Progress in Chemical and Biochemical Research

Journal homepage: www.pcbiochemres.com

Investigation of phytochemicals in methanolic leaves extracts of *Abutilon pannosum* and *Grewia tenax* by Q-TOF LC/MS

Mital K. Aadesariya [a], Vijay R. Ram [a], Pragnesh N. Dave [a,b] *

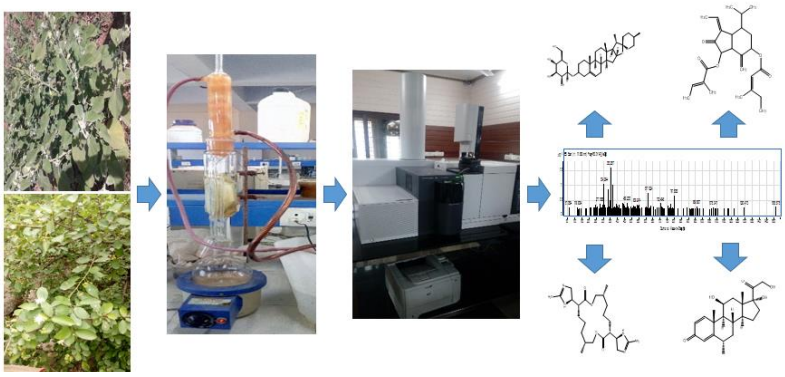
[a] *Department of Chemistry, KSKV Kachchh University, Bhuj, Kachchh- 370001*

[b] *Department of Chemistry, Sardar Patel University, Vallabh Vidynagar-388 120 (Gujarat), India*

ARTICLE INFO

Article history:
Received: 06 February 2019
Accepted: 10 February 2019
Available online: 13 February 2019
Manuscript ID: PCBR-1810-1022

GRAPHICAL ABSTRACT



KEYWORDS

Abutilon pannosum
Grewia tenax
Phytochemicals
LC-Q-TOF-MS
Soxhlet extraction

ABSTRACT

The current study was carried out on the extraction, isolation, and identification of phytochemical constituents existing in the *Abutilon pannosum* and *Grewia tenax* leaves extract collected from Kachchh region of Gujarat state, India. The main aim of this study was to expose important polar phytochemicals in locally available medicinal plants using LC-Q-TOF-MS analytical techniques. The main analytical tool is liquid chromatography quadrupole time of flight mass spectrometry (LC-Q-TOF-MS) which is one of the most sophisticated and sensitive instruments that gives qualitative as well as quantitative information accurately with respect to constituents present in the sample. The continuous extraction of plant leaves was commenced by Soxhlet extractor utilizing methanol as a solvent. The crude methanolic extracts were inserted in LC-Q-TOF-MS for identification and isolation of useful phytochemicals. The results of phytochemical analysis displayed that there were significant phytochemicals found in methanol extracts of *A. pannosum* leaves (APL) such as alkaloids, photoproteins, antibioticand in *G. tenax* leaves (GTL) like alkaloids, photoproteins, glycoside, terpenoids, fatty amides, steroids, fatty alcohols, saponins, flavones, flavonols, steroid etc. Hence, methanolic extract of GTL gives good medicinal activity as compared to the APL.

* Corresponding author: Tel: +91-02692-226856
E-mail: pragnesh7@yahoo.com
†Electronic Supplementary Information (ESI) available.

1. introduction

Plants spices have been utilized to cure many diseases for a long time. In today's time, despite the fact that synthetic drugs are promptly accessible and exceedingly powerful in curing different diseases, there are persons who still incline toward utilizing traditional pharmaceuticals on account of their less harmful impacts [1]. There are a wide diversity of compounds, particularly auxiliary metabolites, which are confined from plants having antibacterial, anticancer, anti-inflammatory, analgesic, antiviral antitumor, and many other activities to a major or minor extent. Well-known examples of these phytochemical compounds incorporate phenols, glycosides, flavonoids, phenolic, saponins and stilbenes, cyanogenic glycosides, nitrogen compounds (betalains, amines, alkaloids), tannins, terpenoids, and few different endogenous metabolites [2].

LC-Q-TOF-MS has quickly been integrated by the investigative network as intense and strong instruments with extraordinary abilities. Specifically, they syndicate the high performance of TOF analysis in both the MS and tandem MS (MS/MS) modes, with the recognized and broadly utilized systems of electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) [3].

Abutilon pannosum (family Malvaeae) is commonly known as Kanghi or khapat [4]. Its extract is used for getting rid of thirst, curing bronchitis, dysentery, gonorrhea, diarrhea, inflammation of the bladder, reducing fever and various other diseases [5, 6].

Fig. 1. *Abutilon pannosum*

Fig. 2. *Grewia tenax*

Grewia tenax (Family: Tiliaceae), commonly known as Gangeti and or guddaim is a valuable plant species in Kachchh region [7, 8]. *G. tenax* is presumed to cure distress of stomach, skin and intestinal infections, fever, diarrhea, cough, dysentery, hepatic disorders, jaundice, rheumatism and have calm antibiotic properties [9, 10]. Leaves branches of *G. tenax* are significant element of traditional medicine for the curing of tonsillitis, trachoma and are used as a poultice to treat swelling [11]. The plant species have free radical scavenging activities which might be in charge of the remedial action against tissue damage [12,13].

2. Materials and Methods

2.1. Collection of plant samples

APL and GTL were collected from Padmavati temple farm, Asambiya road, and Punitvan, Bhuj, Gujarat, India.

2.2. Preparation of samples

The fresh leaves of *A. pannosum* and *G. tenax* were cleaned with water to take out dirt and foreign materials and legitimately dried in shade for 2-3 weeks. Lastly, crispy leaves were ground in a mortar grinder, screened through the mesh screen and put away in the air tight pack.

2.3. Materials and glasswares required

APL and GTL powder, Methanol, condenser, Thimble, RBF (round bottom flask), measuring flask, and heating mantle.

2.4. Preparation of Extracts

15 mg of leaves powder was extracted with 2-3 liter of 95 % methanol (60-64 °C) utilizing Soxhlet apparatus by continuous hot percolation method. Later, it was clarified and the elimination of solvent was done by the distillation process. The extract was collected in the quartz tube. The sample methanolic crude was dissolved in 0.9 ml methanol and 0.1ml 0.1% formic

acid in glass tubes. Examination and detection of the compounds were done by LC-Q-TOF-MS instrument. The mass spectrometer was equipped with an ESI Jet Stream source.

2.5 LC-Q-TOF-MS Method

The separation of the extracts has been performed using an Agilent LC-Q-TOF-MS 6540, Ultra-High Definition (UHD).

2.5.1. LC-Parameter

The infused test volume was 10 µL. Versatile phases A and B were water and acetonitrile with 0.1% formic acid correspondingly. The flow rate was 0.6 mL/min. A 16 min run time was utilized for every examination. The streamlined chromatographic method held the initial mobile phase composition (10% B) consistent for 0 min, trailed by a straight inclination to 100% B after 14 minutes, and returned back (10% B) at 14 minutes. The framework included a binary pump and vacuum degasser, well-plate auto-sampler with a six-port micro-switching valve and a thermostated column compartment. Tests were stacked onto a Reprosil C_{18} column (2.0 mm x 150 mm, 2.5 µm–Dr Maisch, Germany) for metabolite partition.

2.5.2 Q-TOF Parameter

The LC system was associated with an Agilent 6450 Q-TOF equipped with dual electrospray jet stream technology operating in the positive ion mode. The working parameters were listed as below:

Capillary voltage: 4000V; nebulizer pressure: 45 psi(N_2); gas temperature:325°C; drying gas: 8 L/min; nozzle voltage: 1000V; fragmentor voltage: 150 V; skimmer voltage: 65V, m/z; 100 to 1700, sheath gas flow 11 L/min and sheath gas temperature: 350°C.

The data documentation was handled with Agilent Mass Hunter software.

3. Result and Discussion

3.1 *A. pannosum*methanol extract

Table 1 represents the important phytoconstituents found in the methanol extract of *A. pannosum*.

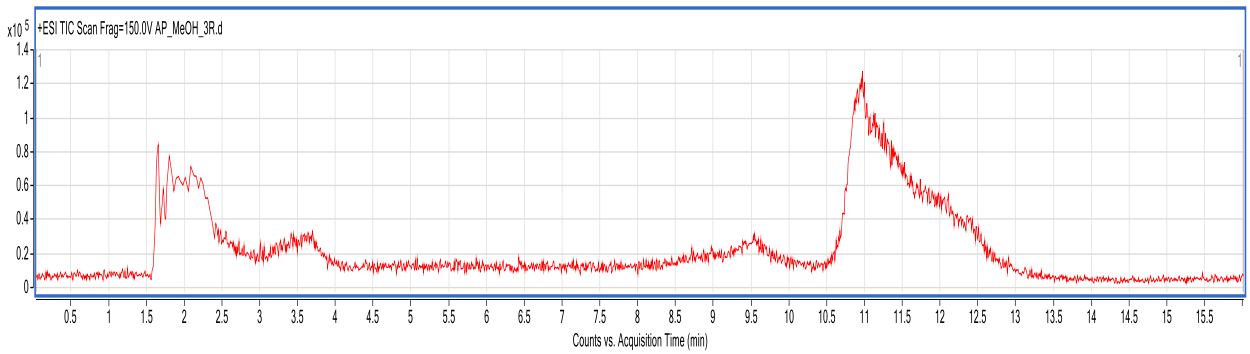


Fig. 3. Chromatogram of methanol leaves extract of *A. pannosum*

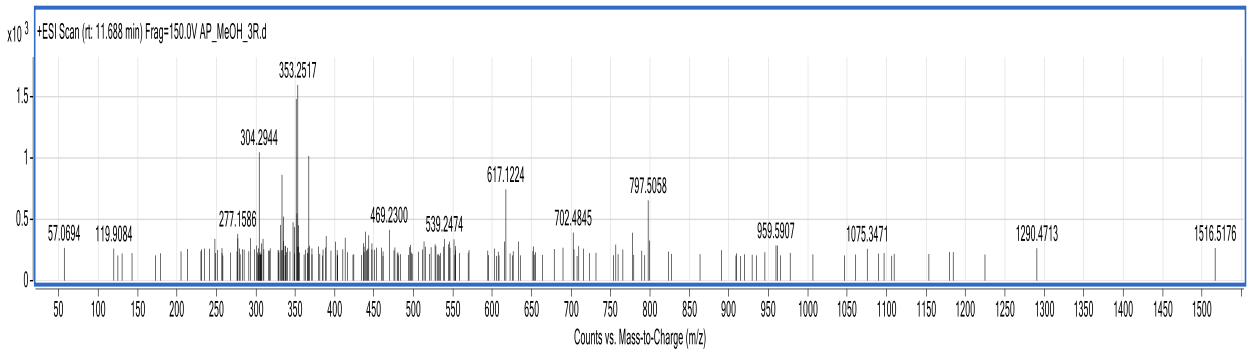
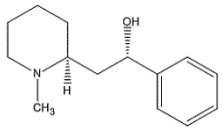
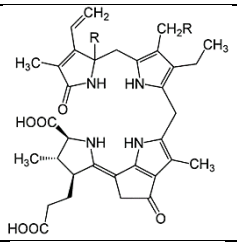
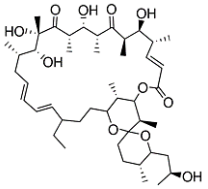


Fig. 4. Mass spectrum of methanol leaves extract of *A. pannosum*

Table 1. Methanol extract of *A. pannosum* leaves

Sr	Name	Formula	RT	Metlin ID	Note	Structure	Ref
1	(-)-Sedamine	$C_{14}H_{21}NO$	11.081	64453	Alkaloids		14
2	Oxidized dinoflagellateluciferin	$C_{33}H_{38}N_4O_7$	12.078	73309	Photoproteins		15
3	Oligomycin A	$C_{45}H_{74}O_{11}$	12.348	68981	Macrolides (antibiotics)		16

3.2 Methanol extract of *G. tenax* leaves

Table 2 represents methanol extract of *G. tenax*.

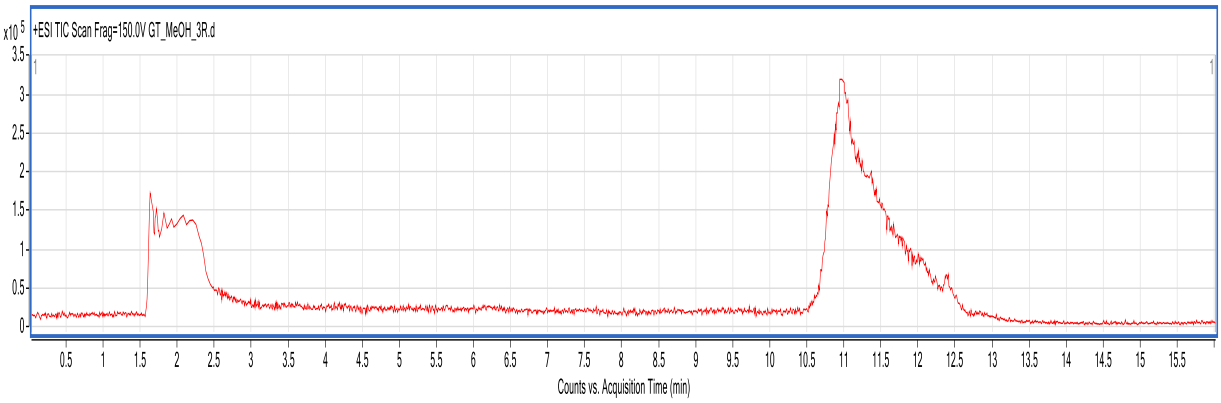


Fig. 5. Chromatogram of methanol leaves extract of *G. tenax*

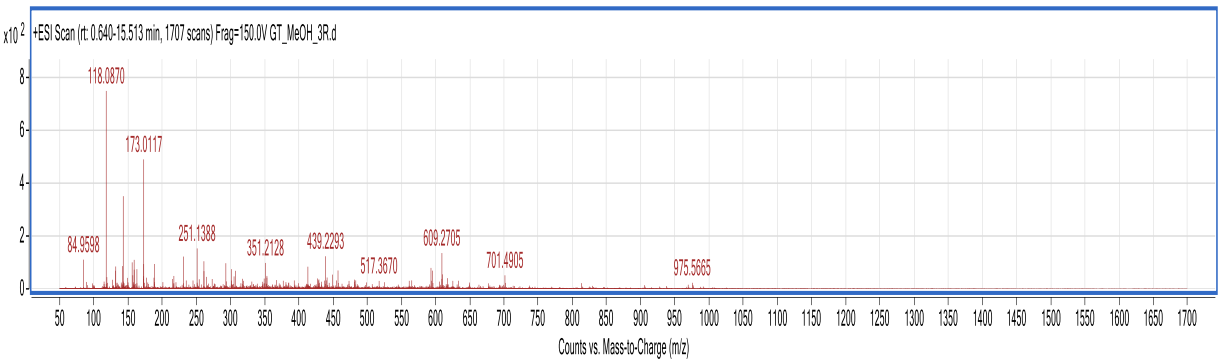
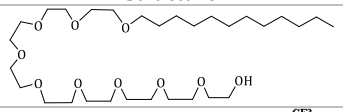
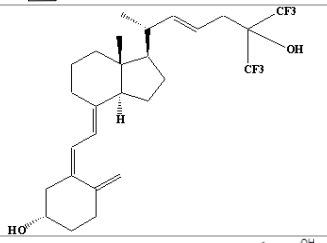
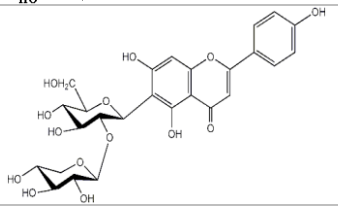
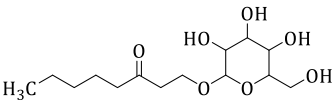
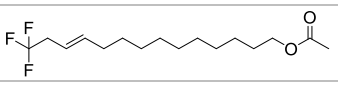
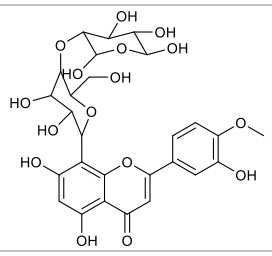
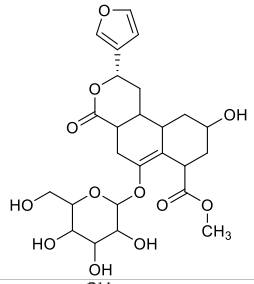
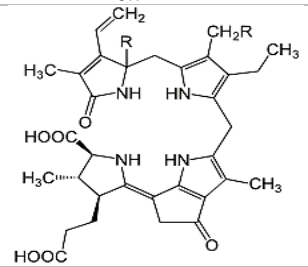
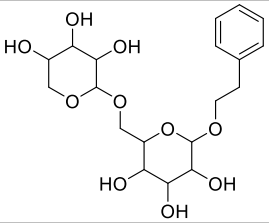
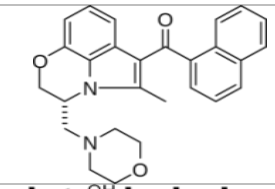
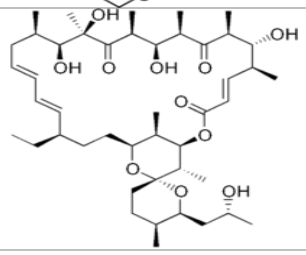
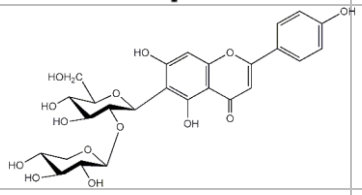
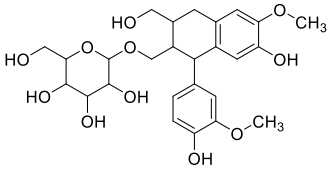
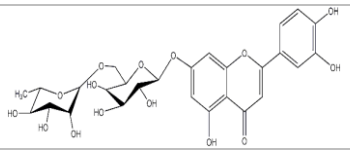


Fig. 6. Mass spectrum of methanol leaves extract of *G. tenax*

Table 2. Methanolic extraction of *G. tenax* leaves sample

Sr	Name	Formula	RT	Metlin ID	Note	Structure	Ref.
1	Polidocanol	$C_{30}H_{62}O_{10}$	12.385	69582	Alkaloids		17
2	(22E)-26,26,26,27,27,27-hexafluoro-25-hydroxy-22,23-didehydrovitamin D3	$C_{27}H_{36}F_6O_2$	12.109	42021	Secosteroids		18
3	Isovitexin 2''-O-xyloside	$C_{26}H_{28}O_{14}$	11.014	48672	Flavonoid, cardio-tonic, diuretic and in prostate diseases		19
4	1-(beta-D-Glucopyranosyloxy)-3-octanone	$C_{14}H_{26}O_7$	10.785	87578	Fatty acyl glycosides		20
5	14,14,14-Trifluoro-11E-tetradecenyl acetate	$C_{16}H_{27}F_3O_2$	11.51	46326	Fatty Ester		21
6	8-C-Glucosyldiosmetin 4''-O-rhamnopyranoside	$C_{28}H_{32}O_{15}$	11.171	49237	Flavones		22
7	Diosbulbinoside F	$C_{26}H_{34}O_{12}$	10.937	91803	fatty acyl glycosides		23
8	Oxidized dinoflagellateluciferin	$C_{33}H_{38}N_4O_7$	12.078	73309	Photoproteins		24
9	Phenylethylprimeveroside	$C_{19}H_{28}O_{10}$	10.937	95688	o-glycosyl		21

10	(Â±)-WIN 55,212	$C_{27}H_{26}N_2O_3$	10.832	96657	Cannabinoid receptors		25
11	Oligomycin A	$C_{45}H_{74}O_{11}$	12.348	68981	Macrolide antibiotics, Inhibitor of ATP synthase		26
12	Isovitexin 2''-O-xyloside	$C_{26}H_{28}O_{14}$	11.014	48672	Flavonoid		27
13	Isolariciresinol 9-O-beta-D-glucoside	$C_{26}H_{34}O_{11}$	11.034	88815	Nutrient		21
14	Luteolin 7-rhamnosyl(1->6)galactoside	$C_{27}H_{30}O_{15}$	10.975	49141	Flavonoid		28

Terpenoids were present only in *G. tenax* leaves extraction. Terpenoids are helpful in the treatment of a few diseases, including cancer. Terpenoids which have additionally antimicrobial, antifungal, anti-inflammatory, analgesic, antiviral, anti-hyperglycemic, anti-parasitic, antispasmodic, anti-allergenic, and immunomodulatory properties [29]. Furthermore, terpenoids can be utilized as defensive substances in storing farming items as they are referred to possess insecticidal properties as well [30]. **Steroids** were present in both plant leaves extraction. It ought to be noticed that steroidal compounds are of much significance and of interest in pharmacy because of their association with sex hormones and anti-bacterial activity. Plant sterols may also possess anti-oxidative properties. Steroids are responsible for cholesterol-reducing properties. Steroids also help in regulating the immune response [31]. **Flavonoids** are absent only in methanolic extract of *A. pannosum*. It is a potent water-soluble antioxidant and has strong anticancer activity [32]. **Carotenoids** might perform as antioxidants and might exhibit anticancer impacts insome specific animal models, using specific carcinogens [33]. MeOH extract of *G. tenax* containing **glycosides** are recognized to exert a useful action on the immune system by improving body strength, thus are valuable as dietary supplements. Glycosides additionally have broad medicinal efficacy as they are found in relatively most medicinal plant [34]. **Fatty acid** present in the *G. tenax* leaves powder methanol extract. It may have been used as an antibacterial and antifungal activity [35]. **Alkaloids** are present in all extract of the sample. It represents a class which influences the central nervous system, diminishes appetite and performs as a diuretic [14]. **Polidocanol** is a local anaesthetic and antipruritic component of ointments, relieves itching caused by eczema and dry skin, also used as a sclerosant, an irritant injected to treat varicose veins [17]. **Proteins** are present in *A. pannosum*and*G. Tenax* methanolic extract. Antioxidants and antimicrobial properties of extracts from several plants have recently been of great importance in both research and the food industry [35].

4. Conclusion

The present study reveals the important phytochemicals from the methanolic extract of APL and GTL by the use of powerful, sophisticated and

advance instrument LC-Q-TOF-MS. The qualitative and quantitative analysis was precisely done helping us to find active constituent in plant sample. From the above results it can be concluded that *A. pannosum* and *G. tenax* have the potential to work as a source of useful drugs due to the existence of numerous phytochemical active components. It gives alkaloid, flavonoid, terpenoid, carotenoid, sterol lipid, photoproteins, secosteroids, flavones, and flavanol. GTL exhibited decenttherapeutic activity in comparison to the APL. Though further studies may be used for the advancement of conventional medicines and to introduce novel active compounds from the therapeutic plants they may produce a novel way to treat several incurable illnesses.

Acknowledgement

Our sincere gratitude to the Center of Excellence in chemical science & Technology, KSKV Kachchh University, Bhuj. We are also grateful to Junagadh Agriculture Food Testing Laboratory for their guidance and allowing us to use the facilities.

5. Reference

[1] Iqbal E., Salim K. A., Lim L. B. L., *J. King Saud Univ.Sci.*, 2015,**27**:224
[2] Nostro A., Germanò M. P., D'angelo V., Marino A., Cannatelli M. A.,*Lett.ApplMicrobiol*, 2000,**30**:379
[3] Shevchenko A.A., Chernushevich I.V., Ens W., Standing K.G., Thomson B., Wilm M, Mann M.,*Rapid Commun Mass Spectrom.*,1997, **11**:1015
[4] Abedin S.,*Pak. J. Bot*., 1980,**12**:43
[5] Sammia Y., Studies on bioactive natural products of selected species of family Malvaceae,*Ph. D. Thesis, Department of Chemistry GC University*, Lahore, 2008
[6] Anup A.,*Bioscience Discovery*, 2012,**3**:317-320
[7] Habiba U., Anwar M., Hussain I., Rais M.,*J. AnimalPlantSci.*, 2015,**25**:650
[8] Aboagarib E., Yang R., Hua X., Siddeeg A., *J. Food Nutrit. Res.*,2014,**2**:187
[9] Tan H. Y. et al., *Evidence-based Complement. Altern. Med.*, 2016
[10] Sharma N., Patni V., *Asian J. Pharm.Clin. Res.*, 2012, **5:**28
[11] Khemiss F., Ghoul-Mazgar S., Moshtaghie A., Saidane D., *J. Ethnopharm.* 2006,**103**:90
[12] Gebauer J., Patzelt A., Hammer K., Buerkert A., *Genet. Resour. Crop Evol.*, 2007, **54**:1153
[13] DaffallaH. M., ElsheikhA. M., AliH. A., and KhalfalaM. M., *Environ. Exp. Biol.*, 2016, **14**:75
[14] El Ghazali G.E.B., El Tohami M.S., El Egami A.A.B., Abdalla W.S., Mohammed M.G.,*Omdurman Islamic University Printing and Publishing House*, Omdurman, 1997
[15] Mishra D. N., Thorat A. K., *Int. J. ChemTech Res.*, 2017, **10:**723
[16] Lebreton J., *Synthesis (Stuttg)*, 2002, 9192
[17] Doganci S. et al., *Eur. Rev. Med. Pharmacol. Sci.*, 2016, 1
[18] Aadesariya M. K., Ram V. R., Dave P. N., *Int. J. Adv. Res. Chem. Sci.*, 2017, **4**(10); 1
[19] Abdelhady M. I. S., Motaal A. A., *Brazilian J. Pharmacogn.*, 2016, **26:**763
[20] A. Maroyi,The Scientific World Journal, 2018, **2018**:1
[21] Qingwei W. et al., *Molecules,* 2012, **17**:12330
[22] De Sousa E. A., Da Silva A. A. C. A., Cavalheiro A. J., Lago J. H. G., Chaves M. H., *J. Braz. Chem. Soc.*, 2014, **25**:704
[23] Varsha. G. J., *Int. J. Environ. Sci. Nat. Resour.*, 2017, **4**:1
[24] Tapondjou L.A., Jenett-Siems K., Bottger S., Melzig M.F.,*Phytochemistry*, 2013, **95**:341
[25] Hastings J.W., *J. Mol. Evol.*,1983, **19**:309
[26] Akpabio U. D., Udo U. E., Akpakpan A. E., *Asian J. Plant Sci. Res.*, 2012, **2:**607
[27] Symersky J., Osowski D., Walters D. E., Mueller D. M., *Proc. Natl. Acad. Sci.*, 2012, **109:**13961
[28] Plazonić A., *Molecules*,2009, **14**:2466
[29] Pattanaik B., Lindberg P., *Life*, 2015, **5:**269
[30] Linn C., Lakshmi V., Aa M., Kumar R., *Herb. Med.*, 2017, 1–5
[31] Aadesariya M. K., Ram V. R., Dave P. N., *Int. J. Adv. Res. Chem. Sci.*, 2017, **4**:1
[32] Asif M., Khodadadi E., *J. Paramed. Sci.*, 2013, **4**:119
[33] Geetha T. S., Geetha N., *Int. J. PharmTech Res.*, 2014, **6:**521
[34] Krishnaiah T., Bono A., Sarbatly R., *J. Med. Plants Res.*, 2009, **3**:67
[35] Murugan M., Mohan V. R., *J. Med. Plants Stud.*, 2014, **2**:51