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Phytochemical analysis of leaf extract of *Balanites aegyptica* L. by HRLC-MS analysis

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Abstract
Phytochemical analysis of leaves of *Balanites aegyptica* L. were carried out by using HRLC-MS spectra method. It is highly sensitive and used for rapid identification of bioactive compounds. These mass spectra are fingerprint of that compound which can be identified from the data library. It may be the first report of its kind to analyze the bioactive compounds of *Balanites aegyptica* L. The phytochemical study indicates the presence of important bioactive compounds which can be used to treat many diseases in medical field. Phytochemical analysis of methanolic leaf extract of *Balanites aegyptica* L. spectrum profile shows total 100 compounds, out of that 23 major compounds were confirmed on the basis of their retention time, mass, molecular formula and mass per charge ratio (m/z). The findings of this study suggested that, *Balanites aegyptica* L. is a source of important bioactive compounds. It may be contributing natural antimicrobial, antioxident, anti-inflammentary activity and further investigations which may lead to the development of drug formulation.

Keywords: phytochemical, antioxident, anti-inflammentary activity and bioactive

1. Introduction
Phytochemicals are naturally present in the plants and play significant role to defend themselves against various pathogenic microbes by showing the antimicrobial activity by inhibition or killing mechanisms. These compounds were varying from plant to plant, some produce large and some produce in small quantity. Recently plant-derived substances became great interest to know their various applications (Baris, 2006) [3]. Ethno pharmacologists, botanists, microbiologists, and natural-product chemists are searching the earth for phytochemicals which could be developed for the treatment of infectious diseases (Tanaka, 2006) [17] especially in drug-resistant microorganisms and to produce more effective antimicrobial agents.
Balanites aegyptiaca L. is one of the most common but neglected wild plant species of the dry land areas. The leaves, bark and unripe fruits of this plant are reported to have anthelmintic, antifertility, purgative and antidysentric properties. The use of this plant is described in many folk books including Ayurveda and different biologically active phytoconstituents were isolated from plant (Kumawat, 2012) [11]. Saboo (2014) [16] revealed that chemically *Balanites aegyptiaca* is very enriched plant; present the number of Chemical constituents like protein, lipid, carbohydrate, alkaloid, saponin, flavonoid, and organic acid quercetin-3-rutinoside;furanocoumarin bergapten and dihydrofuranocumarin D- marmesin, beta-sitosterol, bergapten, marmesin, and beta-sitosterol glucoside,balanitin-1,-2, and -3, balanitoside yamogenin. Scientifically this plant is reported as good anthelmintic, antibacterial, antivenin, anticancer, anti-inflammatory and analgesic activity in vitro. It also shows antioxidant, xanthine oxidase and acetylcholinesterase inhibitory, antinociceptive, antidiabetic, antiviral, wound healing, Hypocholesterolemic and Diuretic activity. Keeping this in view, phytochemical analysis of leaves of *Balanites aegyptica* L. were carried out by using HRLC-MS spectra method.

2. Materials and Methods
2.1 Preparation of sample
Fresh and healthy leaves of *Balanites aegyptica* L. was collected from Shendra MIDC, Aurangabad (MS). The leaves were collected in sterile polythene bags and brought to the laboratory and washed with running tap water. These were dried in shade at room temperature till it gets constant weight. The dried material were crushed in mortar - pestle and then in mixture grinder to make fine powder.

2.2 Preparation of extracts

The prepared powder samples were filtered through muslin cloth and 25 gm of fine powder were extracted with methanol as a solvent by using soxhlet extractor for 18 hours at 65^0C. These extracts were filtered through Whatman filter paper no. 42 and concentrated at 40^0C by using an evaporator and store in umber bottle at 4^0C. These extract were send for HR-LCMS (High Resolution Liquid Chromatography and Mass Spectroscopy) analysis to Sophisticated Analytical Instrumentation Facility, Indian Institute of Technology, Powai, Mumbai, India, for the detection of various phytochemicals.

2.3 HRLC-MS analysis

Equipment and conditions Identification of metabolites from an active sub-fraction of methanol extract was carried out at SAIF, IIT Powai, and Mumbai. Samples were analyzed on a LC-ESI-Q-TOF-MS (Agilent Technologies 6550 i-Funnel) system equipped with a G4220B pump, G4226A auto sampler and G1316C, and a diode array detector (DAD). The elution solvent consisted of a gradient system of 0.1% formic acid in water (A) and acetonitrile (B) at a flow rate of 0.3 ml/min. The gradient system started with 95% A: 5% B reaching 5% A: 95% B in 50 min. then back to initial composition 95% A: 5% B in 10 min which was held at same composition for 5 min. The MS analysis was carried out by ESI positive ionization mode. MS source conditions were as follows: capillary voltage 3500 V, Gas temperature 250 C, drying gas flow 13 L/min, sheath Gas temp 300, sheath Gas Flow 11, nebulizing gas pressure 35 (psig), fragmentor 175 V, Skimmer 65 V, OctopoleRFPeak 750 V, and mass range m/z 50–1000. The resolution was 40,000 FWHM. Metlin database was used to structure confirmation.

3. Results and Discussions

The chemical profile of *Balanites aegyptica* L. was characterized by using HRLC-MS spectra. The relative concentrations of various compounds getting eluted as a function of retention time gives in chromatogram. The relative concentration of the bioactive compounds present in the plants was indicated by the height of peak. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. These mass spectra are fingerprint of that compound which can be identified from the data library. The results are relevant to HRLC-MS analysis of methanolic leaf extract of *Balanites aegyptica* L. spectrum profile (fig.1) shows total 100 compounds present. Out of that 23 major compounds were confirmed on the basis of their retention time, molecular formula and mass. These active compounds are presented in Table 1. The findings of this study suggested that, *Balanites aegyptica* L. is a source of important bioactive compounds. It may be contributing natural antimicrobial, antioxident, anti-inflammentary activity and further investigations which may lead to the development of drug formulation.

This result was confirmed by many authors. Farid (2002) [8] observed that antifungal activity may be due to presence of several triterpene saponins and steroidal saponins in *B. aegyptiaca*. *B. aegyptiaca* acts as a potential natural larvicidal agent against mosquito larvae due to the larvicidal activities present in the saponin rich extracts in the various tissues such as fruit pulp, kernel, root, bark and leaf studied by Weisman (2006). Doughari (2007) [7] observed the leaf extracts of *B. aegyptiaca*, prepared in aqueous and organic solvents were tested for their antibacterial activity against *Salmonella typhi*, by using the disc diffusion method. Ethanolic extracts demonstrated higher antibacterial activity, while the aqueous extracts has shown the least activity. The preliminary phytochemical analysis revealed the presence of saponins, tannins, phenols and anthraquinones in the extracts, and these were considered for antibacterial activity.

Yadav and Panghal (2010) [19] and Daya and Vaghasiya (2011) [6] reviewed traditional uses, phytochemistry and pharmacological properties of *Balanites aegyptiaca*. Six flavonides, glycosides identified as quercetin 3-glucosides, quercetin 3-rutinoside, 3-glucoside, 3-glucosides, 3-rutinoside, 3-7 diglucoside and 3-rhamnogalactosides of isorhamnetin have been extracted and identified from the leaves and branches of the Egyptian plant species. Peter (2011) studied antimalarial activity of seeds of *Balanites aegyptiaca* in vitro and in vivo. Compounds of the extract show growth inhibition and activity against *plasmodial* aminopeptidase. These results suggested that the detected 6-phenyl-2(H)-1, 2, 4-triazin-5-one oxime might contribute to the antiplasmodial activity. Roland (2011) [15] studied antibacterial and anti-inflammatory activities of galls and leaves from *Balanites aegyptiaca* (L.) Del.

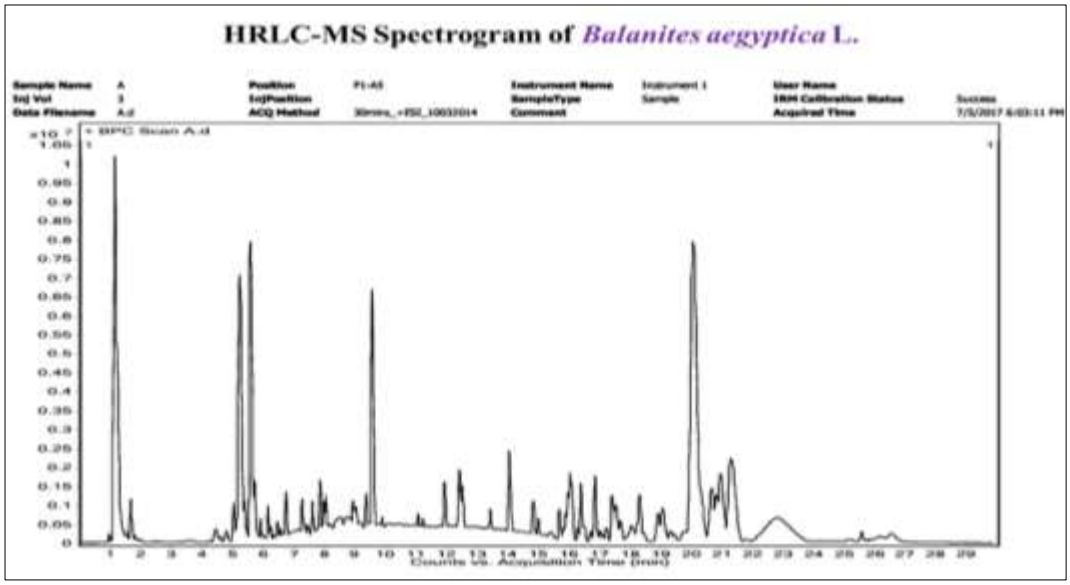

Fig 1: HRLC-MS Spectrogram of *Balanites aegyptica* L.

Table 1: Major Phytochemicals from leaves of *Balanites aegyptica* L. by HRLC-MS

Sr. No.	Name	RT	Mass	Formula	m/z
1	Methyl N-(a- methylbutyryl) glycine	1.169	173.10	$C_8H_{15}NO_3$	156.10
2	Carnitine	1.203	162.11	$C_7H_{16}NO_3$	144.10
3	Neuraminic acid	1.676	267.09	$C_9H_{17}NO_8$	268.10
4	Carisoprodol	5.14	260.17	$C_{12}H_{24}N_2O_4$	265.15
5	13-Ethyl-6a,17-dihydroxy- 18,19-dinor-17a-pregna- 4,9,11-trien-20-yn-3-one	5.242	324.17	$C_{21}H_{24}O_3$	307.17
6	Tetrahydrotrimethylhispidin	5.655	292.13	$C_{16}H_{20}O_5$	297.11
7	Petunidin	6.246	317.06	$C_{16}H_{13}O_7$	317.06
8	1-Hydroxyvitamin D3 3-D- glucopyranoside	7.464	576.36	$C_{33}H_{52}O_8$	577.37
9	Meptazinol	7.614	233.17	$C_{15}H_{23}NO$	216.17
10	10-nitro,9Z,12Z- octadecadienoic acid	7.868	325.22	$C_{18}H_{31}NO_4$	308.22
11	Dihydrodeoxystreptomycin	9.569	567.28	$C_{21}H_{41}N_7O_{11}$	568.29
12	Acarbose (M7)	9.97	552.24	$C_{24}H_{40}O_{14}$	557.22
13	Usnic acid	13.58	344.08	C18H16O7	327.07
14	1alpha, 25-dihydroxy-22-thia- 20-epivitamin D3	14.05	434.28	C26H42O3S	457.27
15	Lactone of PGF-MUM	14.83	296.16	C16H24O5	301.14
16	9,13-dihydroxy-12-ethoxy-10- octadecenoic acid	16.08	358.26	C20H38O5	341.26
17	Hyderocortisone-17- Butyrate	16.49	432.25	C25H36O6	437.23
18	Presqualene diphosphate	16.85	586.32	C30H52O7P2	587.33
19	Dodecaprenyl phosphate- galacturonic acid	17.46	614.32	C31H51O10P	615.32
20	Cholic acid glucuronide	18.25	584.31	C30H48O11	585.31
21	11-(4-acetoxymethyl phenyl)- 1α, 25-dihydroxy-9, 11- didehydro vitamin D3	19.00	562.36	C36H50O5	585.35
22	Trandolapril glucuronide	20.07	606.28	C30H42O11	607.29
23	3 β,6alpha,7α-Trihydroxy-5β-cholan-24- oic Acid	20.96	408.28	C24H40O5	413.26

Table 2: Biological properties of the bioactive compounds of *Balanites aegyptica* L**.**

S. No	Compound	Biological activity	References
1	Methyl N-(a- methylbutyryl) glycine	Associated with autism and mental retardation	PubChem CID:193872
2	Carnitine	Antioxidant activity, Antimicrobial	Katarzyna et el (2010) , Olgun *et al* (2004)
3	Neuraminic acid	Regulation of the immune response and the progression of colon cancer.	Dall'Olio F. (2000)
4	Carisoprodol	Skeletal muscle relaxant	PubChem CID:2576
5	13-Ethyl-6a,17-dihydroxy- 18,19-dinor-17a-pregna- 4,9,11-trien-20-yn-3-one	Prevention of pregnancy in women	PubChem CID: 6291
6	Tetrahydrotrimethylhispidin	Antioxidant activity	Arti Goggi, Nutan Malpathak (2017)
7	Petunidin	Antioxidant, Anti-inflammatory activity	Miguel, M. G. (2011)
8	1-Hydroxyvitamin D3 3-D- glucopyranoside	Regulation of the calcium balance and the bone metabolism	PubChem CID:5282181
9	Meptazinol	Induction of Analgesia or Narcosis.	PubChem CID: 65483
10	10-nitro,9Z,12Z- octadecadienoic acid	-	
11	Dihydrodeoxystreptomycin	-	
12	Acarbose (M7)	For treatment and management of diabetes type II	PubChem CID: 444254
13	Usnic acid	Anti-inflammatory, analgesic, healing, antioxidant, antimicrobial, antiprotozoal, antiviral, larvicidal and UV protection.	Araujo (2015)
14	1alpha, 25-dihydroxy-22-thia- 20-epivitamin D3	Bone Density Conservation Agents; Calcium Channel Agonist; Vitamins	PubChem CID: 9953732
15	Lactone of PGF-MUM	antimicrobial, cytotoxic, anti-inflammatory, antiviral, antibacterial, and antifungal activities	Chaturvedi (2011); Kreuger *et al* (2012)
16	9,13-dihydroxy-12-ethoxy-10- octadecenoic acid	-	-
17	Hyderocortisone-17- Butyrate	used to treat or prevent skin disorders or for the routine care of skin	PubChem CID: 26133
18	Presqualene diphosphate	-	-
19	Dodecaprenyl phosphate- galacturonic acid	-	-
20	Cholic acid glucuronide	-	-
21	11-(4-acetoxymethyl phenyl)- 1α, 25-dihydroxy-9, 11- didehydro vitamin D_3	Bone Density Conservation Agents; Calcium Channel Agonist; Vitamins	PubChem CID:5280453
22	Trandolapril glucuronide	Mild to moderate hypertension	PubChem CID:5484727
23	(Hyocholic Acid) 3 β,6alpha,7α-Trihydroxy-5β-cholan-24- oic Acid	-	PubChem CID:92805

4. Conclusion

The phytochemical study of the leaves of *Balanites aegyptica* L. using HRLC-MS indicates the presence of fatty acids, organic compounds, phenolics, alkaloids, phytoharmone, coenzyme, aminopyrimidines, dipeptide and tripeptides like important bioactive compounds. It may be contributing

natural antimicrobial, antioxident, anti-inflammentary activity (Table 2) and further investigations which may lead to the development of drug formulation.

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