



Qualitative and Quantitative Evaluation of Phytochemicals in Leaf Extract of *Alstonia scholaris* (L.) R. Br. By GC-MS Technique

¹Mamta Goyal and ^{*2}Laxmikant Shinde

¹Research Scholar and ²Associate Professor

Applied Parasitology Research laboratory, Department of Zoology, JES College, Jalna (431203) Maharashtra, INDIA.

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*Corresponding Author Email: kantshinde@gmail.com

Abstract

The aim of present research was to evaluate the various phytochemicals present in leaf extract of *Alstonia scholaris* (L.) R. Br. The essential oil was extracted by Soxhlet extraction method using methanol as solvent. To achieve qualitative and quantitative evaluation of secondary metabolites, extract was analyzed by gas chromatography and mass spectroscopy. GC-MS evaluation of methanolic leaf extract of *Alstonia scholaris* (L.) R. Br revealed the presence of 31 identified components. It constitutes of hydrocarbon moiety (42.44%), flavonoids (24.24%), tannins (9.7%) hydrocarbon derivatives (8.65%), alkaloid (3.61%), sesquiterpenes (2.98%), fatty acids (2.93%), phenols and alcohols (2.6%), sesquiterpenoid (1.36%), coumaran and ketones (1.73%), sugar moiety (0.62%), terpenoid (0.13%). These secondary metabolites (phytochemicals) provide evolutionary advantage to the plant in the form of protection against fungi, insects, microbes and environmental stress. The major components 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%); squalene (2.53%); 1,3-propanediol, 2-(hydroxymethyl)-2-nitro- (2.23%); 9,12-Octadecadienoic acid (Z, Z)- (2.13%); pentane, 2-methyl-(1.40%); pentane ,3-methyl-(1.38%); cyclopentane, methyl-(1.37%); and phytol (1.10%). The majority of phytochemicals in *Alstonia scholaris* found to possess numerous medicinal attributes viz. antifungal, antimalarial, antimicrobial, anti-fertility, antidiabetic, anthelmintic, antiparasitic activities. The plant also possesses broad spectrum of medicinal attributes against many ailments.

Keywords

Alstonia scholaris, GC-MS, phytochemicals, essential oil.

1. INTRODUCTION:

Nature has blessed us with its never ending source of medicinal plants. These plants are enriched in phytochemicals having immense therapeutic power to rescue many critical diseases [18, 27]. 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 [10]. 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 [26].

India is one of the 12 mega-diversity hot spot regions rich with flora and fauna biodiversity. 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 [8].

Alstonia scholaris commonly known as "saptarni" 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 [9, 27].

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 [3, 23, 24, 25]

The objective of present study was to evaluate the qualitative and quantitative of phytochemicals in leaf extract of *Alstonia scholaris* by most accurate and sophisticated GC-MS technique.

2. MATERIAL 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 Essential oil:

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) with methanol (Merck 99.9%) as solvent. The temperature of mantle was kept at 55°C. The solvent with thimble was run for 7-8 hrs in Soxhlet apparatus till solvent becomes decolourised [4].

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°C and 250°C 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°C; it was held isothermal for 3mins and then increased the column temperature up to 320°C at the rate of 30°C/min with intermediate hold time at 200°C (2 min.), 250°C (4 min), 300°C (4min.), 320°C (1 min). The duration of one complete program was 23 min. MS transfer line temperature: 250°C 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.

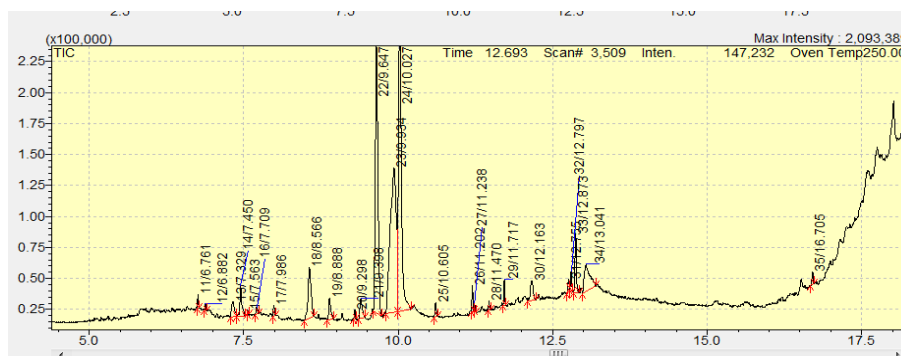


Fig.1: Showing GC-MS chromatogram of methanol leaf extract of *Alstonia scholaris*

3. RESULTS:

Identification of chemical component 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 leaf extract of *Alstonia scholaris* (L.) R. Br revealed the presence of 35 bioactive components. The composition of essential oil comprises of hydrocarbon moiety (42.44%), flavonoids (24.24%), tannins (9.7%) hydrocarbon derivatives (8.65%), alkaloid (3.61%), sesquiterpenes (2.98%), fatty acids (2.93%), phenols and alcohols (2.6%), sesquiterpenoid (1.36%), coumaran and ketones (1.73%), sugar moiety (0.62%), terpenoid (0.13%). Table 1 include details as chemical group of compound, name of compound, retention time,

molecular formula, molecular weight, percent composition with respect to peak area. The major components identified by GC-MS 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%); squalene (2.53%); 1,3-propanediol, 2-(hydroxymethyl)-2-nitro- (2.23%); 9,12-Octadecadienoic acid (Z, Z)- (2.13%); pentane, 2-methyl-(1.40%); pentane ,3-methyl-(1.38%); cyclopentane, methyl-(1.37%); and phytol (1.10%).

Irrespective of amount or concentration whether high or low in which these compounds were found to be present in oil. These bioactive compounds possess some pharmacological significance as well as biological activity.

Table 1: showing group of chemical compound, retention time, compound name, molecular weight, molecular formula, percent area composition of bioactive phytochemical identified using GC-MS technique.

Group of chemical compound	Retention time	Name of compound	Mol.wt.	Mol. Formula	Area %
Saturated hydrocarbon	1.99	n- hexane	86	C ₆ H ₁₄	37.82
	1.867	Pentane, 2-methyl-	86	C ₆ H ₁₄	1.4
	1.927	Pentane, 3-methyl-	86	C ₆ H ₁₄	1.38
	2.17	Cyclopentane, methyl-	84	C ₆ H ₁₂	1.37
	6.76	Nonane, 3,7- dimethyl-	156	C ₁₁ H ₂₄	0.17
Flavonide	2.423	Cyclohexane	84	C ₆ H ₁₂	0.3
	9.933	α-methylmannofuranoside	194	C ₇ H ₁₄ O ₆	12.26
	10.027	2-o-methyl-D-mannopyranosa	194	C ₇ H ₁₄ O ₆	11.98
Tannins	9.647	Quinic acid	192	C ₇ H ₁₂ O ₆	9.7
Hydrocarbon derivative	1.557	Ethane, 1-chloro-1-fluoro-	82	C ₂ H ₄ ClF	8.47
	1.673	Acetonitrile	41	C ₂ H ₃ N	0.16
	2.083	Acetaldoxime	59	C ₂ H ₅ NO	0.02
Alkaloid	8.567	1,3 propanediol,2-hydroxymethyl 2-nitro	151	C ₄ H ₉ NO ₅	2.23
	1.497	1-Alanine ethylamide,(S)-	116	C ₅ H ₁₂ N ₂ O	0.38
Sesquiterpene	18.52	Squalene	410	C ₃₀ H ₅₀	2.53
	11.47	Neophytadiene	278	C ₂₀ H ₃₈	0.45
Unsaturated fatty acid	13.04	9,12 octadecadienoic acid (Z,Z)-	280	C ₁₈ H ₃₂ O ₂	2.13
	12.797	8,11,14Docasatrienoic acid methyl ester	348	C ₂₃ H ₄₀ O ₂	0.3

Sesquiterpenoid	12.873	Phytol	296	C ₂₀ H ₄₀ O	1.1
	16.703	Bis(2-ethylhexyl)phthalate	390	C ₂₄ H ₃₈ O ₄	0.26
Coumaran	7.45	Benzofuran, 2,3- dihydro	120	C ₈ H ₈ O	0.88
	12.163	Methanol[6,8,9-trimethyl-4-(1-propenyl)-3-oxab	236	C ₁₅ H ₂₄ O ₂	0.73
Alcohol	1.633	Ethanol	46	C ₂ H ₆ O	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 ₅ H ₁₀ O ₄	0.04
	9.397	3 deoxy-d-mannoic lactone	162	C ₆ H ₁₀ O ₅	0.65
Ketone	7.987	4-Hydroxy-2-methyl acetophenone	150	C ₉ H ₁₀ O ₂	0.2
	7.33	Catechol	110	C ₆ H ₆ O ₂	0.63
	7.71	Hydroquinone	110	C ₆ H ₆ O ₂	0.15
Phenolic compound	10.603	3 Hydroxy-1-propenyl, 2-methoxyphenol	180	C ₁₀ H ₁₂ O ₃	0.24
	6.883	Phenyl ethyl alcohol	122	C ₈ H ₁₀ O	0.09
Sugar	8.887	.beta.-D-glucopyranose 1,6 anhydro	162	C ₆ H ₁₀ O ₅	0.62
	11.717	Hexadecanoic acid methyl ester	270	C ₁₇ H ₃₄ O ₂	0.36
Saturated fatty acid	9.297	6-Methoxythymyl, 2- methyl butarate	264	C ₁₆ H ₂₄ O ₃	0.14
Terpenoid	11.237	Pentadecane, 1-methoxy-13 methyl	256	C ₁₇ H ₃₆ O	0.13

4. DISCUSSION:

In present investigation, varieties of phytochemicals have been identified. It has been reported that phytochemicals act 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 [5]. 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 [25]. 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 [18]. 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 [23] and same author in a year 2012 performed experiments on mice using leaf extract of *Alstonia scholaris* and proved its analgesic and anti-inflammatory effects [25]. 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 [21]. Sarkhel S. and Ghosh R. (2017) correlated the traditional healer and demonstrated antivenom potential of aqueous *Alstonia scholaris* Linn. in the treatment of snakebite [22]. Surendran S. et al (2012) justified and proved in-vitro cytotoxic and antiproliferative effect of leaf extract on cancer cells [1]. 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* [2]. Hamdiani S. et al (2017) reported the presence of four alkaloids in leaf extract of *Alstonia scholaris*, these were akuammidine, nicotine, strictamine, and voacristine [19] in contrast to this, in present study authors found highest percent content of hydrocarbons (42.44 %) followed by flavonoid (24.24 %), tannins (9.7 %), sesquiterpenes (2.98%) and fatty acids (2.93 %). In present study, chemical constituents like neophytadiene, phytol, squalene, tannins. resembles to GC-MS results from previous study by Swamy N.T. et al [11]. 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 [12,13,28]. The biological activities contributed by phytol include antimicrobial, anti-inflammatory, anticancer, antimalarial and antifungal [15, 16, 20]. Squalene served to possess antimicrobial, anti-oxidant, pesticide, antitumour, cancer preventive, immunostimulant,

chemopreventive, and lipoxygenase inhibitor ^[17]. while neophytadiene shown to possess antimicrobial activity ^[14]. Pharmacological activities like antioxidant, antialgal, antifungal and antibacterial activities were contributed by benzofuran ^[29, 30].

CONCLUSION:

The GC-MS study of *Alstonia scholaris* depicted presence of bio active secondary metabolites which serves innumerable inexpensive, effective, eco-friendly health benefits to humans and environment.

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