Comparative GC-MS analysis of Alstonia scholaris (L.) R. Br leaf extract using methanol and hexane solvent.

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ABSTRACT:

The present study aimed to find out the variation in solubility of phyto-chemical content in two solvent extract. For this, leaf extract of *Alstonia scholaris* (R.Br.) was prepared and analyzed by GC-MS. The GC-MS analysis of *Alstonia scholaris* in methanol and hexane revealed presence of 35 and 29 compounds. The compounds like quinic acids; 1,3 propanediol 2- hydroxymethyl 2-nitro; beta D- glucopyranose 1, 6 anhydro; benzofuran 2,3 dihydro; catechol; hydroquinone; pentadecane; alpha methyl mannofuranoside, 2-O-methyl mannopyranosa1-methoxy-13 methyl obtained in methanolic extract only while linoleic acid ethyl ester and p-xylene in hexane extract. Hydrocarbons like pentane 2- methyl; pentane 3 methyl, cyclopentane methyl, cyclohexane; squalene; neophytadiene; phytol were common to both. It was evaluated that *Alstonia scholaris* showed more solubility to various groups of compounds in methanol whereas hexane was found to be best choice for extraction of hydrocarbon moieties.

Key words: *Alstonia scholaris*, GC-MS, methanol, hexane.

1. INTRODUCTION

Our planet earth is bestowed with huge biodiversity of plants. Plants are the only producer in food chain and food web in any ecosystems. Since from ancient time, humans were solely dependent on plants for food and medicines. The plants extract are also enriched with bioactive compounds benefitting us in innumerable ways [1, 2]. These phytochemicals are actually bio-macromolecules produce by plants to protect themselves from environmental stress and weather changes including infectious attack by insects, fungi and bacteria [3]. These phytochemicals provide many health benefits to plant. Recent research on use of phytochemicals revealed the fact that

phytochemicals are secondary metabolites and also served innumerable benefits to human against various diseases and pest control strategies [4].

The bioactive phytochemicals in plant parts can be analysed by GC-MS technique. It is best used to make an effective chemical analysis. This type of qualitative and quantitative analysis is must before proceeding for elaborated study using plant extract. The solubility of phytochemicals shows remarkable variation with respect to choice of solvent used for extraction of plant material.

Screening of various phytochemicals and its used in herbal therapy to cure many diseases once again explored plant based medicines as eco-friendly, safe, effective and inexpensive. For instance: *Alstonia scholaris*, one of the best known indigenous medicinal plants [5].

Alstonia scholaris commonly known as "saptparni" in Marathi means group of seven leaves. It belongs to family Apocynaceae. This plant found to be distributed in deciduous and evergreen forest, Western Ghats and Western Himalayas of India. It has been declared as state tree of West Bengal (India) [6]. It grows up to 17-20m in height, leaves are in whorls of 4-8 in upper exiles, upper surface is dark green and lower green white [7]. During October to December, the plant bears beautiful blossom. The flowers are small, 7-10 mm long greenish white in appearance and umbellately branched. It is commonly used for plantation on roadside [2,8].

Alstonia scholaris has been in used since from traditional ayurvedic science to today's recent research as antifungal, antimalarial, antiparasitic, antioxidant, antimicrobial, anthelmintic, analgesic , antidiabetic, antihyperlipidemic, hepatoprotective and antifertility [9,10,11,12].The objective of present study was to evaluate the effect of solvent on miscibility of phytochemicals used for extraction of plant materials.The miscibility of phytochemicals differs in polar and non polar solvent, hence choice of proper solvent is most important task for experiments aimed with the intention of studying activity of phytochemical[13].To achieved this, we analysed leaf extract of Alstonia scholaris in methanol-a polar solvent and hexane as non polar solvent and run the samples for gas chromatography and mass spectra for further studies.

2. MATERIALS AND METHODS

2.1 Collection of plant sample:

The leaves of *Alstonia scholaris*(L.) R. Br. was collected during March-April 2015 and 2016 from Mahyco colony, near Hotel Amber, Jalna. The plant sample was identified and authenticated by Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (M.S.). The herbarium was also submitted in Botany Dept. The accession number 0666 was assigned to *Alstonia scholaris* (L.)R. Br.

2.2 Extraction of plant material:

The leaves washed with tap water and shade dried at room temperature for one week. The dried leaf sample was ground using stainless steel blade in grinder to make fine powder and sieved through wheat mesh to remove coarse granules of sample. To prepare the leaf extract, 40 gm of powdered leaf sample of *Alstonia scholaris* (L.) R. Br. was packed in thimble (Whatman's filter paper no. 1) and placed in Soxhlet extractor (Make: Borosil, Glass and glassware) sequentially with hexane and methanol (Merck 99.9%) as solvent. The temperature of mantle was kept at 55^0 C. The solvent with thimble was run for 7-8 hrs in Soxhlet apparatus till solvent becomes decolourised [14].

2.3 Spectral analysis:

Well defined information in qualitative examination can be achieved by GC-MS. (Gas chromatography mass spectroscopy). GC-MS analysis of essential oil was performed by using Shimadzu GCMS coupled to QP 2020 instrument operating in electron impact (EI) mode with MS voltage 0.96 kV with the following specifications of program. Carrier gas; Helium with column flow rate of 0.99 ml/min and inlet flow pressure: 52.7KPa; column oven temperature and injector temp 50^0C and 250^0C respectively. Injection mode: split and split ratio 1:50, purge flow: 3 ml/min .Sample size: 1µl for 1 minute. Column SH-RXI-5silMS (30m x 0.25 mm x 0.25µm) was used. The program for column oven temperature was automated as follows: At 50^0C; it was held isothermal for 3mins and then increased the column temperature up to 320^0C at the rate of 30^0C/min with intermediate hold time at 200^0C (2 min.), 250^0C (4 min), 300^0C (4min.), 320^0C (1 min).The duration of one complete program was 23 min.MS transfer line temperature: 250^0C with acquisition mode scan type and scan range 35 -500 m/z. The mass spectral survey and identification was performed by using the NIST library of mass spectral search program [15].

3. Results and Discussion

Identification of chemical compound was based on details of peak area, retention time, molecular weight, molecular formula, mass spectral details and use of NIST library. GC-MS evaluation of methanolic and hexane leaves extract of *Alstonia scholaris (L.) R. Br* revealed the presence of 35 and 29 bioactive components respectively. The composition of methanolic and hexane extract comprises of hydrocarbon moiety (42.44 and 99.37%), hydrocarbon derivatives (8.65 and 0.03%), sesquiterpenes (2.98 and 1.65%), fatty acids (2.93 and 0.25%), sesquiterpenoid (1.36 and 0.20 %) respectively. The compounds like flavonoids (24.24%), tannins (9.7%), alkaloid (3.61%), phenols and alcohols (2.6%), coumaran and ketones (1.73%), sugar moiety (0.62%), terpenoid (0.13%) were extracted only in methanolic leaf extract of Alstonia scholaris whereas phenylethyl icosanoate (0.06%), linoleic acid(0.065), and unsaturated hydrocarbons (0.05%) in hexane extract. Table 1: Depicting comparative phytochemical analysis in leaf extract of Alstonia scholaris in polar solvent methanol and non polar hexane alongwith group of chemical compound, retention time, compound name, molecular weight, molecular formula, percent area composition of bioactive phytochemicals identified using GC-MS technique.

The comparative major components identified by GC-MS in both methanol and hexane include pentane,2-methyl-(1.40 and 31.30%); pentane,3-methyl-(1.38 and 31.09%); cyclopentane, methyl-(1.37 and 41.21%); and phytol (1.10 and 0.07%), squalene (2.53 and 1.63%) respectively.The other major medically important compounds identified only in methanolic extract includes n-hexane (37.82%); alpha Methyl mannofuranoside (12.26%); 2-O-methyl-D-mannopyranosa (11.98%); quinic acid (9.70%); ethane,1- chloro-1-fluoro(8.47%); 1,3-propanediol, 2-(hydroxymethyl)-2-nitro- (2.23%) and 9,12-Octadecadienoic acid(Z,Z)- (2.13%) whereas in hexane extract, bioactive compound cyclohexane (20.70%) was only identified.(Fig 1 , Fig 2 shows GC-MS chromatogram of methanol and hexane leaf extract of *Alstonia scholaris* respectively*).*

In present investigation, varieties of phytochemicals have been identified in methanolic leaf extract as compared to hexane extract. Irrespective of amount or concentration whether high or low in which these compounds were found to be present in extract. These bioactive compounds possess some pharmacological significance as well as biological activity.

It have been reported that phytochemicals acts as secondary metabolite and plays an important role in the treatment of diseases. Phukan P. and Phukan S.N. (2014) reported antimicrobial and antioxidant property of *Alstonia scholaris* plant [16]. According to Arulmozhi et al (2012) plants produce many bioactive components such as phenolics and flavonoids which possess antimicrobial, antioxidant property and also provide protection

from free radical scavenging activity [12]. Chakraborty P. et al (2016) determined the antimicrobial activity of *Alstonia scholaris* plant parts in methanol and hexane extract and showed that methanolic extract of plant parts has more potent antimicrobial activity as compared to hexane extract [1]. In the study by Arulmozhi S. (2011) suggested ethanolic extract of *Alstonia scholaris* has prominent antiarthritic action which may be attributed to its analgesic, anti-inflammatory, immunosuppressant and anti-oxidant activities [10] and same author in an year 2012 performed experiments on mice using leaf extract of *Alstonia scholaris* and proved its analgesic and anti-inflammatory effects [12]. Singh R. et al (2013) explained role of *Alstonia scholaris* leaf extract for its anticonvulsant and sedative action on swiss albino rats. He concluded that the chemical constituents present in ethanolic leaf extract of *Alstonia scholaris* have excellent antiepileptic and sedative potential [17]. Sarkhel S. and Ghosh R. (2017) correlated the traditional healer and demonstrated antivenom potential of aqueous *Alstonia scholaris Linn.* in the treatment of snakebite [18]. Surendran S. et al (2012) justified and proved in-vitro cytotoxic and antiproliferative effect of leaf extract on cancer cells [19]. Recently Malick Abdul and Hedge Karunakar (2018) also evaluated and concluded significant anticancer potential in one more species of *Alstonia* viz. ethanolic leaf extract of *Alstonia venenata* [20].

Hamdiani S.et al (2017) reported the presence of four alkaloids in leaf extract of *Alstonia scholaris*, these were akuammidine, nicotine, strictamine, and voacristine [21] in contrast to this, in present study authors found highest percent content of hydrocarbons fatty acids in both extract though more quantitatively in hexane. Compounds flavonoid, tannins, sugar, ketones, coumaran, phenols in methanol only. In present study, chemical constituents like neophytadiene, phytol, squalene, tannins resembles to GC-MS results from previous study by Swamy N.T. et al [22].The corresponding contribution of identified constituents for various biological activities can be summarized as under. The bioactive components such as hydrocarbons and fatty acids possess larvicidal activity [23,24,25].The biological activities contributed by phytol include antimicrobial, anti-inflammatory, anticancer, antimalarial and antifungal [20,26,27]. Squalene served to possess antimicrobial, anti-oxidant, pesticide, antitumour, cancer preventive, immunostimulent, chemopreventive, and lipoxygenase inhibitor [28] while neophytadiene shown to possess antimicrobial activity [29] . Pharmacological activities like antioxidant, antialgal, antifungal and antibacterial activities were contributed by benzofuran [30,31].

4. Conclusion

The comparative GC-MS study of *Alstonia scholaris* depicted presence of bio active phytochemicals in methanol- a polar solvent and hexane –non polar solvent best aimed for hydrocarbon rich phytochemical experimental actvities. These phytochemicals serves innumerable inexpensive, effective, eco-friendly health benefits to humans and environment. **References**

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3 Authorship and Acknowledgement

3.1 Authorship

M HG and LVS both the authors are equally involved in experimental design and manuscript preparation.

3.2 Acknowledgement

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3.3 Conflict of interest

We have no conflict of interest.

3.4 Funding Sources

We have no funding for this study, It is self financed.

4 Tables and figures

Table 1-: Depicting comparative phytochemical analysis in leaf extract of *Alstonia scholaris* in polar solvent methanol and non polar hexane alongwith group of chemical compound, retention time, compound name, molecular weight, molecular formula, percent area composition of bioactive phytochemicals identified using GC-MS technique.

Group of chemical compound	Retention time	Name of compound	Mol. wt.	Mol. Formula	Area %	
					Methanol	Hexane
Unsaturated hydrocarbon	1.495	Propyne	40	C_3H_4	-	0.02
	4.891	p-Xylene	106	C_8H_{10}	-	0.01
	5.54	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	136	$C_{10}H_{16}$	-	0.01
	8.627	(E)-.beta.-Famesene	204	$C_{15}H_{24}$	-	0.01
Saturated hydrocarbon	1.778	Butane, 2,2-dimethyl-	86	C_6H_{14}	-	0.07
	1.867	Pentane, 2-methyl-	86	C_6H_{14}	1.4	31.3
	1.927	Pentane, 3-methyl-	86	C_6H_{14}	1.38	31.09
	1.99	n- hexane	86	C_6H_{14}	37.82	-

	2.17	Cyclopentane, methyl-	84	C_6H_{12}	1.37	14.21
	2.35	Pentane, 3,3-dimethyl-	100	C_7H_{16}	-	0.05
	2.423	Cyclohexane	84	C_6H_{12}	0.3	20.7
	2.496	Hexane,3-methyl-	100	C_7H_{16}	-	0.25
	2.604	Cyclopentane, 1,3 dimethyl-	98	C_7H_{14}	-	0.03
	3.092	Cyclohexane, methyl-	98	C_7H_{14}	-	0.02
	6.76	Nonane, 3,7- dimethyl-	156	$C_{11}H_{24}$	0.17	-
Flavonide	9.933	α-methylmannofuranoside	194	$C_7H_{14}O_6$	12.26	-
	10.027	2-o-methyl-D-mannopyranosa	194	$C_7H_{14}O_6$	11.98	-
Tannins	9.647	Quinic acid	192	$C_7H_{12}O_6$	9.7	-
Hydrocarbon derivative	1.557	Ethane, 1-chloro-1-fluoro-	82	C_2H_4ClF	8.47	-
	1.673	Acetonitrile	41	C_2H_3N	0.16	-
	2.083	Acetaldoxime	59	C_2H_5NO	0.02	-
	16.159	14-Oxabicyclo[10.3.0]pentadecane, 2-chloro-	244	$C_{14}H_{25}ClO$	-	0.03
Alkaloid	8.567	1,3 propanediol,2-hydroxymethyl 2-nitro	151	$C_4H_9NO_5$	2.23	-
	1.497	1-Alanine ethylamide,(S)-	116	$C_5H_{12}N_2O$	0.38	-
Sesquiterpene	18.52	Squalene	410	$C_{30}H_{50}$	2.53	1.63
	11.47	Neophytadiene	278	$C_{20}H_{38}$	0.45	0.02
Unsaturated fatty acid	12.797	8,11,14Docasatrienoic acid methyl ester	348	$C_{23}H_{40}O_2$	0.3	-
	13.207	Linoleic acid ethyl ester	308	$C_{20}H_{36}O_2$	-	0.06
	13.04	9,12 octadecadienoic acid (Z,Z)-	280	$C_{18}H_{32}O_2$	2.13	-
	13.253	9,12 ,15 -octadecatrienoic acid (Z,Z)-	306	$C_{20}H_{34}O_2$	-	0.07
Sesquiterpenoid	12.754	(R)-(-)-14-Methyl-8-hexadecyn-1-ol	252	$C_{17}H_{32}O$	-	0.02
	12.873	Phytol	296	$C_{20}H_{40}O$	1.1	0.07

	16.205	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	884	$C_{57}H_{104}O_6$	-	0.02
	16.703	Bis(2-ethylhexyl)phthalate	390	$C_{24}H_{38}O_4$	0.26	0.03
	16.821	9,12-Octadecadien-1-ol, (Z,Z)-	266	$C_{18}H_{34}O$	-	0.01
	17.301	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12 3-(115-7m1e thylethy 0l).-	306	$C_{20}H_{34}O_2$	-	0.05
Coumaran	7.45	Benzofuran, 2,3- dihydro	120	C_8H_8O	0.88	-
Alcohol	12.163	Methanol[6,8,9-trimethyl-4-(1-propenyl)-3-oxab	236	$C_{15}H_{24}O_2$	0.73	-
	1.633	Ethanol	46	C_2H_6O	0.57	-
	12.757	R-(-)-14methyl-8-hexadecyn-1-ol	252	$C_{17}H_{32}O$	0.18	-
	7.563	1,2,3 Propanetriol, 1-acetate	134	$C_5H_{10}O_4$	0.04	-
Ketone	9.397	3 deoxy-d-mannoic lactone	162	$C_6H_{10}O_5$	0.65	-
	7.987	4-Hydroxy-2-methyl acetophenone	150	$C_9H_{10}O_2$	0.2	-
Phenolic compound	7.33	Catechol	110	$C_6H_6O_2$	0.63	-
	7.71	Hydroquinone	110	$C_6H_6O_2$	0.15	-
	10.603	3 Hydroxy-1-propenyl, 2-methoxyphenol	180	$C_{10}H_{12}O_3$	0.24	-
	6.883	Phenyl ethyl alcohol	122	$C_8H_{10}O$	0.09	-
Sugar	8.887	.beta.-D-glucopyranose 1,6 anhydro	162	$C_6H_{10}O_5$	0.62	-
Saturated fatty acid	9.297	6-Methoxythymyl, 2- methyl butarate	264	$C_{16}H_{24}O_3$	0.14	-
	11.717	Hexadecanoic acid methyl ester	270	$C_{17}H_{34}O_2$	0.36	0.1
	12.795	Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopr 1o6p2y5l)7m ethyl]cy 0c.l0o3p ropyl]1m26et2h7y l]cyclopropyl]methyl]-,	314	$C_{25}H_{42}O_2$	-	0.03

	13.844	3-Methylbutyl hexadecanoate	326	$C_{21}H_{42}O_2$	-	0.01
	17.05	Eicosanoic acid, phenylmethyl ester	402	$C_{27}H_{46}O_2$	-	0.01
	17.681	Phenethyl icosanoate	416	$C_{28}H_{48}O_2$	-	0.06
Terpenoid	11.237	Pentadecane, 1-methoxy-13 methyl	256	$C_{17}H_{36}O$	0.13	-

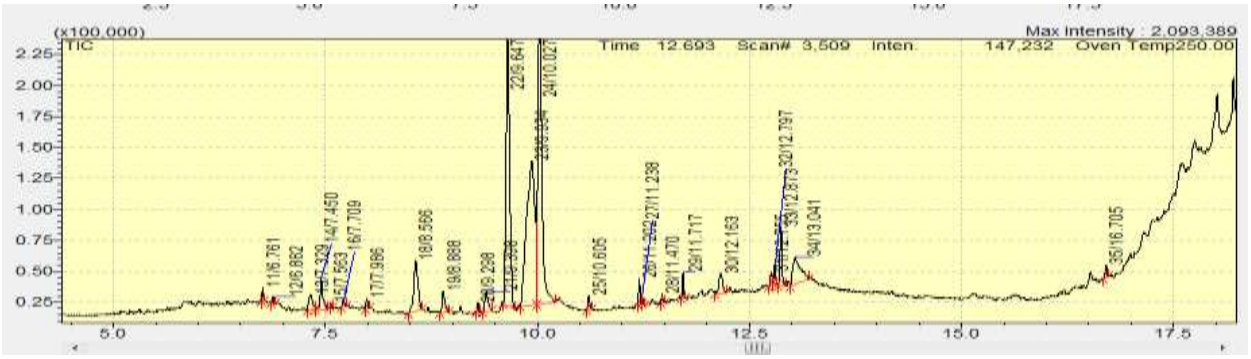

Fig.1: Showing GC-MS chromatogram of methanol leaf extract of *Alstonia scholaris*

Fig.2: Showing GC-MS chromatogram of hexane leaf extract of *Alstonia scholaris.*