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GC MS analysis of the bark of *Holarrhena antidysenterica*

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Abstract
Holarrhena antidysenterica commonly called kutaja belongs to the family Apocynaceae. Kutaja is a medicinal herb used for the treatment of bronchitis, asthma, piles, leprosy, and diarrhoea etc. The present study was carried out to identify the phytoconstituents present in the ethanolic extract of the bark of H. antidysentrica by GC MS analysis. From the GCMS twenty two compounds were identified.

Keywords: *Holarrhena antidysenterica*, phytoconstituents, GC MS analysis

1. Introduction
Holarrhena antidysenterica is commonly known as kutaja. It is an important plant used in indigenous system of medicine as a remedy for bronchitis, hematuria, epilepsy, asthma, piles, leprosy, eczema, diarrhoea, fever etc [2].
Holarrhena antidysenterica is a small deciduous tree with white flowers and found throughout the dry forests of India. It is widely used in traditional medicines for a variety of health disorders [3]. It is known to contain steroidal alkaloids, flavonoids, triterpenoids, phenolic acids, tannin, resins, etc. The alkaloids discovered from various parts of the plant include conessine, isoconessine, conessimine, holarrifine, kurchamide etc [4].
Holarrhena antidysenterica contains gut stimulatory and inhibitory constituents, mediating their effects via histaminergic and calcium antagonist pathways respectively which may explain the folklorioc use of the plant in gastrointestinal motility disorders such as constipation, diarrhoea is used in the Mirzapur and Varanasi district of UP for gastric problems. Tribes of Andhrapradesh uses the stem bark of this plant for skin diseases. The Bodo tribes of Assam also uses this plant as a traditional medicine [5]. In Ayurveda, this plant is used in classical formulation kutajarishtam, kutajavaleha etc. It is classically known for curing amoebiasis, diarrhoea, skin disorders etc. It is widely studied for its antidiabetic activity which is found in seed extract and the ethanolic extract of seeds has been proved beneficial. Aquous, petroleum ether and methanolic extracts of kutaja seeds are known to have hyperglycemic and anti-hyperlipedemic activities at the dosage of 250mg per kg body weight in rats [8, 9, 10]. Apart from seeds the ethanolic extract of *Holarrhena antidysenterica* leaves also have anti diabetic properties when administered for 21 consecutive days in diabetic rats. The present study deals with the GC MS analysis of phytocomponents in the ethanolic extract of the bark of *Holarrhena antidysenterica*.

2. Materials and Methods
The bark of antidysentrica were collected. The dried bark powder was successively extracted with petroleum ether, ethyl acetate and methanol using soxhlet apparatus and finally after extraction with above solvent material, from the soxhlet it was taken out and was dissolved in ethanol .It was then subjected to GC MS analysis.

3. GC MS Analysis
GC MS analysis of these extracts were performed using a Varian GC MS system. Gas chromatograph interfaced with a mass spectrometer equipped with a fused silica capillary column, composed of 100% dimethylpolysiloxane. For GCMS detection an electron ionization system with ionizing energy of 70eV was used. Helium gas was used as the carrier gas used at a flow rate of 1ml/min and an injection volume of 1 µL was employed with a split ratio of 1:10 was used. Injector temperature was kept at 280^0C .The oven temperature was programmed with 50^0 Cto 300^0C. Total GC running time was 38 minutes. The relative percentage of each component was calculated by comparing its average peak to the total area.

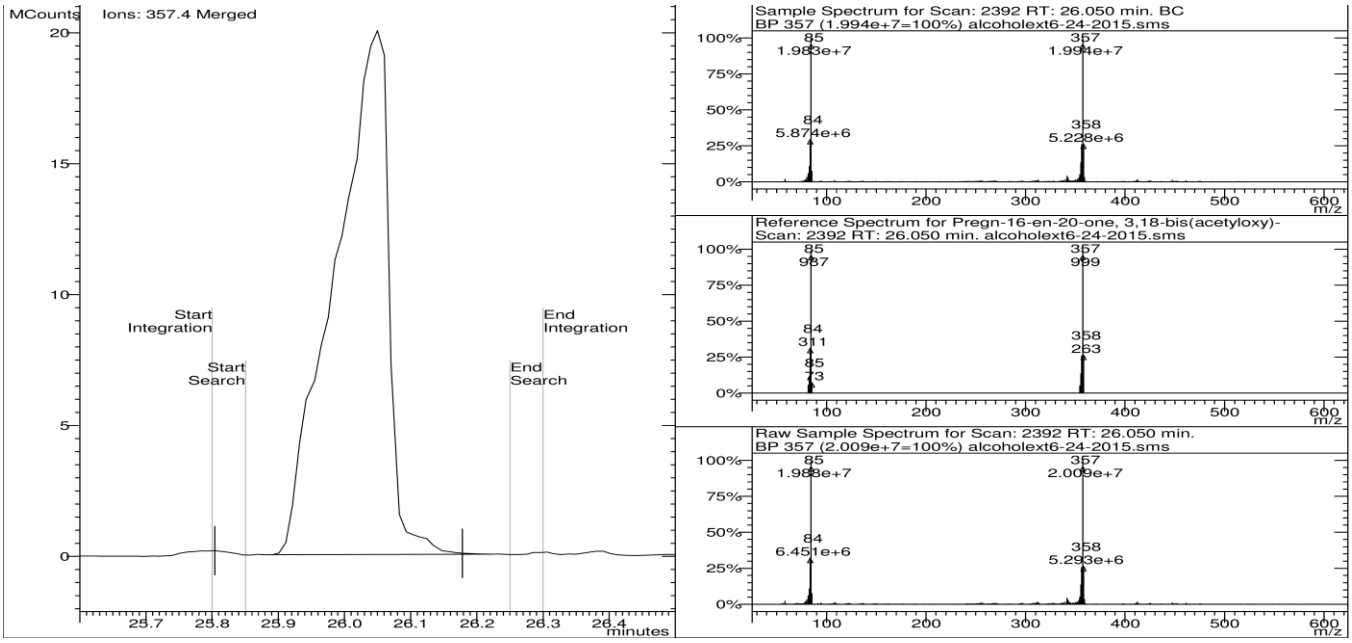
Interpretation on mass spectrum was conducted using the data base of the NIST (National Institute of Standards and Technology). The spectrum of the unknown compound was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of components of the test material was ascertained.

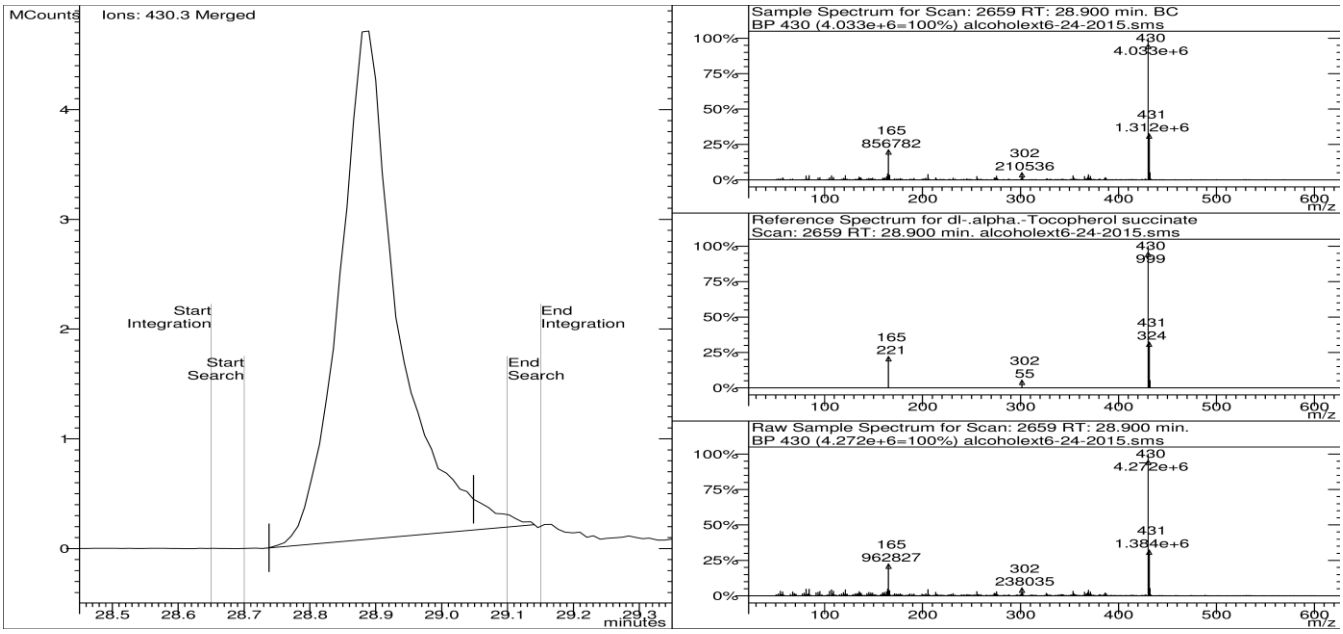
4. Results and Discussions

GC MS analysis was carried out in ethanolic extract of *Holarrhena antidysenterica*. Twenty two phytochemicals were detected. The twenty two compounds along with their retention times and peak area are given in the table 1.The mass spectrum of major compounds are also included and given below. From the GCMS study the compounds that are present in larger amounts are:pregn-16-en-20-one,3,18, bis (acetyloxy) (15.863%), 1-triethylsilyloxyheptadeccane(3.88 %),2H,6H-pyrano[3,2-b]xanthen-6-one,5,9,10(3.17%),dlalpha -Toccopherolsuccinate (4.065%)

Table 1

S No	Name of the compounds	Retention Time	Peak Area %
1	N-2-Carbamoyloxy ethyl carbamic acid	11.54	1.01
2.	3-dimethyl amino Benzene methanol	15.418	2.365
3	Trimethoquinol	16.881	1.0084
4	9-butyl-9,10-dihydro Acridine	17.908	2.125
5	2,7 dimethyl carbazole	18.175	0.732
6	2-phenyl -3-pyridinol,	18.862	0.659
7	pirlindole	19.685	0.201
8	Silanamine1,1,1-trimethyl –N(4-trime	21.55	0.611
9	10,13-Eicosadienoic acid methyl ester	21.842	0.167
10	2-[2-(4-chlorophenyl)-3-morpholin-4yl-3]	22.31	1.70
11	Methyltribenzocentrotriquinanol	24.09	2.2
12	1-[2-(4-bromophenoxy)ethyl]pyrrolidine	24.778	0.625
13	9,10-diphenyl anthracene	25.42	1.076
14	3,18 Bis acetyloxy pregn-16-en-20-one	26.048	15.863
15	4-imino-1,5,5-triphenyl-2-imidazolidinon	26.38	1.596
16	1-triethyl siloxyheptadeccane	26.6	3.88
17	5,9,10 -2H,6H-Pyrano[3,2-b] xanthen-6-one	26.68	3.17
18	dl-alpha-toccopherol succinate	28.85	4.065
19	desmosterol	29.410	0.45
20	campsterol	30.28	0.122
21	Cholesta-22,24-dien-5-ol	30.68	0.73
22	Lanosta-8,24-dien-3-one	31.35	1.939

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430 4.033e+6
431 1.312e+6
165 856782
302 210536
Reference Spectrum for dl-.alpha.-Tocopherol succinate
Scan: 2659 RT: 28.900 min. alcoholext6-24-2015.sms
430 999
431 324
165 221
302 55
Raw Sample Spectrum for Scan: 2659 RT: 28.900 min.
BP 430 (4.272e+6=100%) alcoholext6-24-2015.sms
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431 1.384e+6
165 962827
302 238035
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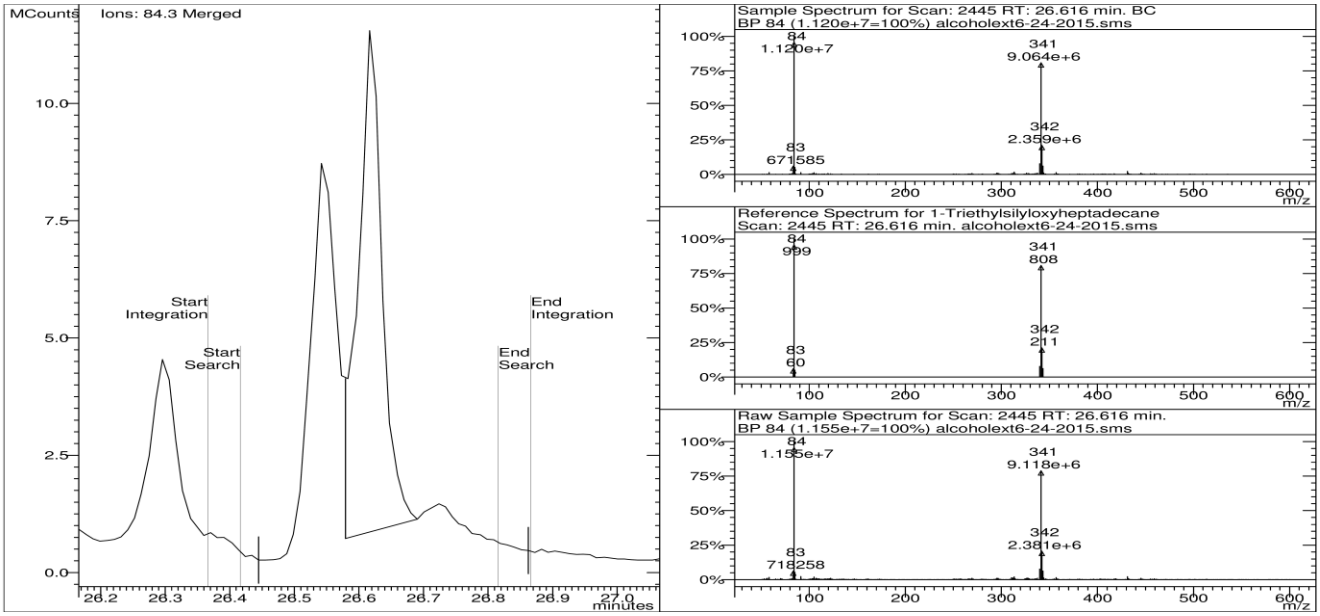
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84 1.120e+7
341 9.064e+6
342 2.359e+6
83 671585
Reference Spectrum for 1-Triethylsilyloxyheptadecane
Scan: 2445 RT: 26.616 min. alcoholext6-24-2015.sms
84 999
341 808
342 211
83 60
Raw Sample Spectrum for Scan: 2445 RT: 26.616 min.
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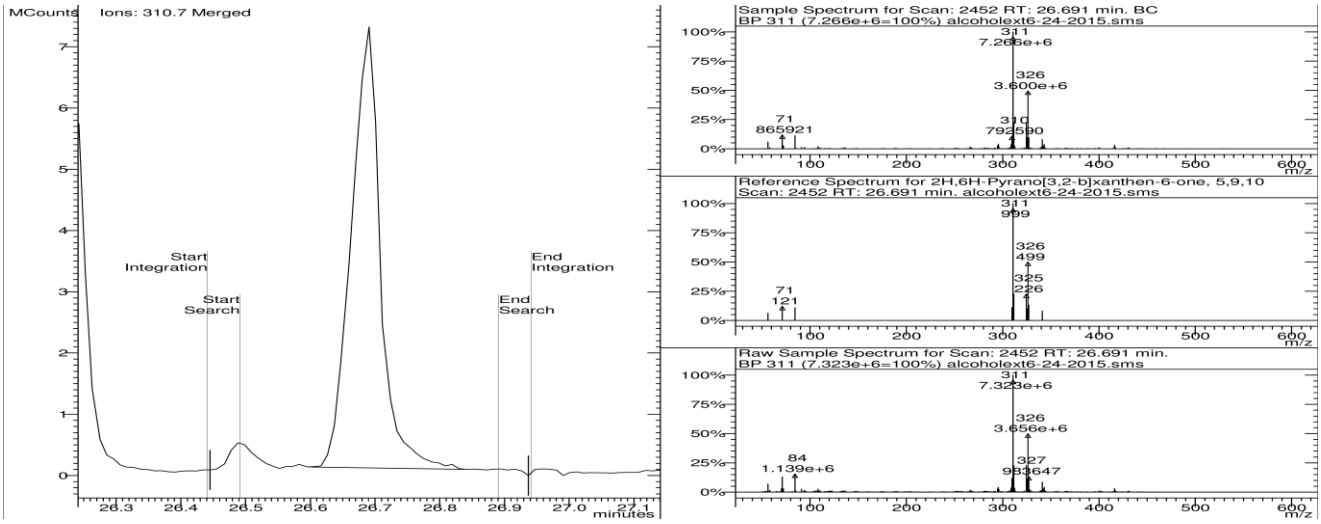
83 718258
m/z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minutes
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BP 311 (7.266e+6=100%) alcoholext6-24-2015.sms
311 7.266e+6
326 3.600e+6
310 792590
71 865921
Reference Spectrum for 2H,6H-Pyrano[3,2-b]xanthen-6-one, 5,9,10
Scan: 2452 RT: 26.691 min. alcoholext6-24-2015.sms
311 999
326 499
325 226
71 121
Raw Sample Spectrum for Scan: 2452 RT: 26.691 min.
BP 311 (7.323e+6=100%) alcoholext6-24-2015.sms
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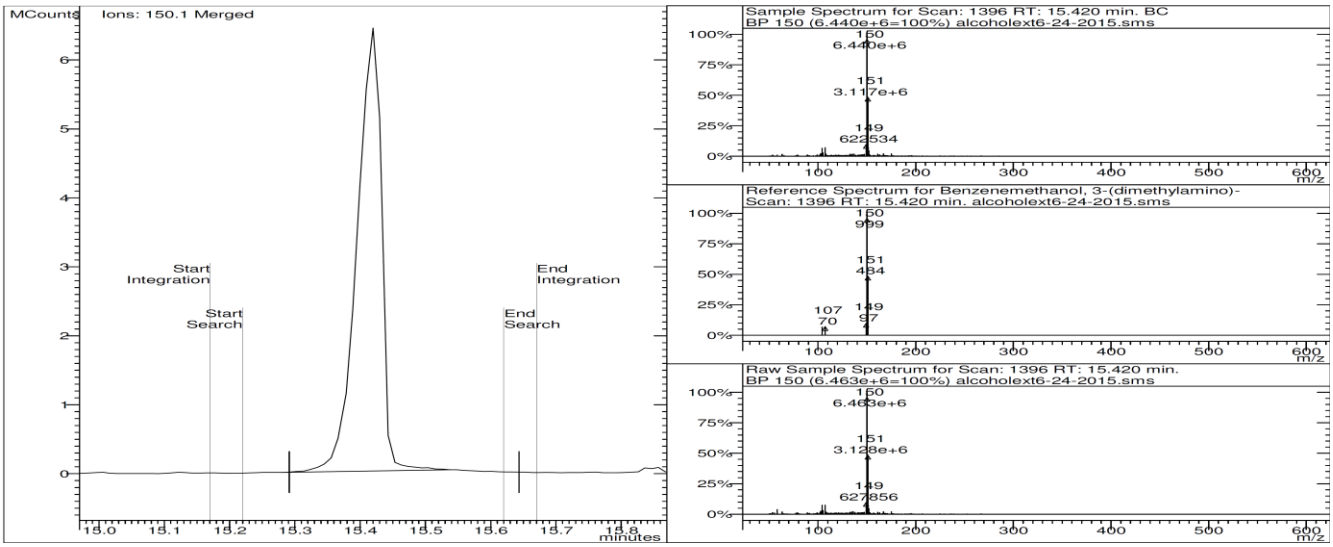

5. Conclusion

Understanding the availability of Phyto chemical compounds and pharmacological properties, GC MS is a valuable tool for reliable and novel identification of phytococmpounds .In the present study 22 compounds have been identified from the ethanolic extract of the bark of *Holarrhena antidysenterica* by GC MS analysis. Thus this type of GC MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study is helpful for further detailed study. Further investigation into the pharmacological properties of *Holarrhena antidysenterica* from various solvent extracts and detailed phytochemistry may add new knowledge to the information in the traditional medical systems.

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