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In-vivo and i*n-vitro* comparative study of primary metabolites and antioxidant activity of *Andrographis paniculata*

Babeet Singh Tanwer*, Ramkishan Choudhary and Rekha Vijayvergia

Plant Pathology and Plant Biochemistry Laboratory, Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India

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Abstract

Andrographis paniculata Nees (Acanthaceae), commonly known as 'Kalmegh' is traditionally used medicinal plant. In present study callus was raised from the seeds. Maximum callus was obtained on MS medium supplemented with 2, 4- D 4.52µM/liter and IAA (5.71 µM/l). The callus and different plant parts were used for primary metabolite quantification and antioxidant activity. Maximum soluble sugars and protein found in callus, maximum amount of starch, lipid and phenolic contents were found in leaf. Leaf extract showed maximum superoxide radical scavenging activity (45.0±1.02%)) than other plant parts while root extract have maximum 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging activity (71.42±1.14%)).

Keywords:, *Andrographis paniculata*, Callus culture, Primary metabolites, DPPH, Superoxide radical.

Introduction

A large number of medicinal plants are being exploited from the natural flora for the commercial production of drugs. In order to conserve the natural flora and meet increasing demand for plant based drugs, alternative methods such as tissue culture has become popular for the production of therapeutic compounds from uniformly vigorous plants [1,2]. Further, plant cell/organ cultures are not limited by environmental, ecological and climatic conditions and cells/organs can thus proliferate at higher growth rates than the whole plant in cultivation [3].

Andrographis paniculata Nees (Acanthaceae), commonly known as 'Kalmegh', has been widely used in India, Thailand, China and Malaysia for the treatment of hepatitis [4, 5].

The plant is reported to possess protective activity against various liver disorders. The primary medicinal constituents of *A. paniculata* are andrographolide and related compounds which are diterpenoids showing antipyretic, antimalarial, anti-inflammatory, immunostimulatory and anticancerous activities [6, 7, 8]. However, no antioxidant activity and primary metabolite study have been done from in vitro raised callus of *A. paniculata*. In the present study, callus of *A. paniculata* was raised and comparative study of primary metabolites and antioxidant activity of root, stem, leaves and callus was investigated.

Material and Methods

Plant material

Healthy plants of *Andrographis panicuata* were collected from pot cultivated plants from University of Rajasthan and authenticated by Herbarium, University of Rajasthan, Jaipur, Rajasthan, India.

Chemicals

All the chemicals and growth regulators were used are analytical grade and purchased from Hi Media Pvt. Ltd. Mumbai, India.

Callus induction

Seeds were surface sterilized by 1 % Teepol for 15min followed by immersion in 70 % ethanol for 1 min and in 0.1 % mercuric chloride for 10 min, and then rinsed thoroughly with sterile distilled water. The seeds were inoculated in the MS medium [9] fortified with different concentrations of 2, 4-D and IAA. The pH of the media was adjusted to 5.8 before autoclaving. All media were autoclaved at 1.06 kg cm-2 and 121ºC for 15 min. The cultures were incubated in growth room at temperature of 25 ± 2 °C and 16-h photoperiod. 20 replicate cultures were established and each experiment was repeated twice and the cultures were observed at regular intervals.

Primary metabolite estimation

Callus, root, stem and leaf parts of *Andrographis paniculata* were evaluated quantitatively to estimate the total levels of soluble sugars, starch, proteins, lipids and phenols following the established methods for the sugars, starch [10], lipid [11], protein [12]and phenol [13]. All experiments were repeated three times for precision and values were expressed in mean ± standard deviation in terms of shade dried material. (Table 2)

Antioxidative assay

The antioxidative activity of the extracts was elucidated by 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging assay [14]. Experiments were initiated by preparing a 0.1 mM solution of DPPH in methanol. Two ml of this solution was added to a sample solution (0.1ml, 1mg/ml in methanol). After 30 min, absorbance at 515nm was measured and the percentage of radical scavenging activity was calculated from the following equation:

% Radical scavenging = (1-Abs.sample/Abs.control)×100

Abs. control is the absorbance of the DPPH solution without sample and Abs. sample is the absorbance of the tested sample.

The superoxide radical scavenging capacity of plant extract was analyzed using a modified method of Beauchamp and Fridovich (1971) [15] as described by Zhishen *et al* (1999) [16]. The 2ml of reaction mixture containing $3x10^{-6}$ mol/l riboflavin, $1x10^{-2}$ mol/l methionine and $1x10^{-4}$ mol/l nitrobluetetrazolium (NBT) in 0.05 M phosphate buffer (pH 7.8) was illuminated with two 20W fluorescent lamps at 25ºC for 25min in an aluminium foil-lined box. The photochemically reduced riboflavin generated O^{2-} which reduced NBT to blue formazan. The unilluminated reaction mixture was used as a blank. The absorbance (A) was measured at 560nm. The plant extracts (0.1 ml, 1mg/ml in methanol) were added to the reaction mixture, which scavenged O^{2-} generation, thereby inhibiting the NBT reduction. Absorbance (A_1) was measured and the decrease in O^{2-} was calculated by $A–A_1$. The degree of the scavenging was calculated by the following equation:

$$\text{Scavenging (\%)} = (A - A_1 / A) \times 100\%$$

Results

Callus induction

MS medium supplemented with different concentrations of 2, 4- D and IAA for callus induction. Seeds showed maximum callus formation on MS medium with 2, 4- D at the concentration of 4.52µM/liter. Callus was compact and greenish yellow colored. But on IAA at 5.71 µM/liter concentration it was fragile and yellowish. Callus obtained after 8 weeks of culture from MS medium supplemented with 2, 4- D (4.52µM/liter) was further evaluated for primary metabolites and antioxidant activity. (Shown in table1 and Figure 1)

Table-1 Percentage of the callus induction from *Andrographis paniculata* seeds under different levels of 2, 4-D and IAA after 8 weeks of culture

S. No	Growth regulators	Concentration (µM/liter)	Percentage of the callus induction	Nature of callus
1	2,4-D	2.26	0.0±0.0	-
		4.52	89±1.1	Greenish yellow, compact
		6.78	82±0.9	Greenish yellow, compact
		9.05	62.05±0.0	Greenish yellow, compact
2	IAA	2.85	0.0±0.0	-
		5.71	55.0±1.7	Yellow, fragile
		8.56	69.6 ± 1.9	Yellow, fragile
		11.41	50.2± 1.1	Yellow, fragile

Data are presented as mean ±S.E.M (n=20)

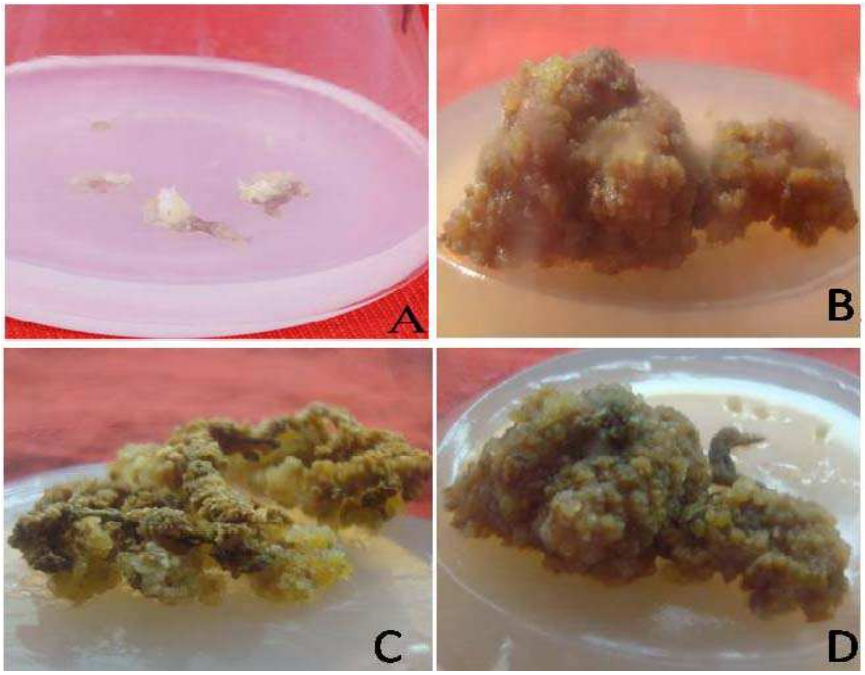


Figure 1 Induction and proliferation of callus from seeds of *Andrographis paniculata*
A. *Direct callus initiation after one week of culture (on MS medium supplemented with IAA 5.71μM)* ***B.*** *Callus after 4 weeks (on MS medium supplemented with 2, 4-D 4.52μM).* ***C.*** *Callus initiation after 6 weeks (on MS medium supplemented with IAA 8.56μM).* ***D.*** *Callus after 8 weeks (on MS medium supplemented with 2, 4-D 4.52μM).*

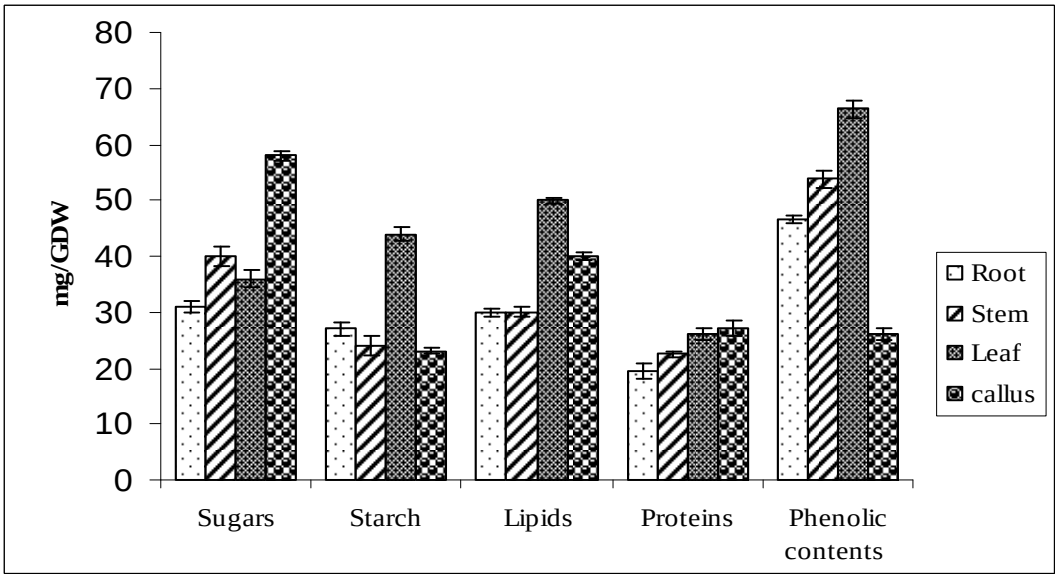
Primary metabolites

Maximum levels of soluble sugars (65±0.84mg/gdw) and proteins (27±1.41 mg/gdw) were found in callus and minimum level of soluble sugar (31±1.14 mg/gdw) and protein (19.5±1.41 mg/gdw) was found in roots. Highest concentration of starch (30±1.64mg/gdw) was in stem and lowest concentration (23±0.49 mg/gdw) in callus. Maximum level of lipid (80±0.45mg/gdw) in stem and phenolic contents (66.4±1.6mg/gdw) in leaves while minimum lipids (30±0.71mg/gdw) in roots and phenolic contents (26±1.09mg/gdw) in callus were found. (Shown in table 2 and graph 1)

Table 2: Primary metabolites of *Andrographis paniculata* in mg/ gram dry weight

Experiments	Root	Stem	Leaf	Callus
Sugars	31±1.14	40±1.58	36±1.41	58±0.84
Starch	27±1.3	24±1.64	44±1.09	23±0.49
Lipids	30±0.71	30±0.84	50±0.45	40±0.71
Proteins	19.5±1.41	22.5±0.49	26±1.02	27±1.41
Total phenols	46.6±0.75	53.8±1.67	66.4±1.6	26±1.09

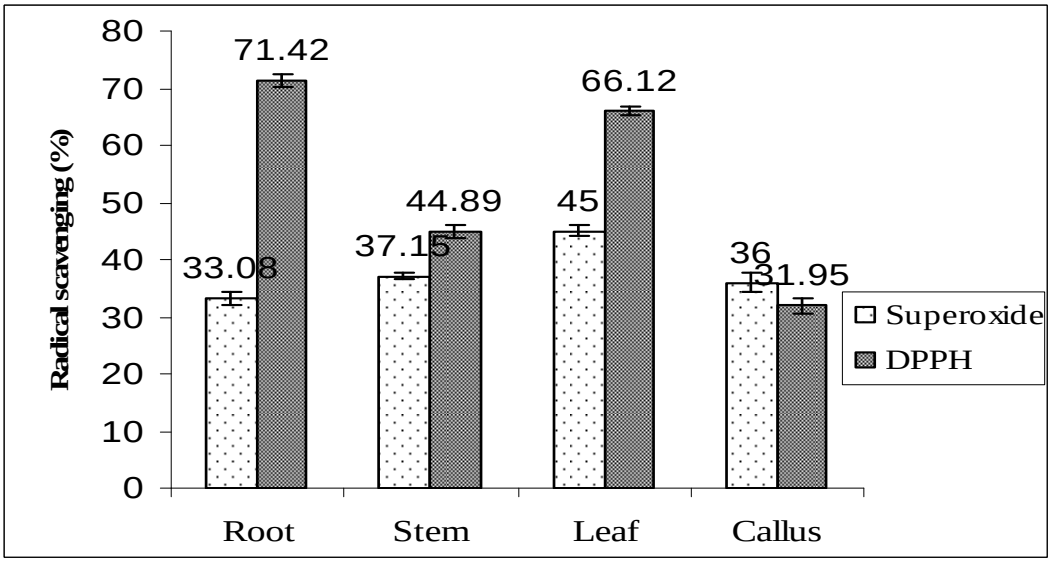
Each value is reported as mean ±SD (n=3)



Graph 1 - Primary metabolites of *Andrographis paniculata* in mg/ gram dry weight

Antioxidant activity

The antioxidant activity of callus and other plant parts of *Andrographis paniculata* was measured using 2, 2-diphenyl-1-picryhydrazyl (DPPH) free radical and superoxide radical scavenging assays. The results showed that all the parts exhibited antioxidative activity. In DPPH assay at 0.1mg/ml (in methanol) displayed comparable activity and the highest radical scavenging activity (71.42±1.14%) in root and lowest in the callus (31.95±1.41%).While the highest superoxide radical scavenging activity was highest in leaf (45.0±1.02%) and lowest in root (33.08±1.09%). However callus showed significant antioxidant activities superoxide radical scavenging (36.0±1.6%) and DPPH radical scavenging (31.95±1.41%) (Shown in graph 2)



Graph 2 Antioxidant activities of callus, roots, stem and leaves of *Andrographis paniculata*

Discussion

In vitro raised callus are being widely used. The callus was raised from explants of cotyledonary leaf and root segments of *Carthamus tinctorius* [17] and *Allium sativum* [18] were efficient for differentiation. Callus formation from axillary meristem explants of *Withania somnifera* in MS medium with 2, 4- D (2mg/L) [19]. In our study 2, 4- D 4.52µM and IAA 8.56µM both auxin concentrations showed callus formation but 2, 4-D at 4.52µM concentration showed maximum yield.

Nerium indicum Mill. *Acalypha indica* Linn and some plants of euphorbiaceae family have investigated for their primary metabolites [10, 21, 22]. Comparative *in vitro* and *in vivo* biochemical performance has been evaluated in *Adhatoda vasica* [23]. In present study callus was showed highest soluble sugars and proteins but less phenolic contents starch and lipids than *in vivo* plant parts. *In vitro* cells accumulate more sugar due to its easy availability in culture medium and these cells are in highly proliferating stage so they accumulate more primary metabolites than storage metabolites (starch, lipid) and secondary metabolites (phenolic contents). In our study also different plant parts as well as callus had showed superoxide radical scavenging activity according to their phenolic contents since presence of phenolic contents supports antioxidant status of the callus as also reported in Indian herbal tea [24].

References

[1] MR Heble, and G Roja. Trends in plant tissue culture and Biotechnology Eds. LK Pareek and Dr. PL Swarnkar. Agro Botanical Publishers, Bikaner, **1997**; 65-71.
[2] G Hirai; N Kasia and T Horada. *Plant Cell Tiss. Org. Cul*., **1997**, 48 (3), 175-180.
[3] SR Rao and KA Ravishankar. *Biotechnol. Adv*., **2002**, 20, 101–153.
[4] A Sharma; RT Singh; V Sehgal and SS Handa. *Fitoterapia*, **1991**, 62, 131–138.
[5] W Tang and G Eisenbrand. *Chinese Drugs of Plant Origin*, Springer-Verlag, Berlin, **1992**; 97–103.
[6] P Mishra; NL Pal; PY Guru; JC Katiyar and JS Srivastava Tandon. *Int. J. Pharmacogn*., **1992**, 30, 263–274.
[7] S Saxena; DC Jain; RA Bhakuni and RP Sharma. *Indian Drugs*, **1998**, 35, 458–467.
[8] RA Kumar; K Sridevi; NV Kumar; S Nanduri and S Rajagopal. *J. Ethnopharmacol*., **2004**, 92, 291–295.
[9] T Murashige and FA Skoog. *Physiol. Plant*, **1962**, 15, 473– 497.
[10] MK Dubois; JK Gilles Hamilton; PA Rebers and FA Smith. *Nature*, **1951**, 168**,** 167 (1951).
[11] J Jayaraman., "Laboratory manual in biochemistry." New Delhi: Wiley Eastern Limited, New Delhi. 1981
[12] OH Lowry; NJ Rosebrough; AL Farr and RJ Randall. *J. Biol. Chem*., 1951, 193, 265-275.
[13] HG Bray and WV Thorpe. *Meth Biochem. Anal.*, 1954, 1, 27-52
[14] S Prachayasittikul; T Suksrichavalit; C Isarankura-Na-Ayudhya; S Ruchirawat and V Prachayasittikul. *EXCLI J*., **2008**, 7, 63–70.
[15] C Beauchamp and I Fridovich. *Analytical Biochemistry*, **1971**, 44, 276-287.
[16] J Zhishen; T Mengcheng and W Jianming. *Food Chemistry*, **1999**, 64, 555-559.
[17] KJ Rani; TN Rao; G Raghunatham and PV Rao. *Ind. J. Genet.*, **1996,** 56, 458–461.
[18] JM Myers; PW Simon. *Plant Cell Rep.*, **1998**, 17, 726–730.

[19] G Roja; MR Heble; AT Sipahimalani. *Phytother. Res*., **1991**, 5, 185–187
[20] R Vijayvergia; J Kumar. *Asian J. Exp. Sci.*, **2007,** 21 (1), 123-128.
[21] T Singh; R Vijayvergia; S Sharma. *The Bioscan*, **2008**, 3(3), 337-339.
[22] R Vijayvergia; S Sharma; T Singh. *J Indian Bot. Soc.*, **2009**, 88 (1&2), 116-119.
[23] S Chauhan; Y Vimala. *J Indian Bot. Soc.* **2009**, 88 (1&2), 54-57.
[24] V Naithani; S Nair; P Kakkar. *Food Res. Inst.*, **2006**, 39 (2), 176-181.