess antihyperglycemic and antilipidemic effects suggesting their beneficial effect in the management of diabetes associated with abnormal lipid profile and related

cardiovascular diseases. [75] Alkaloids are used in medicines for reducing headache and fever. These are ascribed for antibacterial and analgesic properties. [76] Some alkaloids are present in both plant i.e, (-)-Sedamine and N-Methyl-(R,S)-tetrahydrobenzylisoquinoline. When Horhammericine Ceanothine D, Terminaline and (-)-Folicanthine are present in G.T leaves sample. Terminaline act as a steroidal alkaloid, it is used in cardiovascular, cancer therapy, hepatoprotectiv, cholesterol-reducing, and also give antioxidant activity [38] and (-)-Folicanthine is also provide antifungal activities, weak antiviral activities. [46]

All-trans-heptaprenyl diphosphate act as a terpenoids which was shown in G.T. Terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anticancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities. [77] Terpenoids are very important in enticing valuable mites and consume the herbivorous insects. [78] Furfural diethyl acetyl present in A.P. It is one type of heterocyclic compounds which is uses as flavour additive and biofuel liquid. [17] Trolox were existing in both plant leaves sample. Trolox is antioxidant agent thus, it is used in biological or biochemical applications to reduce oxidative stress or damage. [20, 44] 7α-(Thiomethyl) spironolactone is heterocyclic compound i.e., present in G.T. It is use for convenient in diuretic, treatment of hypertension and metabolite of Spironolactone. [36]

G.T contained 25 hydroxyergocalciferol 25-glucuronide i.e., triterpene glycosides. Triterpene glycosides are well-known for their cytotoxic, antimicrobial, anticoagulant, hemolytic, antiviral, antiparasitic and antitumor properties. Some glycosides can prevent the growth [79] survival, invasion [80, 81] and metastasis [82] of cancerous cells; others possess immunomodulatory activity, [83] or inhibit the sodium–potassium ATPase, [84] and even elicit apoptosis [85] G.T also have red chlorophyll catabolite i.e., one type of protein molecule [30] and N-carbamylglutamate, mupirocin and N-stearoyl proline are compound which have antimicrobial activity.[25,40,41] Mupirocin is also useful as an antibiotic, skin disorders, nasal infections, and wound healing.[40]

5. CONCLUSION

The present study was conducted on DCM extracts of *A. pannosum* and *G. tenax* evaluated in this work has different varieties of phytochemicals i.e., sterol lipids, alkaloids, phenolic compounds, sterol glycosides, triterpene glycoside, steroids, carotenoid, tannins, fatty acids and triterpenoids, that could be considered as responsible for medicinal activities. According to the above result exhibited in table 1 & 2, we have found that both plant showed different types of phytochemicals. The presence of *G. tenax* give good medicinal activity compared to the *A. pannosum*, since of G.T has contain higher amount of phytochemicals except sterol glycoside. This study also leads to the further research in the way of isolation and identification of the active compound from the selected fern using chromatographic and spectroscopic techniques. According to the literature search, there is no work that has been done on analysis of DCM extracts of *A. pannosum* and *G. tenax* by the researcher.

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