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Phytochemical Analysis of Andrographis elongata (Vahl) T. Anderson- An Endemic Medicinal Plant

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Abstract

Andrographis elongata (Vahl) T. Anderson isan endemic medicinal plant. It is used as an 'ottamooli' in old Travancore. The main objective of this study is to identify the scientific basis of the usage of this plant by local physicians.The preliminary phytochemical assays revealed that, the methanol extract of root, stem and leaves contain large number of secondary metabolites like phenols, glycosides, flavonoids, alkaloids and tannins. The HPLC analysis of root, stem and leaves detected twelve compounds- 5-hydroxy-7, 8, 2', 6'-tetramethoxyflavone, 5, 7, 8, 2'-tetramethoxyflavone, 5, 4'-dihydroxy-7, 8, 2', 3'-tetramethoxyflavone, Andrographidine A, Andrographidine B, Andrographidine C, Neoandrographolide, Andrographolide, Trans-cinnamic acid and Oleanolic acid. The HPLC analysis revealed the presence of flavonoids and terpenoids as the main bioactive compounds and the methanol extract of leaves possessed maximum number of bioactive compounds.

KEYWORDS:*Andrographis elongata*, HPLC, Bioactive compounds

Introduction

India is very rich in ethno botanical heritage due to its cultural diversity (Jain, 1991). Majority of the people in developing countries give priority for traditional herbal medicine. But the anthropogenic activities like urbanization, industrialization; deforestation etc. vanishes abundant of our valuable plants. Medicinal plants play an important role in the health care needs of tribal communities.

Andrographis elongata(Vahl) T.Anderson comm only called 'mullurinjipachila' belongs to Acanthaceae family.It is used as an 'ottamooli' for removing the thorns from body parts. Hence it is known as mullurinji. *Andrographis elongata* T. And. is an endemic medicinal plant found in Pachamalai hills (Ahmedulla and Nair, 1986), naturally occurring in moist shady places. The various plant parts such leaf, stem and root were used in traditional manner for curing diseases such as diabeties, snake bite, skin problems *etc* (Alagesaboopathy*etal*., 2007). Medicinal plants have been used to treat a number of diseases, though the recovery is gradual, the therapeutic application is becoming familiar because of its ability to principle side effects and antibiotic resistant microorganisms (Rawat, 2003).

Materials and Methods

The plant,*Andrographis elongata* (Vahl) T.Anderson was collected from the home garden inParassala of Thiruvananthapuram district.The plant was identified with the help of flora (Gamble, 1936).

Preparations of Extract

The phytochemicals present in the plant material was extracted by soxhlet apparatus. Different solvents like petroleum ether, acetone, chloroform and methanol were used. . The root, stem and leaves were separated from the whole plant. About 1Kg of plant material was weighed and dried under the shade for 10 days. The dried materials (root, stem and leaves)were powdered and 50 gm. of powdered sample was packed in a thimble and kept in soxhlet apparatus. The solvents used were siphoned by 2 times. The whole apparatus was kept over a heating mantle and heated continuouslyfor 8 hrs at boiling point. The extracts were concentrated to dryness and weighed, the residues were transferred to a preweighed sample bottles and stored in a desiccator for further studies (Harbone, 1973).

Preliminary Phytochemical Analysis

All the plant part extracts viz.root, stem and leaves were screened for the presence of various bioactive compounds such as alkaloids, glycosides, tannin, flavonoids, terpenoids and phenol by standard procedures (Harbone, 1998).

HPLC Analysis

Based on the qualitative analysis, the methanol extracts of root, stem and leaves samples were prepared for HPLC analysis.

Preparation of Sample Solutions

A super critical fluid extractor SFE-2 (Applied Separation,USA) which is capable of pressure up to 680 bar and temperature up to 350^{0}C,static and dynamic extraction with flow from 0-10 L "min (gaseous carbon dioxide) and extraction vessels from 5ml to 11ml were used. An Agilant 1200 liquid chromatograph system (Agilant technologies, CA, USA) consisting of binary pump, an auto-sampler and diode – array detector was used.The column configuration consisted of an AgilantZorbax Extend reversed –phase C 18 column (250mm x 4.6 mm, 5 µm).Detection wavelength was set at 220 nm.The mobile phase consisted of A (Methanol) and B (deionized water), using a linear gradient: 0-40 min (85% A), 40-60 min (85% A-95% A).The flow rate was 1.0 ml " min.The column temperature was maintained at 30 ^{0}C.

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The extract sample of test samples under optimized conditions (Extraction pressure: 30 mpa, Extraction Temperature: 350^{0}C, Extraction time: 1 hour, 20 ml 95% ethanol modifier was used). After evaporating ethanol to dryness by a rotatory evaporator, residue was dissolved in methanol in a 25 ml. flask and then filtered througha 0.45 micro –m Millipore filter before HPLC injection. Three aliquots of the solution (20 µl were injected to RPHPLC DAD system).

HPLC Conditions and Standards Used

A mixed standard stock solution containing Andrographolide, Neo andrographolide, Andrographiside, Andrograpahnin, Apiginin,Onysilin, Methylwogonin,Coumarin,Hydroxycoumarin,Catechin, Cubenol,Ergosterol,Stigmasterol,Sitostrol,Caryophyllene, Trimethylbenzaldehyde,Sequiphellandrene,Lauric acid,Cinnamic acid, Panicolin,Methyl benzoate and Capric acid were prepared in methanol. Working standard solutions were prepared by diluting standard solution with methanol to give fifteen different concentrations with in the ranges (a) 3.8 – 45.6 µg/ml (b)2.1- 36.76 µg/ml (c) 3.2- 45.36 µg/ml (d)4.5- 56.92 µg/ml (e) 2.7-34.56 µg/ml (f) 3.8-34.98 µg/ml (g) 1.3- 23.67 µg/ml (h) 1.4-23.58µg/ml(i) 0.56- 9.43µg/ml (j) 1.2 -4.9 µg/ml(k) 0.67 -5.23 µg/ml (l) 0.23 -2.9 µg/ml (m) 0.21 - 2.65 µg/ml(n) 0.58- 0.97 µg/ml (o) 0.12- 2.43µg/ml. for calibration curves. The standard solutions were filtered through a 0.45 µm membrane prior to injection. The standard stock and working solutions were stored at 4 ^{0}C.

Result and Discussion

The phytochemical constituents of *Andrographiselongata* tested were summarized in the Table -1.The methanol extract of plant parts showed maximum number of phytochemicals related to other solvents.

On HPLC analysis of methanol extract twelve compounds were detected using appropriate standard. They include 5-hydroxy-7, 8, 2', 6'-tetramethoxyflavone, 5, 7, 8, 2'-tetramethoxyflavone, 5-hydroxy-7, 8, 2', 3', 4'-pentamethoxyflavone, 5, 2', 6'-trihydroxy-7-methoxyflavone, 5, 4'-dihydroxy-7, 8, 2', 3'-tetramethoxyflavone, Trans-cinnamic acid,Oleanolic acid, Andrographidine A,Andrographidine B, Andrographidine C, Neoandrographolide and Andrographolide. The three samples (root, stem and leaf) showed somewhat similar results with regards to their constituents. But they showed certain differences also. The three samples showed common compounds to certain extend. They belong to the category of diterpenoids, polyterpenoids and sesquiterpenoid compounds. They also possessed the abundance of flavonoids, polypropanoids and also lactones. The polyproponoids were found only in the leaves (Table 4). A few compounds were undetected due to the lack of appropriate standard. All of these bioactive compounds possessed antimicrobial (Santhi*et al*., 2006), anti-oxidant (Puupponen*et al.*,2001 and Prabhu*et al.*, 2011)and antiproliferative activities (Pourmorad*et al.*, 2006 and Ugwu*et al.*, 2013).

Table1: Phytochemical analysis of different extracts of An*drographis elongata*(Vahl) T. And.

Chemical constituents	Petroleum ether extract			Acetone extract			Chloroform extract			Methanol extract			Distilled water extract		
	L	S	R	L	S	R	L	S	R	L	S	R	L	S	R
Alkaloids	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
Steroids	+	+	+	-	-	-	-	-	-	+	+	+	-	-	+
Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
Saponin	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-
Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Glycosides	-	-		-	-	-	-	+	+	-	+	+	-	-	+
Tannin	-	-	-	+	+	+	-	-	-	+	+	+	+	-	-
Coumarin	-	-	-	+	+	+	+	+	+	+	+	+	+	-	-
Phenol	-	-	-	-	-	-	-	+	+	+	+	+	-	-	-
Anthra quinones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phlobatannin	-	-	-	-	-	-		-	-	-	-	-	-	-	-
Iridooids	-	-	-	-	-	-		-	-	-	-	-	-	-	-

L- Leaf, S- Stem, R- Root(+) Present, (-) Absent

Table 2: Compounds identified from methanolic root extract of An*drographis elongata*(Vahl) T.

Sl.No	Compound	Type	Percentage
1	5-hydroxy-7, 8, 2', 6'-tetramethoxyflavone	Flavanoid	2.03%
2	5, 7, 8, 2'-tetramethoxyflavone	Flavanoid	18.78%
3	5, 4'-dihydroxy-7, 8, 2', 3'-tetramethoxyflavone	Flavanoid	0.45%
4	Andrographidine A	Flavanoid	8.56%
5	Andrographidine B	Flavanoid	1.42%
6	Andrographidine C	Flavanoid	0.34%
7	Neoandrographolide	Diterpenoid	7.96%
8	Andrographolide	Diterpenoid	8.53%

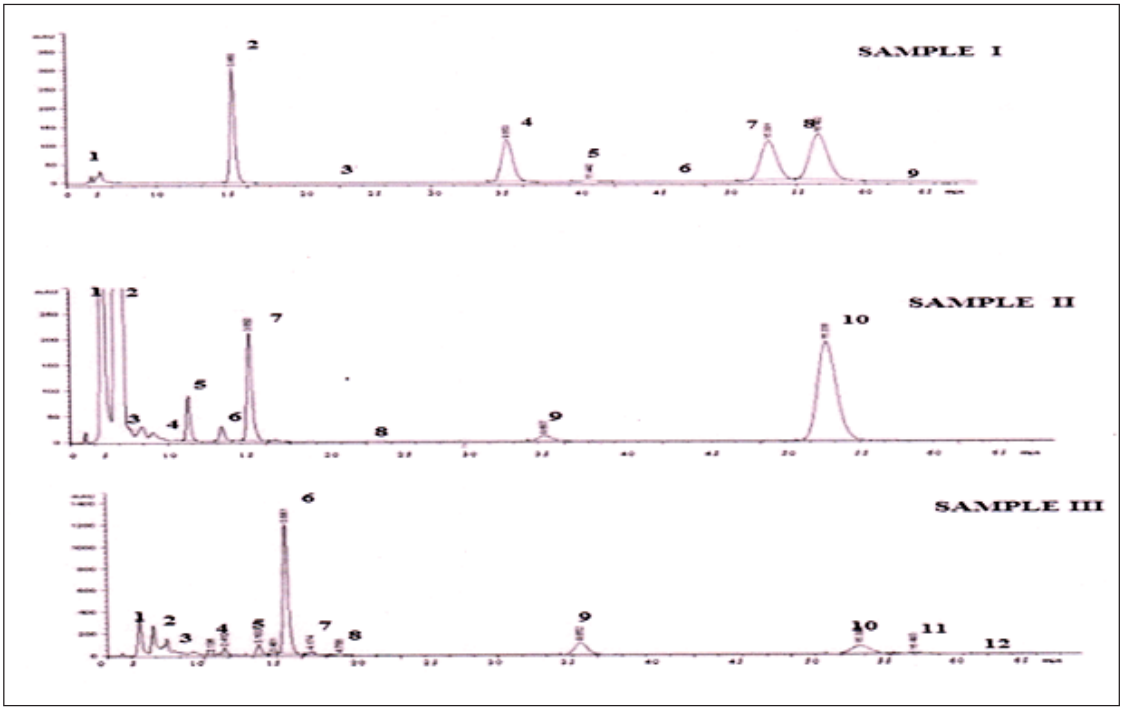
Table 3: Compounds identified from methanolic stem extract of An*drographis elongata*(Vahl) T.

Sl.No	Compound	Type	Percentage
1	5-hydroxy-7, 8, 2', 6'-tetramethoxyflavone	Flavanoid	20.43%
2	5, 7, 8, 2'-tetramethoxyflavone	Flavanoid	20.43%
3	5-hydroxy-7, 8, 2', 3', 4'-pentamethoxyflavone	Flavanoid	1.45%
4	5, 2', 6'-trihydroxy-7-methoxyflavone	Flavanoid	3.45%
5	5, 4'-dihydroxy-7, 8, 2', 3'-tetramethoxyflavone	Flavanoid	2.15%
6	Andrographidine A	Flavanoid	1.56%
7	Andrographidine B	Flavanoid	8.42%
8	Andrographidine C	Flavanoid	0.34%
9	Neoandrographolide	Diterpenoid	7.96%
10	Andrographolide	Diterpenoid	8.53%

Table 4: Compounds identified from methanolic leaf extract of *Andrographis elongata*(Vahl) T.

Sl.No	Compound	Type	Percentage
1	5-hydroxy-7, 8, 2', 6'-tetramethoxyflavone	Flavanoid	10.43%
2	5, 7, 8, 2'-tetramethoxyflavone	Flavanoid	6.72
3	5-hydroxy-7, 8, 2', 3', 4'-pentamethoxyflavone	Flavanoid	2.71
4	5, 2', 6'-trihydroxy-7-methoxyflavone	Flavanoid	2.02%
5	5, 4'-dihydroxy-7, 8, 2', 3'-tetramethoxyflavone	Flavanoid	3.12%
6	Trans-cinnamic acid	Phenylpropanoids	28.13%
7	Oleanolic acid	Phenylpropanoids	1.34%
8	Andrographidine A	Flavanoid	6.56%
9	Andrographidine B	Flavanoid	2.42%
10	Andrographidine C	Flavanoid	1.34%
11	Neoandrographolide	Diterpenoid	0.96%
12	Andrographolide	Diterpenoid	0.53%

Fig 1. HPLC graph of methanol extracts of An*drographis elongata*(Vahl) T.



Sample I: root; Sample II: stem; Sample III: leaf

Conclusion

Andrographis elongata consist of a large number of bioactive compounds. Further studies are needed with this plant to isolate and characterize the structure of the bioactive constituents of this plant for industrial drug formulation. Due to the high medicinal potential properties this endemic plant should be conserved for future generation.

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