IJPSR (2014), Vol. 5, Issue 12 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 30 April, 2014; received in revised form, 18 July, 2014; accepted, 08 August, 2014; published 01 December, 2014

PHYTOCHEMICAL AND ANTIOXIDANT STUDIES ON AN IMPORTANT INDIGENOUS MEDICINAL PLANT-*ANDROGRAPHIS PANICULATA* (BURM.F) NEES

U. Umadevi and M. Kamalam

Department of Botany, PSGR Krishnammal College for Women, Coimbatore, Tamil Nadu, India

Keywords:

Androgrpahis paniculata, Physico-chemical analysis, pharmacogonstical studies, TLC, HPLC, Andrographolide, Antioxidant property

Correspondence to Author:
M. Kamalam

Associate Professor,
Department of Botany,
PSGR Krishnammal College for Women, Coimbatore, Tamil Nadu, India

E-mail: kamaluma12@gmail.com

ABSTRACT: *Andrograhis paniculata* is a wonder drug using in traditional systems of medicine for multiple clinical applications. This research article deals with the phytochemical and antioxidant properties of *A. paniculata* leaves. Qualitative and quantitative phytochemical analyses were carried out by TLC and HPLC method. Physico-chemical analysis was done to standardize the plant drug material so as to ensure the use of genuine material. The result of phytochemical studies confirmed the presence of various secondary metabolites like alkaloids, glycosides, flavonoids, proteins, tannins, phenols, saponins, terpenoids, quinones and steroids. TLC studies of chloroform extracts showed 3 spots and methanol extracts showed 5 spots at different Rf values. HPLC analysis confirmed the presence of the major active constituent andrographolide. Effect of age on quantity of andrographolide content was studied and it was found to be higher in 120 day old plants. Antioxidant property was determined by reducing power method. Maximum antioxidant activity was observed in pure andrographolide followed by methanol and water extracts of leaf powder.

INTRODUCTION: *Andrographis paniculata*, commonly known as Siriyanangai in Tamil, belongs to the family Acanthaceae is widely used in the Indian traditional system of medicine. The major component of *A. paniculata* is andrographolide is a bitter, colorless, and crystalline in appearance, is called diterpene lactone[1]. It is used to treat poisonous bites, diabetes and respiratory tract infection. The plant possesses anti-inflammatory, antipyretic, antiviral, immune stimulatory, anticancer, antihyperglycemic and antioxidant properties[2]. Many of the plants are rich in secondary metabolites and are potent source of drugs.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(12).5240-44

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(12).5240-44

They have been widely screened for their antioxidant properties in order to find out an efficient remedy for diseases associated with oxidative stress and infections. Hence, the work is undertaken with the aim of studying the phytochemical constituents and antioxidant property of *A. paniculata.*

MATERIALS AND METHODS:

The leaves of *A. paniculata* were collected from Kanjampatty village of Coimbatore (Dt), Tamil Nadu. They are shade dried, powdered and extracted using soxhlet apparatus with different solvents like petroleum ether, benzene, chloroform, acetone, methanol and water and stored in refrigerator in air tight containers for further studies.

Qualitative phytochemical analysis

Qualitative phytochemical analysis of alkaloids, glycosides, flavonoids, terpenoids, tannins, phenols, fixed oils, fats, gums, mucilage

and saponins were carried out following the procedure of Harborne[3] (1998).

TLC studies

Chloroform and methanol extracts of leaf powder and standard andrographolide were used for TLC studies[4]. Silica gel was used as an adsorbent. The mobile phase of Chloroform: methanol: Ethyl acetate, used at the ratio of 80:15:10. Phytochemical constituents were identified based on their Rf values.

HPLC

HPLC studies were carried out to confirm the presence of Andrographolide compound using the method of Manoj kumar pandey *et al.,*[5]. The system used for analysis was Shimadzu SP 20A model with C-18 column and Methanol: water (65:35) used as a mobile phase. The injector volume was 20µl and the data is reported.

Quantitative estimation of Andrographolide

Estimation of Andrographolide is done following the method prescribed by Azhar Ali farooq and Sree Ramu[6]. The seeds were collected from pasulamali hills, Theni (Dt), Tamil Nadu and raised the seedlings in the plains of Kanjampatty village in Coimbatore (Dt.). The Andrographolide content was estimated in fresh and dried leaves of 30, 60, 90, 120, 150 and 180 day old plants and the values are expressed in percentage.

Study of antioxidant activity

The antioxidant property of the plant was studied by reducing power assay method[7]. Methanol and water extracts of leaf powder and pure Andrographolide (dissolved in methanol and water) were used to determine the reducing power.1 ml of each extract was taken in different concentration (20, 40, 60, 80 and 100µg/ml) mixed with 2.5ml of phosphate buffer (0.2M, pH 6.6) and 2.5 gm of potassium ferricyanide. The mixture was incubated at 50^{o}C for 20 minutes. 2.0 ml of TCA was added to the mixture and centrifuged at 3000 rpm for 10 minutes. 2.5 ml of upper layer of solution was taken and mixed with 2.5 ml of distilled water and 5ml of ferric chloride solution ie., in the ratio of 1:1:2 and absorbance were measured at 700nm in UV- Visible spectrophotometer. Ascorbic acid used as a standard reference and phosphate buffer used as a blank solution. Increase in the absorbance value signified the increase in reducing power.

RESULTS AND DISCUSSION:

Qualitative phytochemical analysis

Chloroform, methanol and water extracts of leaf powder of *A. paniculata* showed positive result for the presence of most of the secondary metabolites **(Table 1)**. Except fixed oil and fats, all other phytochemical constituents like alkaloids, glycosides flavonoids, tannins, phenols, saponins, terpernoids and steroids. Earlier reports were also confirmed this result[8]. Generally, plant contains the above said phytochemicals, will have high medicinal value. The flavonoids are reported to possess anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities[9].

TABLE 1: QUALITATIVE PHYTOCHEMICAL ANALYSIS OF LEAF POWDER OF *A. PANICULATA*

Sl. no	Phyto Constituents	Solvents					
		Petroleum ether	Benzene	Chloroform	Acetone	Methanol	Water
1	Alkaloids	-	-	+	-	+	+
2	Glycosides	-	-	+	+	+	+
3	Flavonoids	-	-	-	+	+	+
4	Tannins & phenols	-	-	+	+	+	+
5	Fixed oils and fats	-	-	-	-	-	-
6	Saponins	--	--	--	--	--	+
7	Terpenoids	+	+	+	+	+	+
8	Steroids	+	+	+	+	-	+

--Not performed, + present, - Absent

Alkaloids have been used as ant malarial, pain killer and to manage heart diseases. Glycosides serve as defense mechanism against predation by many microbes[10]. Steroids are known for their cardiotonic activities, insecticidal and antimicrobial properties[11]. Phenols and tannins have antioxidant

properties and Saponins were used in hypercholesterolemia, hyperglycaemia, antioxidant, anticancer, anti-inflammatory and weight loss. Apart from this *A. paniculata* is found to contain the major components like andrographolide, neoandrographolide and andrographanin are reported to have medicinal property[12].

TLC Studies

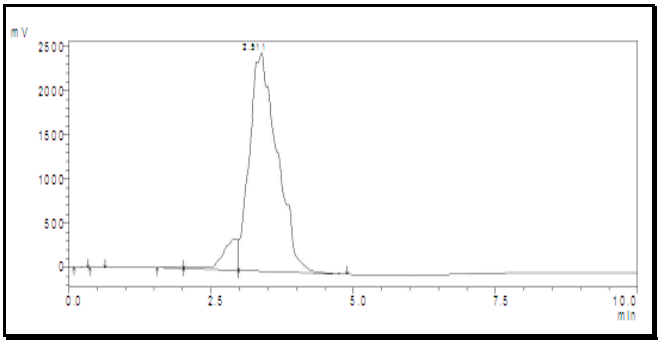
Qualitative phytochemical analysis of chloroform and Methanol extracts revealed the presence of many secondary metabolites in the leaf materials. Methanol extract of *A. paniculata* showed a maximum of five different compounds than chloroform extract **(Table 2)**. The andrographolide was spotted out with the Rf value of 0.53 with other derivatives but in earlier report, the andrographolide was detected with the Rf value 0.58[13].

TABLE 2: TLC STUDIES OF *A. PANICULATA*

S. No	Extracts used	Rf value
1	Andrographolide	**0.53**
		0.6
2	Chloroform	0.76
		0.79
		0.36
		0.4
3	Methanol	**0.53**
		0.76
		0.8

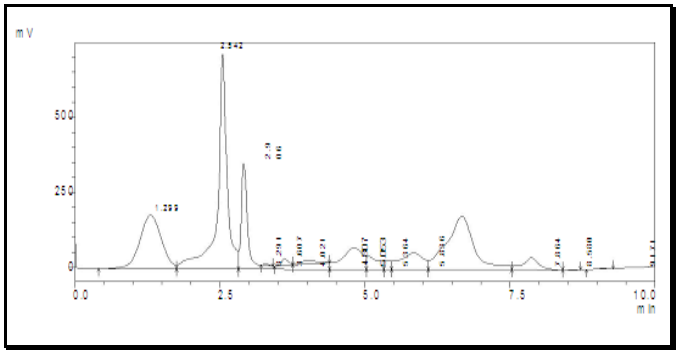
HPLC studies

In HPLC analysis, the methanol extract of the selected plant material was run along with the standard andrographolide, showed the presence of 14 different compounds **(Fig1 & 2).** Among that, andrographolide was detected at the retention time of 2.5 minutes. Similar study was conducted by Meenu Sharma *et al.*, [14] in the same plant and reported the andrographolide content with the retention time of 2.8 minutes.



Peak#	Ret. Time	Area	Height	Area %	Height%
1	0.135	1457	121	0.002	0.004
2	0.432	2815	416	0.003	0.015
3	1.870	218010	11006	0.241	0.388
4	2.511	6805438	353755	7.515	12.477
5	3.384	83525938	2470059	92.239	87.116
Total		90553658	2835357	100.000	100.000

FIG 1: HPLC CHROMATOGRAM OF STANDARD ANDROGRAPHOLIDE



Peak Table

Peak#	Ret. Time	Area	Height	Area%	Height%
1	1.299	4591718	178622	16.686	10.524
2	2.542	8230467	713101	29.909	42.014
3	2.906	3605393	351118	13.102	20.687
4	3.291	34029	6033	0.124	0.355
5	3.607	180776	23194	0.657	1.367
6	4.021	248064	10941	0.901	0.645
7	4.807	1773867	71203	6.446	4.195
8	5.053	599217	43515	2.178	2.564
9	5.364	221100	27723	0.803	1.633
10	5.836	1500457	54277	5.453	3.198
11	6.668	5770279	177115	20.969	10.435
12	7.864	741323	39243	2.694	2.312
13	8.580	2293	242	0.008	0.014
14	9.171	19427	980	0.071	0.058
Total		27518410	1697306	100.000	100.000

Quantitative estimation of Andrographolide

The quantitative estimation of andrographolide content was studied in fresh and dried leaves of 30, 60, 90, 120, 150 and 180 day old plants. The andrographolide content was higher in 120 day old plants **(Fig 3)**. Significant differences were observed in andrographolide content of fresh and dried leaves. The fresh leaves have higher andrographolide content than dried leaf powder. Similar result was reported in the earlier studies[15].

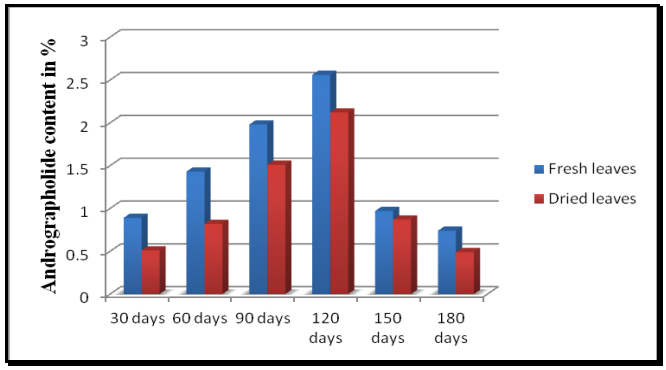


FIG 3: EFFECT OF AGE ON ANDROGRAPHOLIDE CONTENT OF *A. PANICULATA*

Antioxidant activity

Antioxidant may be defined as radical scavengers which protect the human body against free radical that cause pathological condition[16]. When the mechanism of antioxidant protection becomes unbalanced deterioration of physiological functions may occur requiring the system to depend on exogenous antioxidants from natural sources[17]. In this study, antioxidant property of methanol and water extracts of leaf powder and pure Andrographolide compound was studied and the results are recorded in **Table 3** and **Fig 4.**

Among the two extracts, methanol extract exhibited higher antioxidant property than water extract. Comparatively, the antioxidant activity was higher in pure andrographolide component than leaf powder extracts. However, the standard ascorbic acid showed the highest reducing power. As the concentration of the extracts increased the reducing power also increased. The reducing ability of a compound depends on the presence of reductants (antioxidants). The presence of antioxidants in *A. paniculata* causes the reduction of the Fe^{3+} ferricyanide complex to the ferrous form exhibiting its antioxidant potency[18].

TABLE 3: ANTIOXIDANT ACTIVITY OF LEAF POWDER OF *A. PANICULATA*

Sl.No	Concentration in µg/ml	Standard Ascorbic acid OD value at 700 nm	*A. paniculata*		Andrographolide in	
			Methanol extract	Water extract	Methanol	Water
1	20	1.010	1.159	0.832	1.161	1.177
2	40	1.200	1.191	0.945	1.203	1.281
3	60	1.523	1.216	1.016	1.231	1.343
4	80	1.733	1.282	1.124	1.311	1.446
5	100	1.832	1.407	1.320	1.553	1.607

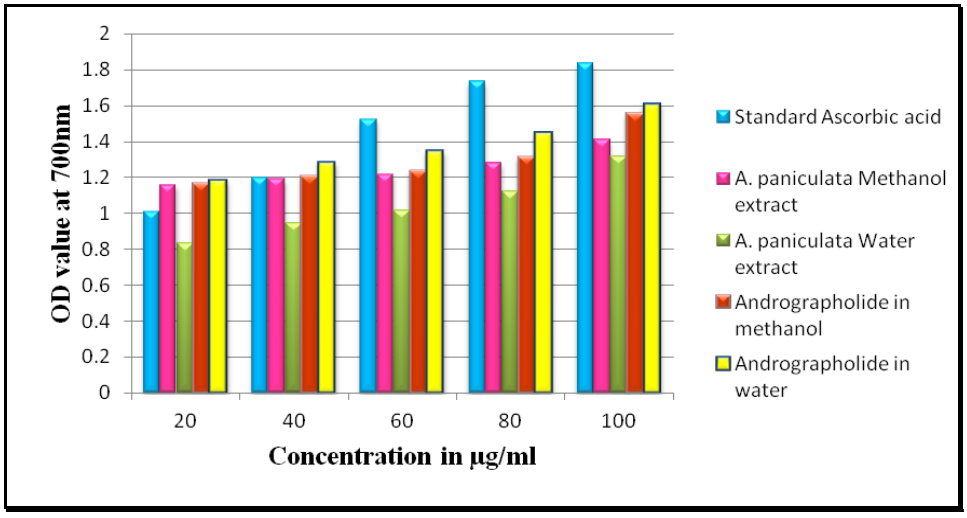


FIG 4: ANTIOXIDANT ACTIVITY OF LEAF POWDER OF *A. PANICULATA*

CONCLUSIONS: The efficacy of any drug depends on the scientific validation of the material. In the present study, proper time of collection of plant material for drug preparation is identified and reported. The correct age and time for collecting the material to obtain maximum amount of andrographolide content is 120 day old plants (just before flowering). The antioxidant potency of the plant is also found to be good. Therefore, it is concluded that this scientific methods can be conveniently adopted for validating the plants for drug preparation.

ACKNOWLEDGEMENTS: The Authors thank UGC for providing financial support (JRF) for this work.

REFERENCES:

1. Siripong P, Kongkathip B, Preechanukool K, Picha P, Tunsuwan K, Taylor W.C., Cytotoxic diterpenoid constituents from *A. paniculata* Nees leaves. J. Sci. Soc. Thailand, 1992; 18: 187-194
2. Katta Vijayakumar, Papolu, B.S. Murthy, Sukalak kannababu, Syamasundar, B., and Gottamukkala V. Subbaraju, Estimation of Andrographolide in *Andrographis paniculata* Herb, Extracts and Dosage

forms. International Journal of Applied Science and Engineering, 2007; 5, 1:27-39
3. Harborne, J.B: Methods of extraction and isolation. In: Phytochemical methods, (Chapman and Hall, London) 1998; 60.
4. Abubacker, M.N., and Vasantha, S., Antibacterial activity of Ethanolic leaf extract of *Andrographis paniculata* Nees. (Acanthaceae) and its bioactive compound Androgrpholide, Drug invention today, and 2010; 2(10):440-442. DTM; Taplin, Zaias, Rebell and Blank, 1969, Arch. Dermatol., 99:203-209.
5. Manoj Kumar Pandey, Singh, G.N., Rajeev Kr Shrama and Sneh Lata., Physicochemcial standardization of *Andrographis paniculata* (Nees): An Ayurvedic Drug, International Journal of Pharmaceutical research and development, 2011; 3(6): 81-89
6. Azhar Ali Farooqi and Sree Ramu, B.S., Cultivation of Medicinal and Aromatic crops, University press, 2001; 151-156.
7. Savitha, S and Rathna vijaya, C., Minimum inhibitory concentration and antioxidant properties of *Andrographis paniculata* using different solvent extracts, International Journal of Chemical science and technology, 2011; I:1:21-28.
8. Aiyelaagbe, O.O. and Osamudiamen, P.M., Phytochemical screening for active compounds in *Mangifera indica*. *Plant Sci. Res*., 2009; 2(1): 11-13.
9. Oomah, D.B., Isolation, characterization and assessment of secondary metabolites from plants for use in human health. *PBI Bull*., 2003; 13-20.
10. De, M., Krishna De, A. and Banerjee, A.B., Antimicrobial screening of some Indian spices. *Phytother. Res*., 1999; 1: 616-618.
11. Callow R.K. Steroids. Proc. Royal, Soc London Series A., 1936; 157:194.
12. De-Lucca A., Clevaland T., Rajasekara K., Boue S. and Brown Brown. Fungal properties of CAY-1, a plant saponin, for emerging fungal pathogens. 45th *Interscience Conference in Antimicrobial agents and Chemotherapy*, Abstract, 2005; F-490,180.
13. Ji, L.L., Wang Z., Dong F., Zhang F.W. and Wang Z.T. Andrograpanin, a compound isolated from anti-inflammatory Chinese medicine *Andrographis paniculata*, enhances chemokine SDF-alpha –induced leukocytes chemotaxis. *J. Cell. Biochem*., 2005; 95(5): 970-976.
14. Meenu Sharma, Aakansha Sharma and Sandeep Tyagi, Quantitative HPLC analysis of Andrographolide in *Andrographis paniculata* at two different stages of life cycle of plant, Acta Chimica & Pharmaceutica Indica, 2011;2(1): 1-7.
15. Renu Parasher, Anubha Upadhyay, Noor Afshan Khan and Sunil Kumar Dwivedi, Biochemical estimation and quantitative determination of medicinally important Andrographolide in *Andrographis paniculata* at different growth stages, Electronic Journal of Environmental, Agricultural and food chemistry, 2011;10(7): 2479-2486
16. Jain, P.K., and Agarwl, R.K., Antioxidant and free radical scavenging properties of developed Mono and Polyherbal formulation, Asian J.Exp.Sci. 2008; 22:3, 213-220.
17. Basniwal P.K, Sutha, M, Rathore G.S, Gupta R., Kumar V., Pareek A. and Jain D. *In-vitro* antioxidant activity of hot aqueous extract of *Helicteres isora Linn*. Fruits. Natural Product Radiance 2009; 8(5): 483-487.
18. Vlietinck, A.J., Van Hoof, L., Totte, J., Lasure, A., Vanden Berghe, D., Rwangabo, P.C., and Mvukiyumwami, J., Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties, J. Ethnopharmacol. 1995; 46:31-47.

How to cite this article:
Umadevi U and Kamalam M: Phytochemical and Antioxidant Studies On an Important Indigenous Medicinal Plant-*Andrographis Paniculata* (Burm.F) Nees. Int J Pharm Sci Res 2014; 5(12): 5240-44.doi: 10.13040/IJPSR.0975-8232.5 (12).5240-44.

All © 2014 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

Article can be downloaded to **ANDROID OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)