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Phytochemical Investigation of *Allophylus serratus* Kurz Leaves by UV and GC-MS Analysis

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ABSTRACT

Allophylus serratus Kurz is one of the largest genus of family Sapindaceae distributed all over India. The plant has got strong ethnopharmacological background like useful in the treatment of inflammation, gastrointestinal disorders, elephantiasis, osteoporosis etc. The ethanolic extract of the plant was reported for its antiulcerogenic and antiosteoporetic action, beyond its other traditional uses. The present study explored the nature of phytoconstituents present in *Allophylus serratus* Kurz using various modern analytical techniques. Determination of secondary metabolites like total phenolic and flavonoid content were done by UV spectroscopy whereas total tanning substances and foaming index were determined by conventional methods. The fatty acid content of the ethanolic extract was studied by GC-MS analysis. Among the different solvent extracts evaluated, the acetone extract have shown higher phenolic (0.82%) and flavonoid content (0.61%), whereas the ethanolic extract have shown high tanning substances (7.5%) and foaming index (222.3). In GC-MS analysis totally 22 fatty acids were identified by GC and their mass spectra were compared with those compounds from WILEY library. In conclusion, the plant material is found to have rich phenolics, flavonoids, tanning substances and saponins. In GC MS analysis the presence of various fatty acids were also identified.

Key words: *Allophylus serratus*, GC-MS, fatty acids, total phenolics, total flavonoids, tanning substances, foaming index, secondary metabolites

INTRODUCTION

Allophylus serratus Kurz is one of the largest genus of family Sapindaceae found extensively all over India. It is a large shrub or small tree grows upto 10 meters in height. The plant has a distinction of being used in Indian system of medicine (Ayurveda) as an anti-inflammatory and carminative drug and has been used in elephantiasis, oedema, fracture of bones, several gastrointestinal disorders including dyspepsia, anorexia and diarrhoea, wound, ulcers, anorexia and general debility. The fruits are sweet, cooling and nourishing tonic (Gupta *et al*., 2004). The plant is found to be reported for its phytoconstituents like Quercetin, pinitol, luteolin-7-O-β-D-glucopyranoside, rutin, apigenin-4-O-β-D-glucoside (Kumar *et al*., 2010), β -sitosterol and phenacetamide (Hegnauer, 1961). *Allophylus edulis* is reported to have L-Quebrachitol (Diaz *et al*., 2008) and cyanolipids and triacylglycerols from seed oil (Aichholz *et al*., 1997). A novel type α-trans-polyprenols, alloprenols were found in the leaves of *Allophylus caudatus* (Ciepichal *et al*., 2007). A new sesquiterpene 11- acetoxy-4 α-methoxyeudesmane and other known

compounds carissone and apigenin-8-C-β-rhamnopyranoside were reported in *Allophylus laevigatus* (David *et al*., 2004). The seed oil extracted from *Allophylus natalensis* is reported to have triacylglycerol, type I cyanohipids, 1-cyano-2-hydroxymethylprop-2-en-1-ol-diesters with minor amounts of type III CL, 1-cyano-2-hydroxymethylprop-1-en-3ol-diesters (Avato *et al*., 2005). Preliminary chemical characterization of the aqueous extract of *Allophylus cominia* leaves reported total protein concentration, fatty acids like lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid and carbohydrates like arabinose, xylose, galactose and glucose (Rodriguez *et al*., 2005).

The ethanolic extract of *Allophylus serratus* Kurz was reported for its potential anti-ulcerogenic (Dharmani *et al*., 2005) and anti-osteoporotic action (Kumar *et al*., 2010) in the literature. The ethanolic extract of the plant was found to exhibit antiviral activity against Ranikhet disease virus and showed gross effects on central nervous system and hypothermia (Babbar *et al*., 1982).

Thus the plant *Allophylus serratus* Kurz is found to have strong ethnopharmacological background as well as reported potential pharmacological activities like antiulcerogenic and antiosteoporetic actions. From the literature, it was found that the genus *Allophylus* found to possess diverse chemical nature of constituents and the species *Allophylus serratus* Kurz has not been chemically investigated in detail. It is found to be highly unexplored on its nature of phytoconstituents present. In account of its medical uses in the traditional system of medicine as well as its diversified therapeutic uses reported and the unexplored phytoconstituents present in the plant responsible for these activities and therapeutic uses, the present study was undertaken to explore the nature as well as the quantity of secondary metabolites present in the plant extract. Thus the objective of the current study was to explore the various phytoconstituents present in *Allophylus serratus* Kurz using modern analytical techniques.

MATERIALS AND METHODS

Plant material: The whole plant material A.S was collected from Kolli hills, Namakkal district in the period of December to January in the year (2010-2011) and authenticated by the Director of the Raninat herbarium and Centre for Molecular Systematics, St. Joseph's college (campus), Tiruchirappalli, Tamil Nadu, India. The voucher specimen number is RT 001.

Cold extraction: Powdered leaves of *Allophylus serratus* kurz (0.5 kg) were soaked with in a glass percolator with different solvents like ethanol, chloroform, acetone (4 L) and allowed to stand at room temperature overnight. The percolate was collected. This process of extraction was repeated four times. The combined extract was filtered, concentrated at 45°C under vacuum and then dried.

Hot extraction: The powdered leaves of 0.25 g were taken in a round bottom flask and extracted with different solvents like absolute ethanol, chloroform and acetone. The material was extracted for 4 h. The excess solvent present in the crude extracts was removed by distillation and concentrated under vacuum and then dried.

Determination of physicochemical constants: Physicochemical parameters of powdered drug were determined and reported as total ash, water-soluble ash and acid-insoluble ash values. Ether, chloroform and water-soluble extractive values were determined to find out the amount of soluble components in the respective solvents according to the method of Khandelwal (1998).

Estimation of secondary metabolites

Determination of total phenolic content: The total phenolic content of ASE was determined in three different solvent extracts spectrophotometrically according to the Folin-Ciocalteu method (Singleton *et al*., 1999) using Gallic acid as a standard (the concentration range: 0.025 to 0.5 mg mL^{-1}). The total phenolic content was expressed as GAE in milligram per gram dry extract.

Determination of total flavonoid content: The total flavonoid content was determined according to the aluminium chloride colorimetric method (Lin and Tang, 2007). Rutin was chosen as a standard (the concentration range: 0.005 to 0.1 mg mL^{-1}) and the total flavonoid content was expressed as milligram per g of dry extracts.

Determination of tanning substances: The total tanning substances of the different solvent extracts were determined according to the method of WHO (1998) guidelines. The total quantity of tanning substances is expressed in terms of percentage.

Determination of foaming index: The foaming index of various extracts was determined according to WHO (1998) guidelines.

Determination of fatty acid by gas chromatography mass spectroscopy:

- **Sample preparation:** The crude sample was washed with sodium sulphate by using ordinary filter paper. Before the filtration process the funnel was covered with ordinary filter paper and then added the sodium sulphate and allowed the crude sample into the funnel. Finally the purified sample was obtained.
- **Stationary phase:** Fifteen gram of Alumina
- **Solvent:** Mixture of petroleum ether, acetone (9:1) v/v
- **Procedure:** Column was packed with 15 g of alumina, between two sterile cotton swabs and the solvent mixture was allowed to run through the column. After sometime, the filtrate is poured in to the column and pigments were run along with solvent in descending direction. Depending upon the differential stability the pigments was separated at different types of the column. Three different bands were obtained

GC MS conditions: GC-MS analysis was carried out on a Shimadzu QP2010 plus system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: RT x 5 MS column (30 x 0.25 mm ID x 1 μM df, composed of 100% Dimethyl poly diloxane), operating in electron impact mode at 70eV; helium (99.999%) was used as carrier gas at a constant flow of 1 mL min^{-1} and an injection volume of 0.5 μL was employed (split ratio of 10:1) injector temperature 250°C; ion-source temperature 200°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C min^{-1} to 200°C, then 5°C min^{-1} to 250°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70eV; a scan interval of 0.5 sec and fragments from 40 to 450 Da. Total GC running time is 54 min.

Identification of components: Interpretation on mass spectrum GC-MS was conducted using the database of WILEY having more than 62,000 patterns. The spectrum of the unknown

Table 1: Physiochemical constant parameters of *Allophylus serratus* Kurz leaves

Physiochemical parameters	*Allophylus serratus* crude powder
Ash values	
Total ash	12.3
Acid insoluble ash	5.2
Water soluble ash	8.1
Extractive values	
Ether soluble	7.6
Chloroform soluble	8.2
Water soluble	19.4

component was compared with the spectrum of the known components stored in the WILEY library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS

Physiochemical constants: *Allophylus serratus* leaves powder showed (Table 1) total ash content 12.3% among which acid insoluble ash was 5.2% and water soluble ash was 8.1%. Among the extractive values water soluble extractive have shown high content 19.4% and other extractive values like ether soluble and chloroform soluble extractives were 7.6 and 8.2%. This is the first report on the physiochemical constant values of *Allophylus serratus* leaves. Determination of physiochemical constants helps in assessing the quality of the extract. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also helpful in estimation of specific constituents soluble in a particular solvent (Ozarkar, 2005).

Estimation of secondary metabolites

Total phenolic content: The total phenolic content of three different solvent extracts of *Allophylus serratus* Kurz was determined spectrophotometrically according to the Folin-Ciocalteu method and the results are given in Table 2. It is clear from the table that among all the extracts, the acetone extract showed high amount of phenolic content 0.73 and 0.82% in both cold and hot extracts respectively compared to other solvent extracts. The chloroform extract have shown the least phenolic content 0.35 and 0.42% in cold and hot extract respectively.

There is no report on the total phenolics present in the *Allophylus serratus* leaves extract in previous studies. Kumar *et al.* (2010) isolated five flavonoids and flavonoid glycosides from the ethanolic extract of *Allophylus serratus* Kurz leaves, whereas the present study showed acetone extract contains higher phenolic content rather than the ethanolic extract.

Derived polyphenols from plants are of great importance because of their potential antioxidant and antimicrobial properties. Phenolic compounds exhibit a considerable free radical scavenging (antioxidant) activity (Wojdylo *et al.*, 2007). Thus the phenolics present in the plant *Allophylus serratus* might contribute various therapeutics uses of the extract in traditional system of medicine.

Total flavonoid content: The total flavonoid content was determined according to the aluminium chloride colorimetric method in three different solvent extracts of *Allophylus serratus* Kurz and the results are given in the Table 2. The acetone extract showed high amount of flavonoid content in both cold and hot extracts 0.52 and 0.61%, when compared to other solvent extracts. The chloroform extract showed the least flavonoid content 0.14 and 0.19% in cold and hot extracts respectively.

Table 2: Determination of secondary metabolites in various solvent extracts of Allophylus serratus Kurz leaves

	Allophylus serratus Kurz extracts					
	Ethanol		Chloroform		Acetone	
Secondary metabolites	CE	HE	CE	HE	CE	HE
Total phenolic content	0.33	0.54	0.35	0.42	0.73	0.82
Total flavonoid content	0.22	0.35	0.14	0.19	0.52	0.61
Total tanning substances	7.50	7.10	6.40	6.80	6.90	7.10
Total saponius (foaming index)	200	222.3	166.6	166.6	181.8	200

CE: Cold extract; HE: Hot extract

The technique of flavonoid isolation from a plant material, including the type of extracting solvent, depends generally on the type of flavonoid compound and the quantity of plant material. The above results indicate that the flavonoids present in the plant *Allophylus serratus* may be present in the glycosidic form as the polar solvent like acetone and ethanol extract have shown the higher content than the chloroform extract.

Total tanning substances: Estimation of tanning substances was done in various solvents like ethanol, chloroform and acetone. Among all the extracts ethanolic extract showed high amount of tannins 7.5 and 7.1% in both cold and hot ethanolic extract compared to solvent extracts.

Tannins are complex substances; they usually occur as mixtures of polyphenols that are difficult to separate and crystallize. They are easily oxidized and polymerized in solutions; if this happens they lose much of their astringent effect and are therefore of little therapeutic value WHO (1998).

There is no report on tannins present in this plant extract. The present study indicates the presence of tannins in *Allophylus serratus* extract for the first time. Both the ethanolic extract as well as the acetone extract is equally effective in extracting the tannins from the leaves of *Allophylus serratus*. Based on the results the total tannins constitute the major part of the secondary metabolites present in the plant *Allophylus serratus* Kurz.

Foaming index in *Allophylus serratus* Kurz: Estimation of foaming index in various solvents like ethanol, chloroform and acetone was carried out. Among these the ethanolic extract showed high value of foaming index 200 and 222.3 in both cold and hot extracts, when compared to the other solvent extracts.

Many medicinal plant materials contain saponins that can cause persistent foam when an aqueous decoction is shaken. The foaming ability of an aqueous decoction of plant materials and their extracts is measured in terms of a foaming index.

Saponins are secondary metabolites with high polarity, thus its content can easily be extracted using high polar solvents like ethanol, methanol and water. Thus in the present study, only the ethanolic extract have shown the higher amount of saponins when compared to acetone and chloroform.

Total fatty acid content by GC MS analysis: The ethanolic extract of *Allophylus serratus* Kurz was analyzed by GC-MS. The GC chromatogram is shown in Fig. 1. The analyses have shown 22 fatty acid components (Table 3). The fatty acids were separated with retention times starting from

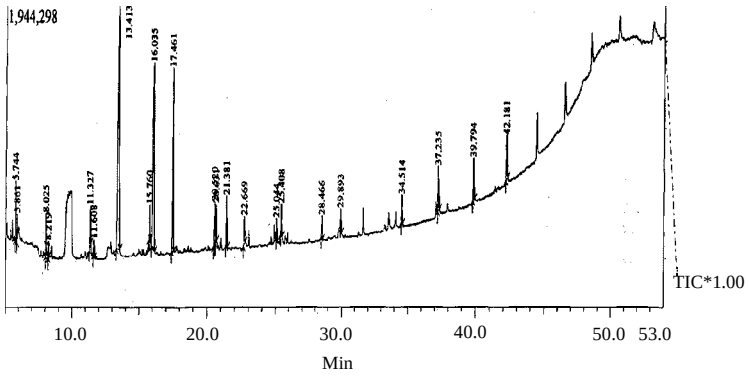

Fig. 1: GC chromatogram of fatty acids in ethanolic extract of Allophylus serratus Kurz leaves

Table 3: GC-MS analysis of fatty acid composition of ethanolic extract of *Allophylus serratus* Kurz leaves

Name of the compound	Retention time	Molecular mass	Molecular formula
Silicic acid tetraethyl ester	5.740	208.32	$C_8H_{20}O_4Si$
Cyclodeca[b]furan-2(3H)-one,3a,4,5,6,7,8,9,11a-octahydro-7,9-dihydroxy-10-methyl-3,6-bis(methylene)-,(3aS,7R,9S,10E,11aR)-	5.861	264.32	$C_{15}H_{20}O_4$
Cyclo octane 1,2 diethyl	8.025	168.31	$C_{12}H_{24}$
2-Undecane, 3-methyl- (Z)-	8.219	168.31	$C_{12}H_{24}$
1-tetradecene	11.327	196.38	$C_{14}H_{28}$
1-Dodecene -2-ethyl	11.608	196.37	$C_{14}H_{28}$
Cycloheptasiloxane	13.413	519.07	$C_{14}H_{42}O_7Si_7$
1-octadecanaol (stenol)	15.760	270.50	$C_{18}H_{38}O$
Diethyl phthalate	16.035	222.24	$C_{12}H_{14}O_4$
Hexasiloxane	17.461	458.99	$C_{14}H_{42}O_5Si_6$
1,5 diphenyl-2H-1,2,4-triazoline -3-thione	20.520	253.32	$C_{14}H_{11}N_3S$
Cyclooctacosane	20.631	392.74	$C_{28}H_{56}$
Heptasiloxane hexadecamethyl	21.381	533.14	$C_{16}H_{48}O_6Si_7$
Phthalic acid,2,7-dimethyloct-7-en-5-yn-4yl	22.669	166.13	$C_8H_6O_4$
Silicone grease	25.044	-	$(C_2H_6OSi)_n$
1-Heneicosyl formate	25.408	340.58	$C_{22}H_{44}O_2$
Tetracosamethylcyclododecasilone	28.466		
1-Eicosanol	29.893	298.54	$C_{20}H_{42}O$
Heptasiloxane, 1,1,3,3,5,5,7,7,9,9, 11,11,13,13-tetradecamethyl-	34.514	505.09	$C_{14}H_{44}O_6Si_7$
Eicosamethylcyclodecasiloxane	37.235	741.5	$C_{20}H_{60}O_{10}Si_{10}$
2,6-dihydroxybenzoic acid	39.794	154.02	$C_7H_6O_4$
1H-Purin-6-amine[(2-fluorophenyl)methyl]-	42.200	243.23	$C_{12}H_{10}FN_5$

5.74, 5.86, 8.02, 8.21, 11.32, 11.60, 13.41, 15.76, 16.03, 17.46, 20.52, 20.63, 21.38, 22.66, 25.04, 25.40, 28.46, 29.89, 34.51, 37.23, 39.79 and 42.2. Among 22 fatty acids, only three were found to be major components like cycloheptasiloxane (13.41, 519.07), diethyl phthalate (15.03, 222.24) and hexasiloxane (17.46, 458.99). There are various reports on different fatty acids present in the

genus allophylus in the literature (Rodriguez *et al*., 2005; Aichholz *et al*., 1997; Diaz *et al*., 2008). But this was the first report on the GC-MS analysis of fatty acids present in *Allophylus serratus*. The method showed good resolution with separation of 22 fatty acid components from the ethanolic extract of AS.

In general the reported fatty acids are used in beverages, perfumes, pharmaceutical dispensing in cosmetic creams, for emulsions, textile oils and finishes.

CONCLUSION

The ethanolic extract of *Allophylus serratus* Kurz leaves was explored for its nature and quantity of phytoconstituents present and the results have shown the presence of phenolics and flavonoids by UV analysis whereas tanning substances and saponins by conventional methods. Further the fatty acid composition of the extract was also carried out by GC-MS analysis and totally 22 fatty acids were found to be present in the extract. The detailed identification and isolation of the individual phytochemicals will be carried out in future.

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