Original research article

GC-MS Analysis of Young Leaves of *Allophylus cobbe* (L.) Raeusch. and *Allophylus serratus* (Roxb.) Kurz.

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ABSTRACT

Background: *Allophylus cobbe*(L.) Raeusch. and *Allophylus serratus* (Roxb.)Kurz. (Sapindaceae) are medicinal plants traditionally used to cure bone fractures and other ailments in India. **Objective:** Present work was aimed at the identification of phytochemical compounds from young leaves of *A. cobbe* and *A. serratus* by applying gas chromatography mass spectrometry. **Method:** GC-MS technique was used for analysis of compounds. **Results:** It indicates 11 compounds from *A. serratus* and seven compounds from *A. cobbe* having mixture of terpenoids, alkanes and fatty acids. The major compounds found in *A. cobbe* were 1,1-diethoxy ethane (82.97%) (RT: 3.192min), phytol (7.07%) (RT: 43.690 min) and hexanoic acid (3.67%) (RT: 29.576 min) while in *A. serratus* 3-methyl butanol (53.16%) (RT: 3.223 min), 2-propenoic acid, 2-(dimethylamino) ethyl ester (27.08%) (RT: 13.94 min) and diisocytyl phthalate (4.49%) (RT: 52.584 min) were found in higher quantity. Phytol was detected from both species. Phytol possesses anti-radical, anti-cancer, antibacterial and anti-inflammatory properties as well as used in artificial synthesis of vitamin E and vitamin K in cosmetics and fragrance industry. **Conclusion:** The abundant presence of phytol in leaves of *Allophylus* species (7.07% in *A. cobbe* and 2.60% in *A. serratus*) suggests that these species may prove helpful in prevention of degenerative diseases and application of these herbs in cosmetics which may prove a cost effective natural alternative to current synthetic cosmetics. Presence of industrially important chemicals like phytol, 1,1-diethoxy ethane, hexanoic acid, diisocytyl phthalate, 2-propeonic acid, 2-(dimethylamino) ethyl ester underlines industrial importance of these species which needs further research on this aspect to focus in detail.

Key words: GC-MS Analysis, *Allophylus Cobbe, Allophylus Serratus*, Young Leaves, phytol, medicinal plants.

INTRODUCTION

Allophylus (family Sapindaceae) having about 255 species found worldwide out of which nine species are found in India.[1] *A. cobbe* and *A. serratus* are species found in Maharashtra state of India.[2] These two species are found in various localities of Kolhapur district of Maharashtra State.[3] Most of the local people in Maharashtra call both the species by a same name- *Tipan* (in Marathi language).[4] The fresh leaves of these species are tied over the bone fractures for a faster recovery.[4] The fresh leaves as well as dry powder of leaves are also taken with small amount of jaggery at early morning with empty stomach, for relief from joint pains.[4] Leaves of *A. cobbe* are used by local people against bone fractures,[5] rashes,[6] and stomach ache.[6] Benzylamide is isolated from leaves of *A. cobbe* (L.) Raeusch[7]. *A. serratus* is used against bone fractures.[8] *A. serratus* (Kurz) leaves contain β-sitostrerol[9] and phenacetamide, an anti-ulcer phytochemical.[10] Kumar *et al.* observed osteogenic activity of rutin isolated from stem of *A. serratus* in *in vitro* studies.[11] Chavan and Gaikwad reported antibacterial activity of *A. serratus* and *A. cobbe*.[12]

In recent years, GCMS is being a helpful tool to identify the phytochemicals from the medicinal plants and various natural products; because it is a non-destructive, direct and fast analytical method

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for identification of terpenoids, fatty acids and other phytochemicals as well as a very less plant material is required.

The phytochemical information of young leaves of these species is scanty. Hence, present investigation was aimed at characterizing chemical components of these species.

MATERIAL AND METHODS

Collection of plant material

Young leaves of *A. cobbe* and *A. serratus* were collected from forest of Panhala (Kolhapur district, Maharashtra State, India). The material was washed with water, blotted to dry and kept in oven at 60° C until gaining the constant weight. The completely dried material was taken out, finely ground with Willey's grinding machine and these powders were kept in airtight containers until use.

Extraction of Plant Material

5 g of dry powders of young leaves of *A. serratus* and *A. cobbe* were taken and extracted at 60 °C in absolute ethanol (Merck) up to 8 hr by using soxhlet apparatus. After completion of extraction, extract was taken out, poured in evaporating dish, kept on water bath (60ºC) and evaporated to dryness. Resulting residue remained at bottom of evaporating dish was dissolved in 5 ml absolute ethanol and used for GC-MS analysis.

Chromatographic analysis

GC-MS analysis was performed on a Shimadzu (Tokyo, Japan) Make QP-2010 with nonpolar 60 M RTX 5MS capillary column, full scan mode, injector mode-split, (split ratio 1:20), quadra pole mass selective detector (MSD), injection temperature 220ºC, GC-MS interface temperature 230ºC, the injection volume was 1µl. Helium was employed as carrier gas, at a pressure of 60 KPa; flow rate was 1ml/min. Mass spectra were detected at 70eV. Temperature programming was set as follows: column temperature was started from 60ºC (held for 2 min) and linearly increased by 5ºC/min to 130ºC (held for 2min); after that it was increased by 4ºC/min to 200ºC (held for 2 min); further it was increased by 8ºC/min. to 250ºC (held for 10 min). Total GC running time was 55 min.

Identification of components

Interpretation of mass spectra of GC-MS was conducted using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns (computer software version 1.10 beta, Shimadzu).

RESULTS AND DISCUSSION

The GCMS data of *A. cobbe* is represented in Table 1 and plate 1. It indicates that it is the mixture of terpenoids, alkanes and fatty acids. A total of seven compounds were identified with the help of their retention indices as well as by mass spectrometric data. The sample of young leaves of *A. cobbe* comprised of 1,1-diethoxy ethane (82.97%), mesostilbene glycol (2.57%), 1,1-diethoxy propane (0.6%), hexanoic acid (3.67%), amyl nitrite (1.13%), eicosanoic acid, ethyl ester (2%) and phytol (7.07%). Major phytochemicals detected were 1,1-diethoxy ethane (retained by 3.192 min.) and phytol (retained by 43.690 min. from column).

From young leaves of *A. serratus*, 11 compounds have been detected as indicated in Table 2 and chromatogram in plate 2. It is the mixture of terpenoids and fatty acids. Eleven co mpounds were identified with the help of NIST library and available data. It comprises 3methyl -1-butanol (53.16%), 2-propenoic acid,2-(dimethylamino) ethyl ester (27.08%), diisocytyl phthalate (4.49%), palmitic acid ethyl aster (3.51%), 1,1,3-triethoxy propane (3.17%), phytol (2.6%), n-hexadecanoic acid (1.68%), Nonanoic acid,9-(3-exanylidenecyclo propylidene)-2-hydroxy methyl ethyl ester (1.17%), octadecanoic acid, ethyl ester (1.15%), 1,1-diethoxy-3-methyl-butane (1.01%) and cyclopentyl 2-n-hexadecylcyclopentane (0.99%). Major compounds found were 3-methyl 1-butanol (retention time-3.223 min) and propenoic acid, 2-(di methylamino) ethyl ester (retention time-13.942 min).

Previous studies by Priya *et. al.* indicated presence of 22 different compounds in ethanolic extracts of leaves of *A. serratus*, identified by GC-MS technique, in which cycloheptasiloxane, diethyl phthalate and hexasiloxane were major compounds.[13] Present study gives different result from that of the study by Priya *et. al.* which may be due to the difference between type of plant material (in present analysis, only young leaves have been used), the temperature programming and solvent extraction procedure. Various phytochemicals have been isolated from different parts of *Allophylus* species (See appendix). An anti-ulcer compound phenacetamide have been detected from leaves of *A. serratus*.[10] Extraction of *A. serratus* leaves followed by column chromatography over silica gel revealed the presence of various compounds like- pinitol, quercetin, rutin, luteolin-7-O-β-D-glucopyranoside, and apigenin-4'-O-β-D-gluco-side which were tested for osteogenic activity.[11] *In vitro*

osteogenic activity was studied using calvarial osteoblasts obtained from Sprague Dawley rats by applying method of Ishizuya *et al.*[11-14] Cultured osteoblasts at ~80% confluence were trypsinised and treated with isolated compounds from plant extracts which indicated increase in alkaline phosphatase activity due to rutin.[11] Chavan and Gaikwad detected antibacterial activity of aqueous and ethanolic extracts of young and mature leaves of *A. cobbe* and *A. serratus*.[12] 25 µl (1 mg/ml) each of extract was tested by agar well diffusion method against bacteria-*Staphyllococcus aureus* and *Bacillus subtilis*.[12] As compared to cefotaxime, both aqueous and ethanolic extracts of young and mature leaves of AC and AS indicated remarkable antibacterial activity against *B. subtilis*.[12] *A. cobbe* exhibited significant nematicidal activity against the root knot nematode *Meloidogynae incognita*.[15]

There are some reports on phytochemical studies of *A. cobbe*. Screening of leaves and barks of *A. cobbe* gave

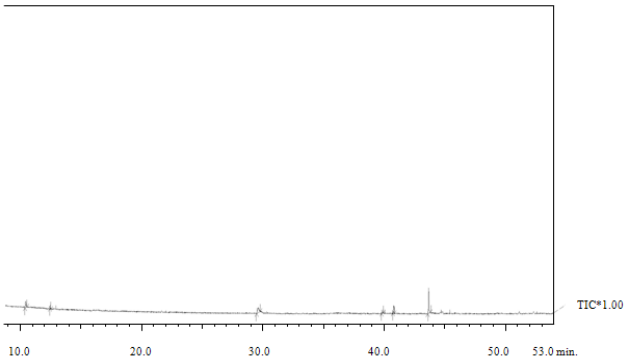

Plate 1: Chromatogram of GCMS analysis of young leaves *A. cobbe*

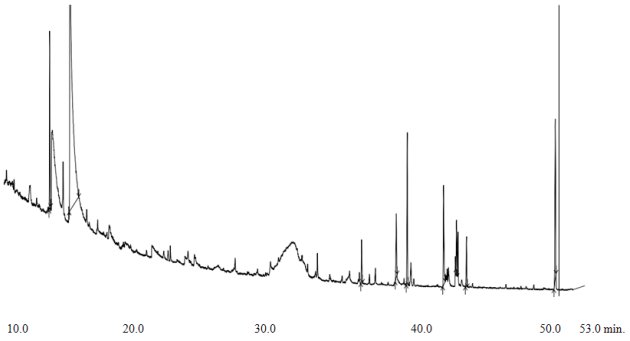

Plate 2: Chromatogram of GCMS analysis of young leaves *A. serratus*

Table 1: GCMS analysis of leaves of *A. cobbe* L. Raeusch

C.No.	RT (min.)	% Conc.	MW	MF	Name	Applications	Ref.
1	3.192	82.97	118.176	$C_6H_{14}O_2$	1,1- diethoxy ethane	flavoring agent in distilled beverages	[29]
2	43.690	7.07	296.53	$C_{20}H_{40}O$	phytol	Phytol is used in the fragrance industry and used in cosmetics, shampoos, toilet soaps, household cleaners, and detergents	[28]
						anti-cancer	[24]
						antimicrobial	[23]
						anti-radical	[22]
						anti-inflammatory	[25]
3	29.576	3.67	116.16	$C_6H_{12}O_2$	hexanoic acid	For manufacture of artificial flavors, hexyl derivatives	[30]
4	10.367	2.57	214	$C_{14}H_{44}O_2$	mesostilbene glycol	*	
5	40.789	2	340.5836	$C_{22}H_{44}O_2$	eicosanoic acid, ethyl ester	*	
6	39.864	1.13	117.15	$C_5H_{11}ONO$	amyl nitrite	used in heart diseases, angina	[31]
						as an antidote in cynide poisoning	[32,33]
7	12.373	0.60	132	$C_7H_{16}O_2$	1,1-diethoxy propane	used as a reducing agent in the aerobic epoxidation of alkenes	[34]

Abbrv-RT- Retention Time, %Conc.-percent concentration, MW—Molecular weight, MF- Molecular formula
*-literature not found.

negative tests for alkaloids when tested in the field, but laboratory assays showed the presence of traces of alkaloids.[16] Large scale extraction of the leaves obtained the crude 'alkaloid' fraction which was found to be an extremely complex mixture of non-alkaloidal material.[16] Benzylamide has been isolated from leaves of *A. cobbe*.[7] In a phytochemical screening of plants in Crocker range, at Sabah, Malaysia, it was observed that leaves of *A. cobbe* contained saponin while it lacked alkaloids, steroids and triterpenes.[17] In Vietnam, an anti-diabetic compound, L-quebrachitol has been isolated from the leaves of *A. cobbe*.[18]

In the present study, it is observed that the leaves of both the species are rich in terpenes, alkanes, alcohols, esters and fatty acids. The major compounds found in *A. cobbe* is 1,1- diethoxy ethane and phytol while the major compound observed in *A. serratus* is 3-methyl butanol. Two phytochemicals (Hexadecanoic acid, ethyl ester and

Table 2: GCMS analysis of leaves of *A. serratus* (Roxb.) Kurz.

C.No.	RT (min)	% Conc.	MW (g/mol)	MF	Name	Applications	Ref.
1	3.223	53.16	88.148	$C_5H_{11}OH$	3 methyl-1-butanol	purification of nucleic Acids	[35]
2	13.942	27.08	143	$C_7H_{13}NO_2$	2-propenoic acid, 2-dimethylamino) ethyl ester	in the manufacture of polymers and copolymers	[36]
3	52.584	4.49	390.56	$C_{24}H_{38}O_4$	diisocytyl phthalate	additive in food packaging plastic	[37]
						component of adhesives, plasticizer of rubbers; used as a penetration agent for fungicides; used in teethers (10.2%) and pacifiers (17.1%)	[38]
						used in insulation in building wire	[39]
4	40.798	3.51	284.5	$C_{18}H_{36}O_2$	palmitic acid ethyl aster	used as lubricant, plasticizer	[40]
5	12.350	3.17	176.2533	$C_9H_{20}O_3$	1,1,3-triethoxy propane	*	
6	43.697	2.60	296.53	$C_{20}H_{40}O$	phytol	used in the fragrance industry, in cosmetics, shampoos, toilet soaps, household cleaners, and detergents	[28]
						phytol has high antimicrobial activity, high stability, low toxicity	[23]
						as cosmetics, hypolipodemic, anxiolytic and antidepressant	[21]
7	39.931	1.68	256.42	$C_{16}H_{32}O_2$	n-hexadecanoic acid	major part of human breast milk	[41]
						food additive	[42]
						anti-inflammatory	[43]
8	44.725	1.17	352	$C_{21}H_{36}O_4$	nonanoic acid,9-(3-hexanylidene cyclo propylidene)-2-hydro xy methyl ethyl ester	*	
9	45.513	1.15	312.53	$C_{20}H_{40}O_2$	octadecanoic acid, ethyl ester	used as marker for excessive alcohol consumption	[44]
						used in cosmetics, candles, soaps, plastics, oil pastels, and for softening rubber	[45]
10	8.316	1.01	160	$C_9H_{20}O_2$	1,1-diethoxy-3-methyl-butane	food flavoring	[46]
						found in beverages and gives flavor	[47]
11	37.174	0.99	362	$C_{26}H_{50}$	cyclopentyl 2-n-hexadecylcyclopentane	compound has been detected in leaves of highly medicinal plant *Ocimum sactum*	[20]

nonanoic acid, 9-(3-hexanylidenecyclo propylidene)-2-hydroxy methyl ethyl ester) from present study have been previously reported from Apricot fruits (*Prunus armeniaca* L.) detected by GC-MS technique.[19] Apricot fruit is having therapeutic properties like antioxidant and antibacterial against gram positive bacteria.[19] Another compound from present investigation-cyclopentyl 2-n-hexadecylcy-clopentane has previously been identified from leaves of highly medicinal plant-*Ocimum sanctum*.[20] Phytol is found common in both the species. Phytol is an important phytochemical compound having properties as an anxiolytic, anti-depressant,[21] anti-radical,[22] antibacterial,[23] anti-cancer,[24] anti-inflammatory.[25] Thus the abundant presence of phytol in leaves of *Allophylus* species suggests that these species may prove helpful in prevention of degeneartive diseases. As well as, phytol is used in artificial synthesis of vitamin E and vitamin K[26,27] Additionally, phytol has various applications in cosmetics and fragrance industry[28] suggests the application of these herbs in cosmetics, which may prove a cost effective alternative to current synthetic chemicals used in current cosmetic industry. Thus, the further detailed studies of each compound will ascertain phytopharmaceutical activity of *A. cobbe* and *A. serratus*. Additionally, to isolate and purify the identified bioactive compounds from *Allophylus* may be useful to formulate the novel drugs for various diseases.

SUMMARY AND CONCLUSION

The major compound found in *A. cobbe* was industrially important compound-1,1- diethoxy ethane (flavor imparter in distilled beverages) while the major compound observed in *A. serratus* was 3-methyl butanol (used in flavors and nucleic acid purification). Presence of a very important compound amyl nitrite (used against heart disorders) in *A. cobbe*, opens up new avenues for investigation in cardio protective role of this species. An abundant presence of phytol in leaves of *Allophylus* species suggests that these species may prove helpful in prevention of degenerative diseases. Phytol is used in

Appendix: Phytochemicals isolated from different parts of species of *Allophylus*

Species	Name of compound isolated	Source	Biological activity of compound	Reference
A. edulis	Quercetin3-β-D glucoside	Branches	Anti-hypertensive	[48]
	L-quebrachitol	Twig	Antidiabetic	[49]
A. cobbe	Rutin	Stem	Osteogenic	[11]
	L-quebrachitol	Leaves	Antidiabetic	[18]
	Benzylamide	Leaves	Nil	[48]
A. laevigatus	11 acetoxy-4α-methoxy-eudesmane	Fruit	Nil	[50]
	Apigenin 8-c-β rhamnopyranoside	Fruit	Nil	[50]
	Carissone	Fruit	Nil	[50]
A. serratus	Phenacetamide	Leaves	antiulcer	[10]
	β-Sitosterol	Leaves	antiulcer	[10]
	Rutin	Leaves	antiosteoporotic	[11]
			hepatoprotective	[51]
			radical scavenger	[52]
	Quercetin	Leaves	hepatoprotective	[51]
			anti-inflammatory, analgesic, aniti-cancer	[53]
			tyrosinase kinase inhibitor	[54]
	Pinitol	Leaves		[11]
			hepatoprotective	[55]
			Anti-cancer	[56]
			Anti-diabetic	[57]
	Luteolin-7-O-β-D-glucopyranoside	Leaves		[11]
			Inhibits cell apoptosis	[58]
	Apigenin-4'-O- β -D-glucoside	Leaves		[11]
			against reflux oesophagitis gastritis	[59]

(Adapted from: Chavan and Gaikwad, 2016)

artificial synthesis of vitamin E and vitamin K. Additionally, phytol has various applications in cosmetics and fragrance industry suggests the application of these herbs in cosmetics, which may prove a natural and cost effective alternative to current synthetic chemicals used in cosmetic industry. Thus, the further detailed studies will ascertain phytopharmaceutical activity of these medicinal species. In addition, the isolation and purification of identified bioactive compounds from *Allophylus* may be useful to formulate the novel drugs for various diseases. In nutshell, some of the phytochemicals detected in a present study are well known about their bioactivities, fewer are known but little is known about their bioactivities while some are known but there are no reports on their biological activities.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ABBREVIATIONS USED

RT: Retention time; **min:** Minutes; **GC-MS:** Gas chromatography-mass spectrometry.

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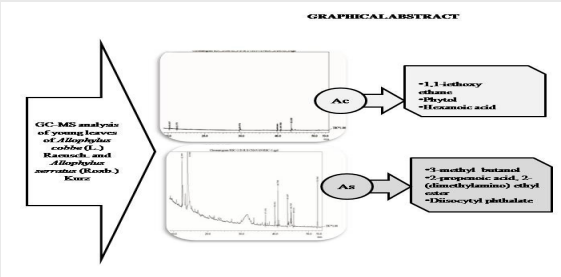
PICTORIAL ABSTRACT



SUMMARY

- GCMS analysis of young leaves of *cobbe* and *A. serratus* investigated
- Phytol present abundantly in both species
- Phytol is effective as an anticancer, antibacterial, anti-inflammatory
- Major compound in *A. cobbe*-industrially important compound-1,1- diethoxy ethane (flavor imparter in distilled beverages)
- Major compound in *A. serratus*-3-methyl butanol (used in flavors and nucleic acid purification).

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