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Preliminary phytochemical screening and GC-MS analysis of methanolic leaf extract of *Abutilon pannosum* (Forst. F.) Schlect. from Indian Thar desert

Ilham Bano and GS Deora

Abstract
Present study was designed to determine the presence of various phytoconstituents in methanolic extract of leaves of the plant *Abutilon pannosum* by preliminary phytochemical screening and to identify possible specific compounds with their concentration through GC-MS analysis. Methanolic extract of leaves of *Abutilon pannosum* was prepared and analyzed qualitatively for presence or absence of various phytoconstituents such as carbohydrates, protein, alkaloid, steroid, phenol, glycosides, terpenoids, flavonoids etc. furthermore GC-MS analysis was performed for identification of phytocomponents. Preliminary phytochemical screening of methanolic extract of *Abutilon pannosum* by common methods revealed presence of carbohydrates, amino acids, alkaloids, phenols, flavonoids, phytosterols, terpenoids, glycosides etc. GC-MS analysis revealed presence of various phytocompounds most of them have reported to have medicinal utility like n-Hexadicanoic acid, Phytol, Vitamin E, Lupeol, 2,3 Dihydrobenzofuran, Stigmasterol, Ergost-5-en-3-ol,(3.beta.,24R),gamma–Sitosterol, Neophytadien, Naphthalene, Tocopherols such as alpha Tocospiro A and alpha Tocospiro B. Presence of various phytochemical compounds in the leaves of *Abutilon pannosum* justifies the medicinal use of the plant. The present study results could provide future insight of this plant to be useful in pharmaceutical industries for welfare of human being.

Keywords: Phytochemical screening *Abutilon pannosum*, alpha Tocospiro A, GC-MS analysis, Phytosterols

Introduction
Plants always play an important role in medicine both in modern and traditional views. Medicinal utility of plants is mainly due to presence of various phytoconstituents such as alkaloid, tannins, flavonoids and phenolic compounds [1]. Phytochemicals are bioactive non nutrient plant derived chemicals which are beneficial to human health and disease prevention [2, 3]. These substance provide characteristic odour and colour to the plant as well as play an important role in plants natural defense mechanism and disease resistance [4]. Phytochemical screening is crucial for validating the traditional use of medicinal plants. Furthermore GC-MS analysis technique can be used to study traditional medicine and to characterize the compounds of interest [5].

Family Malvaceae consist of approximately 244 genera and 4225 species [6]. It is one of the largest families of Angiosperm. Many plants of this family have been used in traditional system of medicine from ancient time; still there are certain medicinal plants whose medicinal properties have not been explored properly.

Abutilon is an important medicinal plant from ancient times its species were used to treat various diseases and ailments such as rheumatism, demulcent, laxative, diuretic etc. [7, 8].Various phytoconstituents such as steroids, ester glycosides, flavonoids, triterpene etc. have been identified from *Abutilon* species [9-11].

Abutilon pannosum commonly known as 'Kanghibunti', is an erect hairy, perennial shrub distributed in open dry places all over Indian Thar Desert area of Rajasthan The whole plant is densely hairy with broadly ovate leaf, Orange yellow flowers and blackish brown schizocarpic awn less fruit [12, 13]. Traditionally Abutilon *pannosum* is used in cleaning wound and ulcer and treating urinary tract infection, diabetes, hemorrhoids and anemia. [14] Although *Abutilon pannosum* is pantropic in distribution yet the species has been explored very little due to lack of knowledge about its phytoconstituents.

Taxonomic treatment of *Abutilon pannosum*
Kingdom : Plantae
Subkingdom : Viridaeplantae (green plants)
Infrakingdom : Streptophyta (land plants)
Division : Tracheophyta (vascular plants)
Subdivision : Spermatophytina (seed plants)
Infradivision : Angiospermae (flowering plants)
Class : Magnoliopsida (Dicotyledons)
Subclass : Dilleniidae
Superorder : Rosanae
Order : Malvales Family: Malvaceae (mallows)
Subfamily : Malvoideae
Tribe : Abutilieae
Genus : *Abutilon Mill* (Indian mallow)
Present study was designed to determine the presence of various phytoconstituents in methanolic extract of leaves of the plant *Abutilon pannosum* by preliminary phytochemical screening and to identify possible specific compounds with their concentration through GC-MS analysis.

Materials and methods

Collection of plant material

Leaves of *Abutilon pannosum* were collected from open field of Baiji Talab road Jodhpur, Rajasthan of Indian Thar Desert area during December, 2017. Plant was identified and authenticated by BSI, Arid Zone Regional Centre, and Jodhpur.

Preparation of extract

Leaves were thoroughly washed in running tap water followed by distill water to remove any trace and dried in shade for 15 days. Dried leaves were made into coarse powder using electric grinder and stored in air tight container till further use.
10g of leaf powder was soaked in 100 ml of HPLC Grade methanol (1:10 w/v) in a beaker for 48hours with occasional stirring. The extract was filtered with sterile muslin cloth followed by Whatmann filter paper No.1.Further the filtrate was centrifuged at 2500 rpm for 15 minutes to get clear solution. The extract thus obtained was evaporated to dryness to obtain crude extract. The extract was preserved in brown bottles at 4◦C till further use [15].

Preliminary phytochemical screening

Preliminary phytochemical screening of methanolic crude extract of *Abutilon pannosum* was carried out using standard methods [16, 17] to evaluate presence or absence of major primary and secondary metabolites such as Carbohydrates, Protein, alkaloid, steroid, phenol, Glycosides, terpenoids and flavonoids etc.

GC-MS analysis and identification of components

For GC-MS analysis crude extract was redissolved in methanol to make stock solution.1 µl of stock solution was used in GC-MS analysis.
Gas chromatography-Mass spectrometry(GC-MS) analysis of the methanolic leaf extract was carried out at USIC, AIRF,JNU, New Delhi with GC-MS- QP 2010 Shimadzu,Japan equipped with thermal desorption system TD 20. Helium gas (99.99%) was used as a carrier gas at a constant flow rate –total flow: 64.7 ml/min. and column flow: 1.21 ml/min. injector and mass transfer line temperature were set at 200 and 240°C respectively. The oven temperature was programmed (column Oven Temp: 80 °C and Injection Temp.: 260.00°C).Total running time of GC-MS was 50 minutes. The relative percentage amount of each component was calculated by comparing its average peak area to the total area, software adopted to handle mass spectra and chromatograms was Turbo mass. The relative percentage of the each extract constituents were expressed as percentage with peak area. The phytoconstituents were determined by comparing their retention time and mass weight with authentic samples obtained by GC as well as from the mass spectra from the Willey libraries and National Institute of Standards and Technology (NIST) database having 62000 patterns.

Results and Discussion

Preliminary qualitative phytochemical screening of methanolic extract of Abutilon *pannosum* by common methods revealed presence of carbohydrates, amino acids, alkaloids, Phenols, Flavonoids, phytosterols, tannins, terpenoids, and glycosides (Table. 1).
GC-MS chromatogram of leaf methanolic extract of the plant *Abutilon pannosum* showed various peaks indicating presence of 50 phytochemical compounds (Fig. 2). The bioactive principles were confirmed and identified on comparison of the mass spectra with the Willey spectral library and NIST database by their retention time and peak area (Table 2). The principle components identified with highest peak area was n-Hexadicanoic acid (13.38) at retention time 25.47 minutes and lowest peak area recorded was of 2-Butanone, 4-(2, 6, 6-trimethyl-1, 3-cyclohexadien-1-yl)-(0.15) with retention time 16.97 minutes. Present study reports phytoconstituents in the spectrum which are known for various biological properties like that Phytol, a diterpene known to possess anti-microbial, anti-cancer, anti-inflammatory and diuretic properties [18]. Phytol shows cytotoxic activity against breast cancer cell line, it is also a precursor of Vitamin E and Vitamin K [19, 20].
Similarly Vitamin E protects the body as an antioxidant, protecting molecules and tissues from harmful free radicals. It plays an important role in skin protection [21]. Lupeol is anti-microbial, anti- inflammatory, anti-cancerous diterpene [22]. Lup-20(29)-en-3-one also known as Lupenone is a lupine type triterpenoid. Phytochemical screening of lupenone revealed many important biological properties including anti-inflammatory, antivirus, anti-diabetes and anti-cancer activities [23].
2, 3 Dihydrobenzofuran is an essential oil used in the treatment of diabetic retinopathy and arthritis [24]. 9, 12-octadecadienoic acid (Z, Z)-, methyl ester has anticancer properties [25]. Biosynthesis of various phytosterols is necessary for normal activity of plants some of these phytosterols are important in medicine such as stigmasterol reported shows thyroid inhibiting, anti- peroxidative and hypoglycemic properties [26]. Ergost-5-en-3-ol, (3.beta.,24R) also known as Campesterol shows antioxidant and anticancerous properties [27]. gamma–Sitosterol has been reported to contain antidiabetic [28] and anticancerous properties [29]. Neophytadien is known to possess antibacterial properties [30]. It is useful in treatment of headache, rheumatism and some skin diseases [31]. Naphthalene is an anti-microbial agent [32]. Similarly 2-Methoxy 4 -vinyl phenol identified is a phenolic compound, reported to have potent anti-inflammatory effect [33]. Squalene has antioxidant, anticancerous and cholesterol lowering properties [34]. Beside these various fatty acids and their derivatives were recorded on GC-MS chromatogram that have importance in medicines such as n-Hexadicanoic acid shows anti-inflammatory properties [35]. and also an antioxidant, hypocholestrolemic,

nematicide,pesticide, antiandrogenic [36]. Tocopherols play an important role as antioxidants and also in maintaining membrane stability in plants [37] Alpha Tocospiro A and alpha Tocospiro B are tocopherols and has been proven to show antioxidant properties [38].

Table 1: Phytochemical Screening in methanolic extract of *Abutilon pannosum*

S. No.	Phytochemical Components	Test	Methanolic extract of *Abutilon pannosum*
1.	Carbohydrates	Molisch's test	++
		Fehling's test	++
2.	Proteins and Amino Acids	Ninhydrin test	++
		Xanthoproteic test	++
3.	Alkaloids	Dragendrof 's test	++
		Wagner's test	++
4.	Phenols	Ferric chloride test	++
		Lead acetate test	++
5.	Flavonoids	Shinoda test	++
		Alkaline reagent test	++
6.	Phytosterol	Salkowski test	++
		Libermann Burchard's test	++
7.	Glycosides	Keller-kilani test	++
		NaOH test	++
8.	Saponin	Froth test	++
		Olive oil test	++
9.	Gums and Mucilages	Alcohol test	--
		Ruthenium red test	++
10.	Oils and Fats	Spot test	++

Table 2: Phytochemical compounds identified in methanolic leaf extract of *Abutilon pannosum*

S. No.	R.T. (min.)	Compound name	Molecular formula	Molecular weight	Peak area %
1	11.497	1-(2-hydroxyethyl)-4-methyl piperazine	$C_7H_{16}N_2O$	144	4.32
2	11.855	1,3-dideoxy-4,5-o-(1-methylethylidene)-2-o-{[2-(trimethylsilyl) ethoxy] Methyl} hexitol	$C_{15}H_{32}O_5Si$	320	0.39
3	12.136	Naphthalene	$C_{10}H_8$	128	8.36
4	12.431	Cyclohexene, 3,3-dimethyl-1-(trimethylsilyloxy)-	$C_{11}H_{22}OSi$	198	0.28
5	12.980	2,3-dihydro-benzofuran	C_8H_80	120	1.91
6	13.942	1-Dimethylvinylsilyloxy-3-methylbenzene	$C_{11}H_{16}OSi$	192	0.64
7	14.483	1-Piperazineethanamine, N,N,4-trimethyl-	$C_9H_{21}N_3$	171	2.58
8	14.690	2-methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	0.49
9	15.375	Phenol, 2,6-dimethoxy-	$C_8H_{10}O_3$	154	0.33
10	16.979	2-Butanone, 4-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-	$C_{13}H_{20}O$	192	0.15
11	17.538	9-desoxo-9-xi-hydroxy-3,7,8,9,12-pentaacetat-ingol	$C_{30}H_{42}O_{11}$	578	0.73
12	17.620	3-Buten-2-one, 4-(3-hydroxy-6,6-dimethyl-2-methylenecyclohexyl	$C_{13}H_{20}O_2$	208	0.68
13	17.875	2-Buten-1-ol, 2-ethyl-4-(2,2,3-trimethyl-3-cyclopenten-1-yl)-	$C_{14}H_{24}O$	208	1.55
14	18.259	5-Octyn-4-one, 2,2,7,7-tetramethyl-	$C_{12}H_{20}O$	180	0.26
15	18.782	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-,	$C_{11}H_{16}O_2$	180	0.54
16	19.328	2-Ethyl-1,3-bis(trimethylsilyloxy)propane	$C_{11}H_{28}O_2Si_2$	248	0.65
17	20.475	Succinic acid, 3,7-dimethyloct-6-en-1-yl nonyl ester	$C_{23}H_{42}O_4$	382	0.40
18	21.069	10,11-dihydroxy-3,7,11-trimethyl-2,6-dodecadienyl acetate	$C_{17}H_{30}O_4$	298	0.38
19	21.273	(4-Methyl-piperazin-1-yl)-acetic acid, fluoren-9-ylidene-hydrazide	$C_{20}H_{22}N_4O$	334	0.46
20	22.299	4-(1-Hydroxyallyl)-2-methoxyphenol	$C_{10}H_{12}O_3$	180	0.78
21	23.614	Neophytadiene	$C_{20}H_{38}$	278	3.41
22	23.692	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	1.18
23	23.974	Neophytadiene	$C_{20}H_{38}$	278	0.95
24	24.247	Neophytadiene	$C_{20}H_{38}$	278	1.26
25	24.893	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1.29
26	25.478	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	13.38
27	26.538	Palmitic Acid, TMS derivative	$C_{19}H_{40}O_2Si$	328	5.14
28	27.196	9,12-octadecadienoic acid (z,z)-, methyl ester	$C_{19}H_{34}O_2$	294	0.41
29	27.279	3,6-octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	0.89
30	27.488	Phytol	$C_{20}H_{40}O$	296	3.58
31	27.845	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	$C_{17}H_{32}O$	252	4.44
32	28.206	Phytol, TMS derivative	$C_{23}H_{48}OSi$	368	7.32
33	28.749	.Alpha.-linolenic acid, tms derivative	$C_{21}H_{38}O_2Si$	350	0.97
34	29.080	Stearic acid, TMS derivative	$C_{21}H_{44}O_2$	356	0.93
35	29.610	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	$C_{12}H_{23}NO_2$	213	1.32
36	29.763	Glycidylpalmitate	$C_{19}H_{36}O_3$	312	0.47
37	30.416	4,8,12,16-Tetramethylheptadecan-4-olide	$C_{21}H_{40}O_2$	324	0.45
38	30.878	Hexanedioic acid, bis(2-ethylhexyl) ester	$C_{22}H_{42}O_4$	370	0.41

39	31.812	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	$C_{12}H_{23}NO_2$	213	0.58
40	33.264	Hexadecanoic acid, 4-[(trimethylsilyl)oxy]butylester	$C_{23}H_{48}O_3Si$	400	1.48
41	35.170	2-ethylhexyl ethylphosphonofluoridoate	$C_{10}H_{22}FO_2P$	224	0.49
42	35.386	O1-Nonanoyl-O3-octanoyl-glycerol, TMS derivative	$C_{23}H_{46}O_5Si$	430	0.68
43	35.593	9-octadecenamide	$C_{18}H_{35}NO$	281	0.35
44	35.956	Squalene	$C_{30}H_{50}$	410	1.22
45	36.294	.Alpha.-Tocospiro A	$C_{29}H_{50}O_4$	462	0.72
46	36.561	.Alpha.-Tocospiro B	$C_{29}H_{50}O_4$	462	1.07
47	37.358	Hexadecane, 1-methoxy-13-methyl-	$C_{18}H_{38}O$	270	0.53
48	40.572	Vitamin E	$C_{29}H_{50}O_2$	430	1.16
49	42.746	Ergost-5-en-3-ol, (3.beta.,24r)-	$C_{28}H_{48}O$	400	1.66
50	43.359	Stigmasterol	$C_{29}H_{48}O$	412	5.85
51	44.937	Gamma.-sitosterol	$C_{29}H_{50}O$	414	4.16
52	46.322	Lup-20(29)-en-3-one	$C_{30}H_{48}O$	424	2.62
53	47.333	Lupeol	$C_{30}H_{50}O$	426	3.76
					100.00

Table 3: Major phytoconstituents identified in methanolic leaf extract of *Abutilon pannosum*and their biological activities

S. No.	Compound name	Compound class	Biological activity of compound
1.	Phytol	Diterpene	Antimicrobial, anticancer, anti-inflammatory properties[18]
2.	Squalene	Triterpene	Anticancerous,antioxidant,hypocholestrolemic.[34]
3.	Lupeol	Diterpene	Antimicrobial, anticancerous, anti-inflammatory.[22]
4.	Lupenone	triterpenoid	Antivirus, antidiabetes, anticancer activities.[23]
5.	2-3 dihydro- benzofuran	Essential oil	Diabetic retinopathy, arthritis.[24]
6.	9,12-octadecadeinoic acid(Z,Z)-,methylester	Fatty acid derivative	Anticancer properties.[25]
7.	n-Hexadecanoic acid	Fatty acid	Anti-inflammatory,anti-oxidant,antiandrogenic,hypocholestrolemic.[35,36]
8.	Stigmasterol	Phytosterol	Thyroid inhibiting, anti-peroxidative, hypoglycemic.[26]
9.	Ergost 5-en-3ol 3beta,24R	Phytosterol	Antioxidant, anticancerous properties.[27]
10.	gamma-Sitosterol	Phytosterol	Antidiabetic, anticancerous properties.[28]
11.	Neophytadiene	Sesquiterpenoid	Antibacterial properties.[30]
12.	Naphthalene	Aromatic hydrocarbon	Antimicrobial agent.[32]
13.	2 Methoxy 4 vinyl phenol	Phenolic compound	Anti-inflammatory.[33]
14.	Vitamin E	Alpha tocopherol	Antioxidant, skin protection. [21]
15.	alpha Tocospiro A	Tocopherol	Antioxidant.[37,38]
16.	alpha Tocospiro B	Tocopherol	Antioxidant.[37,38]

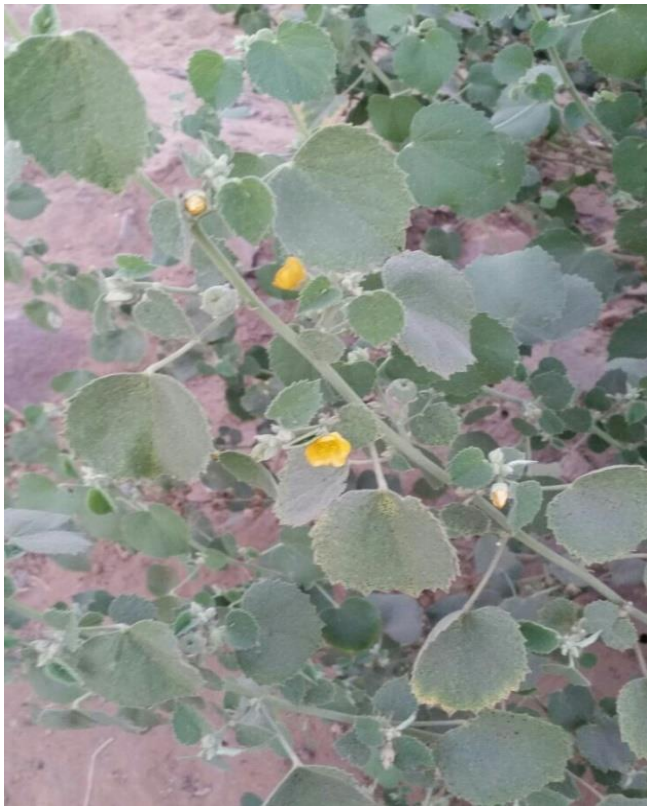

Fig 1: *Abutilon pannosum* field photograph

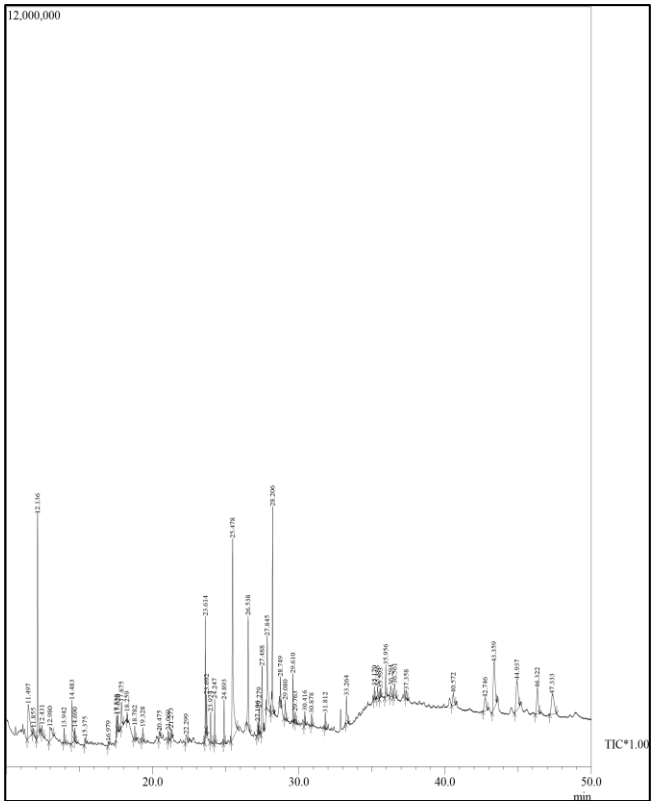

Fig 2: GC-MS chromatogram of *Abutilon pannosum* methanol

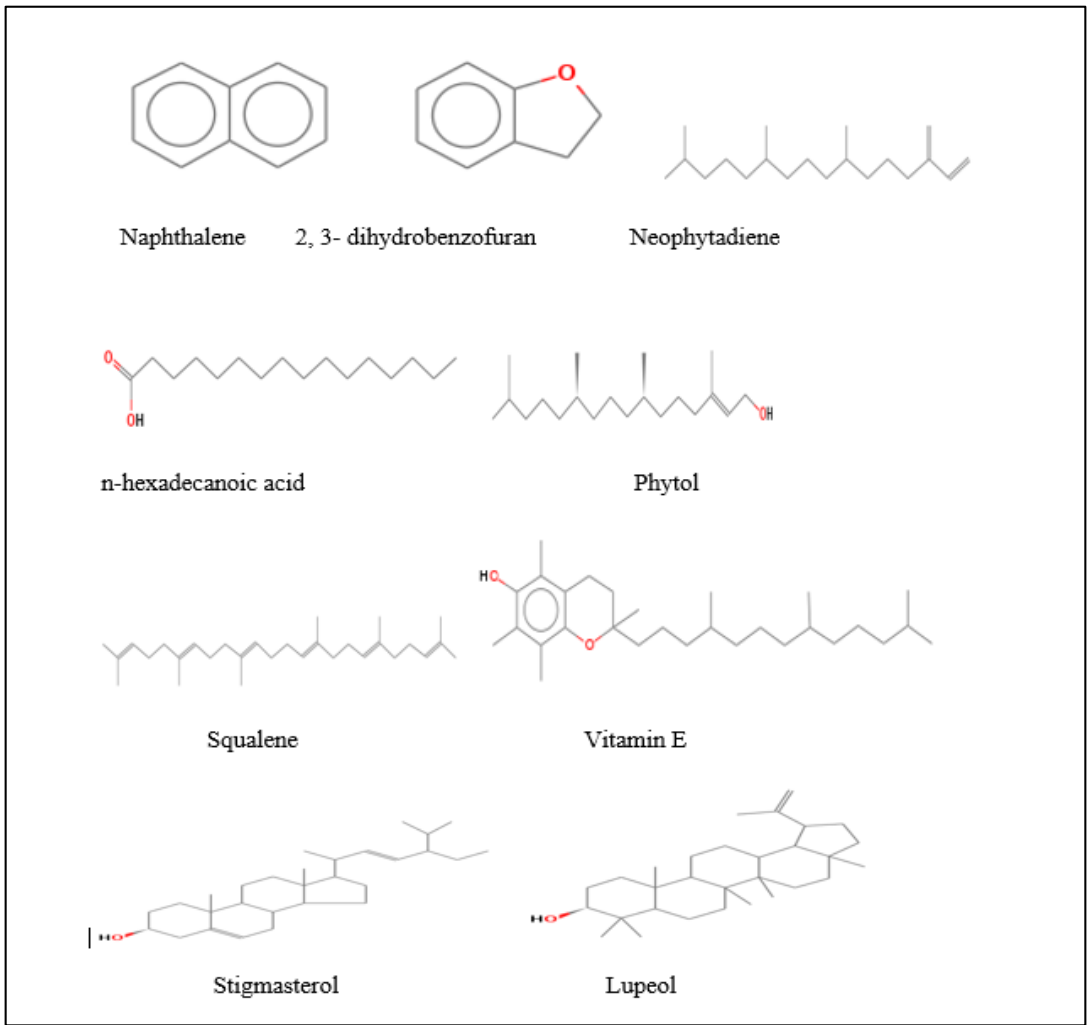

Fig 3: Molecular structure of some phytochemical constituents identified in methanolic leaf extract of *Abutilon pannosum*

Conclusion

Thus presence of various phytochemical compounds in the leaves of *Abutilon pannosum* justifies the medicinal use of the plant. Although other species of *Abutilon* such as *Abutilon indicum* has been proven important medicinally and explored well. *Abutilon pannosum* despite of its wide occurrence as weed has been explored very less so the present study results could provide future insight of this plant to be useful in pharmaceutical industries for welfare of human being after testing of its toxicology.

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