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Original Article

INFLUENCE OF SEASONS AND PLANT PARTS ON THE ESSENTIAL OIL COMPOSITION OF THE ENDEMIC SPECIES *ARISTOLOCHIA KRISAGATHRA* SIVARAJAN AND PRADEEP

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ABSTRACT

Objective: *Aristolochia krisagathra*, a species endemic to the Western Ghats of India is found closely allied to two other species native to India, *A. indica* and *A. tagala*. The plant is medicinally useful and hence may harbor important phytochemical constituents. The phytochemical studies on this plant are less. Hence, the objective of the research was to analyse the oil composition of this endemic plant considering the seasons and plant parts.

Methods: The essential oil analyses of both the leaf and stem oil of the plant was carried out in three different periods, pre-monsoon, monsoon and post-monsoon to analyse the major compounds present and the effect of seasons on the oil composition. Oil extraction of the shade dried leaves and stem were done in a clevenger type apparatus. 4 h of hydro distillation was done and the oil samples were analyzed by Gas Chromatography-Mass Spectrometry (GC-MS).

Results: The stem oil yield was higher than leaves and the oil colour of the leaves and stem varied. The results showed the predominance of sesquiterpenes. Diterpenes were completely absent in the stem oil in all the three seasons but was present in leaf oil in small concentrations. The oil yield from stem was higher than leaves. The major compound in the leaf and stem oil varied. Major compound in the pre-monsoon and post-monsoon remained the same, copaene in the leaf and alloaromadendrene in the stem. The major compound in the monsoon period was delta-cadinene in leaf and spathulenol in stem. Caryophyllene, the sesquiterpene hydrocarbon, was present in both the stem and leaf oil irrespective of the seasons studied.

Conclusion: The study showed that the plant parts and seasons are important factors affecting the oil composition. Hence while collecting medicinal plants and their oils for various bioactivities, these factors need to be considered.

Keywords: *A. krisagathra*, Seasonal variation, Essential oil

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INTRODUCTION

The genus *Aristolochia* commonly known as 'Dutchmans pipe' or 'Birthworts' consists of approximately 500 species, most found in tropical, subtropical and Mediterranean regions having only certain degree of diversity in areas with seasonal climate of the Northern hemisphere [1, 2]. *A. krisagathra* Sivarajan and Pradeep, is one among the medicinally important species of *Aristolochia* reported from India and is endemic to the southern part of Western Ghats in Kerala and Tamil Nadu [3]. Studies on this endemic plant are limited. The juice of leaves and rhizome of *A. krisagathra* is used along with other plants for snakebite by the Kani tribes [4]. The presence of aristolochic acid I in the plant was reported and was found the highest among the four *Aristolochia* species included in the study [5].

Essential oils are important because of their bioactivity as antimicrobial agents [6]. Moreover these are sometimes used for systematic studies. A productive application of essential oil components to scientific plant classification requires knowledge of the causes of variation qualitatively and quantitatively [7]. Though the essential oil composition of *A. krisagathra* has already been reported [8], the oil composition in different seasons has not been studied. Hence the study aims to focus on the influence of two factors, seasons and plant part on the oil composition.

MATERIALS AND METHODS

The source plant *A. krisagathra* was collected from Rosemala near Aryankavu (8 °58′0″N 77 °8′35″E), Kollam district of Kerala, India in three different seasons-pre-monsoon (March-May), monsoon (June-September) and post-monsoon (October-February). The species was authenticated and voucher specimens (*A. indica*-SHC: 07, *A. tagala*-SHC: 08, *A. ringens*-SHC: 09, *A. krisagathra*-SHC: 10) deposited in the herbarium of Sacred Heart College, Thevara. The leaves and stem were separated, shade dried and powdered. 100g each of powdered plant material of leaf and stem was hydrodistilled using a clevenger type apparatus. The time of distillation was 4 h and the distillate obtained was collected and stored in vials under refrigeration (4 °C) prior analysis.

The composition of the volatile constituents was established by GC-MS analyses [9] using GC-6850 network GC system, Agilent technologies with column type HP5MS 30.0 m x 250.00 µm, film thickness 0.25 µm. Split ratio 50:1; carrier gas He at a flow rate of 1.0 ml/min. Temperature was programmed from 60 °C to 180 °C at the rate of 2.5 °C/m and then at the rate of 5 °C at 230 °C. Injection temperature was 200 °C. For GC-MS 5975C VLMSD with triple axis detector, Agilent technologies was used.

The identification of the components was based on comparison of their mass spectra with those of the NIST mass spectral library. Compounds which showed more than 90% similarity was only considered.

RESULTS AND DISCUSSION

The genus *Aristolochia* is generally not treated as an important essential oil yielding plant. But being medicinal, the essential oil composition of the plant has been studied and tested for various activities [10-12].

Table 1: The overall oil composition of the stem and leaf oil of *Aristolochia krisagathra* in pre-monsoon, monsoon and post-monsoon periods

Name of compounds	Percentage composition						*Class of compound*
	Stem			Leaves			
	Post-monsoon	*Pre-monsoon*	*Monsoon*	*Post-monsoon*	*Pre-monsoon*	*Monsoon*	
beta-elemene	0.405	-	-	0.248	-	-	SH
alpha-gurjunene	-	-	0.12	0.113	-	-	SH
caryophyllene	0.29	0.282	0.738	2.731	5.126	0.78	SH
alloaromadendrene	14.512	25.872	2.843	1.05	-	0.723	SH
2-pentadecanone 6,10,14-trimethyl	-	-	-	3.033	3.312	0.454	CDC
beta-selinene	2.544	2.018	-	3.084	2.312	0.537	SH
bicyclogermacrene	-	-	-	2.028	4.462	-	SH
delta-cadinene	0.36	-	6.065	7.244	5.021	5.425	SH
spathulenol	0.549	-	7.1	6.333	5.845	-	OS
caryophyllene oxide	-	-	-	-	3.634	-	OS
hexadecanoic acid, methyl ester	-	-	-	0.22	-	-	FAD
n-hexadecanoic acid	-	-	0.255	4.563	1.555	5.312	FAD
phytol	-	-	-	-	6.388	2.026	Diterpene
alpha-caryophyllene	-	-	0.219	2.537	3.928	0.953	SH
beta-maaliene	-	9.053	-	-	-	1.641	SH
alpha-guaiene	-	-	-	-	0.695	-	SH
isolongifolene, 9,10-dehydro	-	-	-	0.118	-	0.332	SH
gama-cadinene	-	-	1.641	2.319	-	1.17	SH
nerolidol	-	-	-	0.602	0.608	1.659	OS
valencen	-	-	-	0.184	-	-	SH
9-octadecenoic acid	-	-	-	-	1.092	-	FAD
copaene	0.455	-	3.663	10.881	10.687	4.141	SH
isocaryophyllene	-	-	-	3.72	7.233	1.734	SH
alpha-muurolene	-	-	0.932	0.951	0.51	-	SH
alpha-farnesene	-	-	-	0.576	1.131	-	SH
gama-gurjunene	-	-	0.225	-	-	0.527	SH
alpha-cadinene	-	-	-	0.245	-	0.267	SH
1R-alpha-pinene	-	-	-	0.608	-	0.323	MH
beta-pinene	-	4.516	-	0.123	-	-	MH
D-limonene	0.393	-	-	0.207	-	-	MH
linalool	-	-	-	0.295	0.599	-	OM
beta-cadinene	-	-	-	0.5	-	-	SH
delta-heptadecene	-	-	-	0.401	-	-	HC
1H-indene 1-ethylidene octahydro-7a-methyl-cis	-	-	-	0.416	-	-	HC
1R,4S,7S,11R-2,2,4,8-tetramethyltricyclo [5.3.1.0(4,11)]undec-8-ene	-	-	-	0.136	-	-	HC
1,5-cyclododecadiene (Z,Z)	-	-	-	0.267	-	-	HC
isophytol	-	-	-	0.31	-	-	Diterpene
9-octadecenoic acid (Z)-methyl ester	-	-	-	0.205	0.215	1.756	FAD
9,17-octadecadienal (Z)	-	-	-	0.966	-	-	FAD
(+)-4-carene	-	-	-	-	0.718	-	MH
bicyclo[4.4.0]dec-1-ene, 2 isopropyl-5-methyl-9-methylene	-	-	-	-	1.845	3.467	SH
alpha-cadinol	-	-	-	-	1.934	-	SH
active amyl hexanoate	-	-	-	-	-	0.217	FAD
levo-bornyl acetate	-	-	-	-	-	0.883	OM
beta-bisabolene	-	-	-	-	-	0.489	SH
6,7 dimethyl tetralin	-		-	0.372	-	-	HC
gama-muurolene	-	-		-	-	1.981	SH
alpha-elemene	-	-	-	-	-	1.442	SH
(7R, 8S)-cis-anti-cis-7,8-epoxytricneyclo[7.3.0.0(2,6)]dodec	-	-	-	-	-	0.215	HC
naphthalene, 1, 2, 4a, 5, 6, 8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)	-	-	-	-	-	0.809	SH
9, 12-octadecadienoic acid (Z, Z)		-	-	-	-	0.383	FAD
germacrene D	-	-	0.41				SH
alpha-cubebene	-	-	0.467				SH
cyperene	0.22	-	-				SH
cadine 1,4-diene	-	-	0.356	0.259	0.796	0.949	SH
trans-beta-ionone		-	-	0.95	0.266	-	CDC
dodecanoic acid		-	-	0.316	-	0.31	FAD
borneol	-	-	-	0.139	0.219	-	OM

Name of compounds	Percentage composition						
	Stem			Leaves			*Class of compound*
	Post-monsoon	*Pre-monsoon*	*Monsoon*	*Post-monsoon*	*Pre-monsoon*	*Monsoon*	
alpha-terpineol	-	-	-	0.24	0.252	0.26	OM
(-)-myrtenol	7.374	1.842	-	0.298	-	-	OM
tau-cadinol	-	-	1.552	2.24	-	3.536	SH
tetradecanoic acid	-	-	-	0.326	-	0.308	FAD
bornyl acetate	-	0.972	-	0.153	0.873	0.513	OM
alpha-terpinyl isovalerate	-	-	-	0.784	-	-	OM
beta-ionone	-	-	-	0.264	-	0.427	CDC
Total percentage	76.747	61.393	33.184	63.555	71.256	45.949	
Sesquiterpene hydrocarbons	28.726	37.225	24.235	41.028	45.68	30.903	
Oxygenated sesquiterpenes	0.549	-	7.1	6.935	10.087	1.659	
Monoterpene hydrocarbons	18.461	17.728	-	0.938	0.718	0.323	
Oxygenated monoterpenes	26.387	6.107	0.613	1.909	1.943	1.656	
Diterpenes	-	-	-	0.31	6.388	2.026	
Hydrocarbons	-	-	0.113	1.592	-	0.215	
Carotenoid derived compound	-	-	-	4.247	3.578	0.881	
Fatty acid derivative	1.312	0.333	0.96	6.596	2.862	8.286	
Benzotriazine	-	-	0.163				

SH: Sesquiterpene hydrocarbons, OS: Oxygenated sesquiterpenes, MH: Monoterpene hydrocarbons, OM: Oxygenated monoterpenes, HC: Hydrocarbons, CDC: Carotenoid derived compound, FAD: Fatty acid derivative

Leaf and stem oil of *A. krisagathra* exhibited different colors and the oil yield from stem were higher than leaves. Different colors of oil in different developmental stages have been reported [13], but seasons were not found to influence oil color. Stable color of oil was observed throughout. The oil yield did not show significant variation with seasons in contrary to the reports by many [14, 15]. Yield was partly described to be due to the structures associated with secretion; internal secretory structures maintaining a stable yield to external structures [16]. Hence the stable yield of oil with seasons in the present study could be attributed to localization of oil within individual cells. The number of volatiles identified from the leaf oil in different seasons was in the order post-monsoon>monsoon>pre-monsoon. This high number of volatiles in post-monsoon could be attributed to the physiological changes as it corresponds to the flowering period of the plant. However, in the stem oil the number of identified volatiles in the post-monsoon and monsoon was 26 and higher than in the pre-monsoon.

The leaf oil was dominated by copaene in pre-monsoon (10.687%) and post-monsoon (10.887%) period, whereas delta-cadinene (5.425%) was the major compound in the monsoon. GC-MS chromatograms of the leaf oils are given (fig. 1-3). Delta-cadinene was reported as the major compound in the leaf oil [8]. Sesquiterpene hydrocarbons formed the major group in all the seasons. Oxygenated sesquiterpenes represented only a minor percentage. Monoterpene hydrocarbons and oxygenated monoterpenes were present in all seasons in minor quantities. The compounds commonly present in all the seasons in *A. krisagathra* leaves were caryophyllene, alpha-caryophyllene, beta-selinene, 2-pentadecanone 6, 10, 14-trimethyl, n-hexadecanoic acid, delta-cadinene, copaene, nerolidol, isocaryophyllene, alpha-terpineol,, bornyl acetate, cadine 1,4-diene and 9-octadecenoic acid (Z)-methyl ester (table 1).

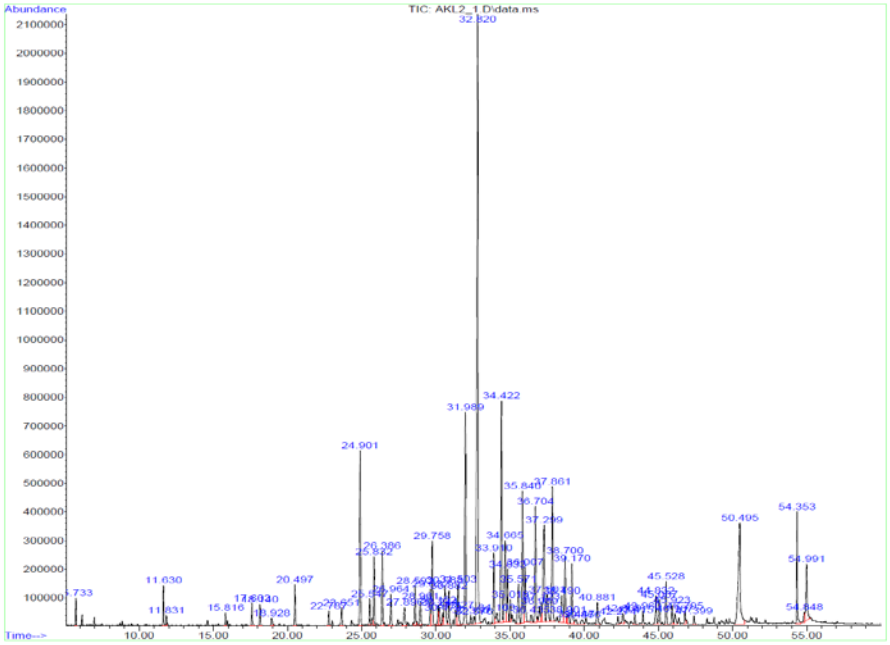

Fig. 1: GC-MS chromatogram of *A. krisagathra* leaves in the pre-monsoon period

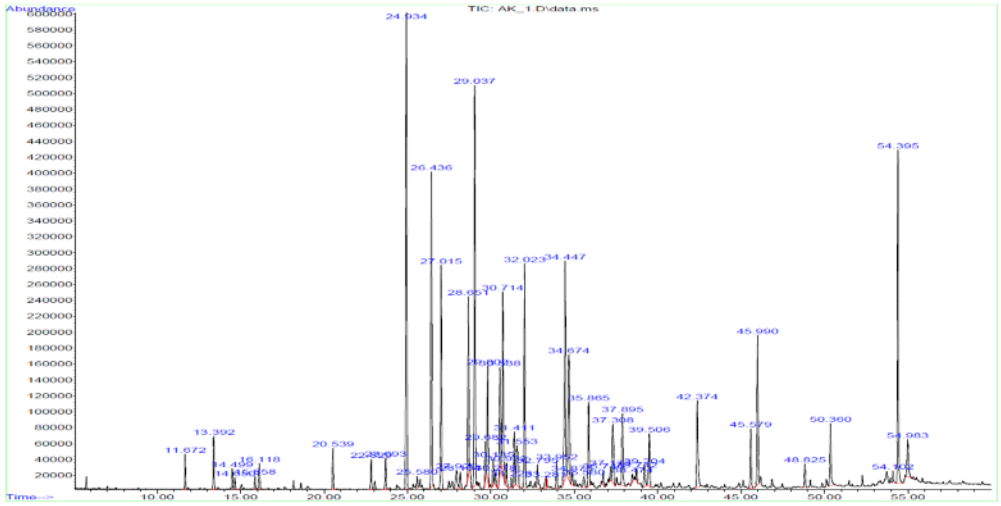

Fig. 2: GC-MS chromatogram of *A. krisagathra* leaves in the monsoon period

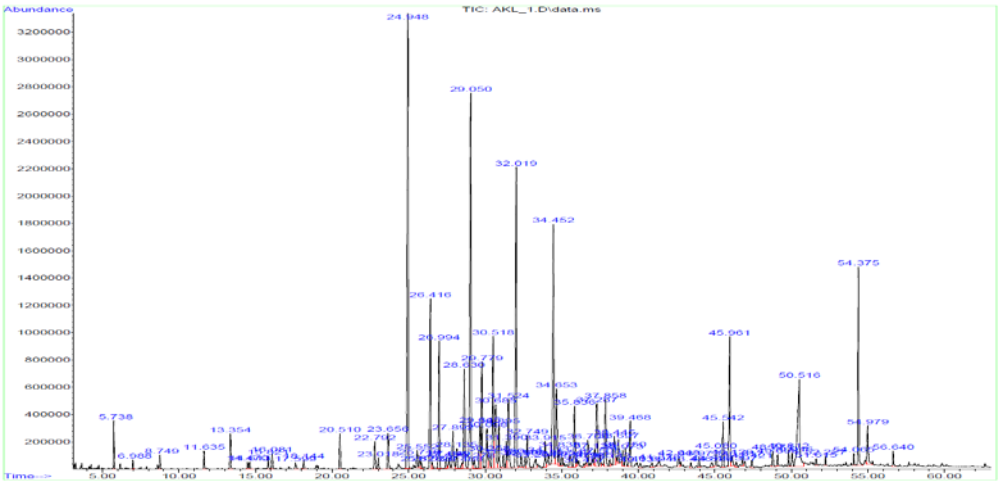

Fig. 3: GC-MS chromatogram of *A. krisagathra* leaves in the post-monsoon period

A. krisagathra stem oil showed alloaromadendrene as the major compound in the pre-(25.872%) and post-monsoon (14.512%) period and spathulenol (7.1%) in monsoon period (table 1). GC-MS chromatograms of the stem oils are given (fig. 4-6). Spathulenol was the major compound in the stem oil of *A. krisagathra* in an earlier study [8]. Only two common compounds, caryophyllene, and alloaromadendrene, were present in the stem oil in all three seasons. Caryophyllene was the only compound present commonly in all the three seasons in both the stem and leaf oils of *A. krisagathra* (table 1).

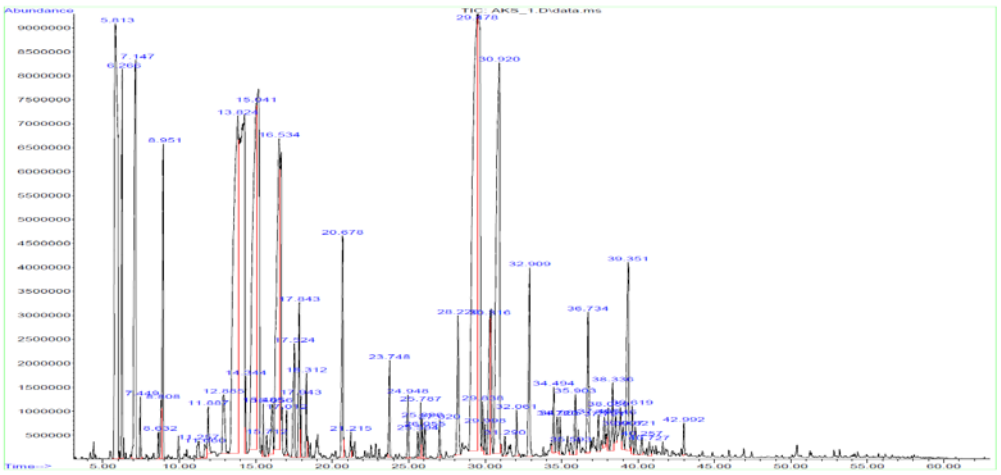

Fig. 4: GC-MS chromatogram of *A. krisagathra* stem in the pre-monsoon period

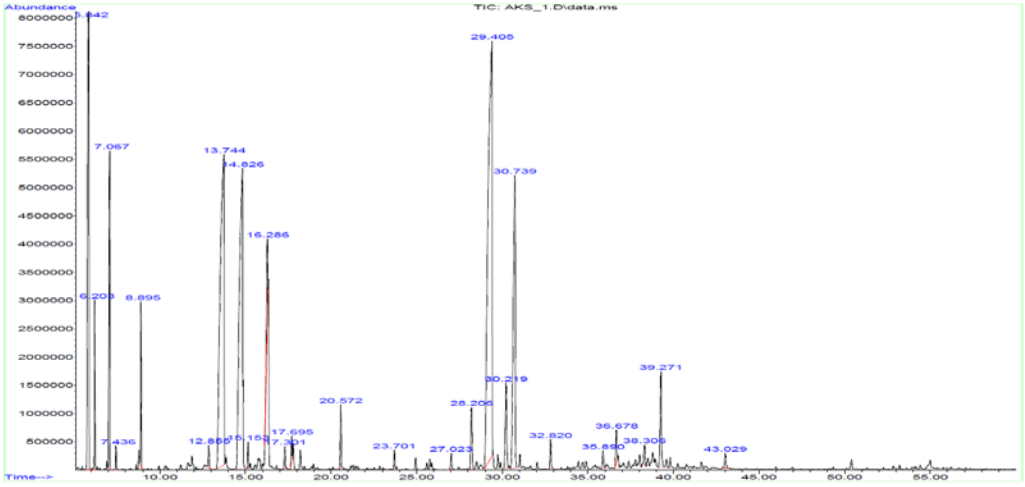

Fig. 5: GC-MS chromatogram of *A. krisagathra* stem in the monsoon period

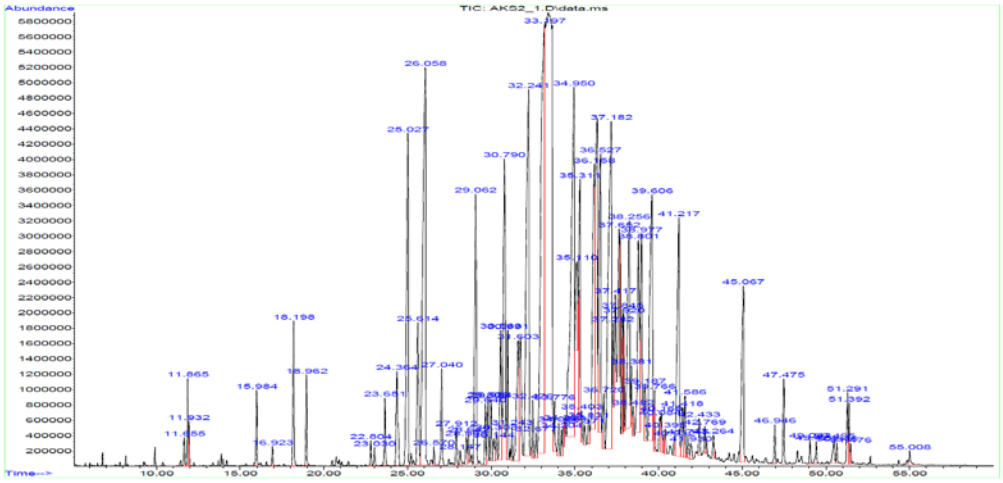

Fig. 6: GC-MS chromatogram of *A. krisagathra* stem in the post-monsoon period

Sesquiterpenes were the dominant class in the leaf oils in all the seasons and in the pre-monsoon and monsoon period in the stem oil. However, monoterpenes dominated in the stem oil in the post-monsoon period. Sesquiterpenes were reported to be the dominant group in leaf and stem oil of *A. gibertii* and *A. triangularis* [17, 18]. Varying nature of dominant compounds with seasons was evident; the pre-monsoon and post-monsoon periods maintained stability in the major compound. The major compound varied in the monsoon period.

Thus, on considering the oil composition with respect to the factors such as seasons and plant parts, they were found to influence the quality of the oil. The accumulation of oil in different seasons and organs did not follow any regular pattern or trend. These variations may be the result of expression of different genes at different developmental stages of the plant and further by the environmental factors arising from seasonal variations [19].

CONCLUSION

Only less than 50% of compounds have been identified in the monsoon period. *A. krisagathra*, being endemic and medicinal with fewer studies on the phytochemistry, can turn out to be a potential plant harboring useful metabolites. Further studies and analyses on this plant are essential from the phytochemical aspect. Moreover, variation on oil composition with seasons has to be looked upon with great significance especially since *A. krisagathra* is medicinal and the essential oil has many activities. The study thereby indicates the importance of seasons in collecting medicinal plants.

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CONFLICT OF INTERESTS

Conflicts of interest declared none by all authors

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