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Research Article

Chemical Profile and Antioxidant Properties of *Andrographis producta* (C. B. Clarke) Gamble

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ABSTRACT

Background: *Andrographis producta* (Acanthaceae) is endemic to Western Ghats, India, traditionally used by native people for the control of various ailments including intestinal worms, to relieve constipation and also used to eliminate phlegm in women during postpartum. **Objective:** To investigate the chemical compounds in root, stem and leaves of *A. producta* and their antioxidant properties. **Method:** The phytochemical contents were determined using spectrophotometric methods and chemical profiling of root, stem and leaf extracts was carried out using GC-MS. Further, extracts were investigated for their antioxidant capacities using in vitro DPPH radical scavenging and FRAP assay. **Results:** The total phenolics (163.61 mg GAE/g), flavonoids (35.11 ± 0.53 mg QE/g) and tannins (84.52 ± 0.07 mg TAE/g) were highest in stem compared to leaf and root. Stem was exerted superior antioxidant capacities in both DPPH (EC_{50} 3.58 mg/ml) and FRAP assays (1.742 ± 0.02 OD at 1mg/ml) and were comparable to standards. GC-MS analysis revealed total 89 chemical compounds including phenolics, flavonoids, terpenoids and organic acids. 2-Methoxy-4-vinylphenol (0.70 %), 2,4-di-tert-butylphenol (9.74 %), phytol (10.32 %), 5-hydroxy-7,8-dimethoxyflavone (11.42 %), gamma-sitosterol (8.32 %), salvigenin (12.09 %), solanesol, (2.92 %), and alpha-terpinene (4.58 %) were important bioactive compounds found in significant amount. **Conclusion:** The present investigations indicate that various parts of *A. producta* can be explored as good source of antioxidants due to the presence of phenolics and flavonoids. The meticulous assessment of bioactive compounds from *A. producta* would be great contribution in field of medicine.

Key words: *Andrographis*, Antioxidants, GC-MS, Methanol extract, 2,4-Di-tert-butylphenol, Salvigenin.

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INTRODUCTION

Andrographis Wall. ex Nees (Family Acanthaceae) consisting 45 species globally according to International Plant Name Index (IPNI)[1] and 28 species in India where most of them have distributional range in South Indian states namely Karnataka, Tamil Nadu, Kerala and Andhra Pradesh.[2,3] *Andrographis paniculata* Nees (Kalmegh) is therapeutically well known species in the genus distributed throughout India and other South East Asian countries. Kalmegh has been used to treat fever, cough, sore throat, gastric infections and other acute diseases and is as prime ingredient in several Ayurvedic formulations.[4] The plant possesses wide range of pharmacological effects including antimalarial, anticancer, anti-oxidative, anti-inflammatory, antidiabetic, immunomodulatory and antiretroviral activities. Andrographolide is major diterpene lactone in the plant and neoandrographolide, 14-deoxy-11,12-didehydroandrographolide, andrographon, 14-deoxyandrographolide are other bioactive compounds contributing to pharmacological activities together with some flavonoids.[4,5]

Andrographis producta (C. B. Clarke) Gamble is an undershrub reaching 150 cm in height. It is indigenous to South Indian states namely Karnataka, Kerala and Tamil Nadu[2] and grows frequently in the edges of shola forests and often high elevated hill forests. The leaves are lanceolate-elliptic, acute-acuminate, sub-membranous, strigose on adaxial surface, 10-20 cm in long. The inflorescence is branched, rather terminal panicle with purplish white flowers. Corolla decurved, with a prominent ventricose protuberance.

Modern technologies and scientific advancements have been facilitating the discovery of synthetic drugs which helped in the treatment and control of even dreadful diseases but prolonged medication of synthetic drugs may lead to toxic side effects in human. The demand for the herbal based medicines, pharmaceuticals, nutraceuticals, food supplements and herbal cosmetics is growing worldwide due to the numerous benefits including non-toxicity, less/no side effects, better compatibility with human physiology and affordable costs over synthetic drugs.[6] Hence, identification of ethnopharmacologically important plants and extraction, isolation and characterization of chemical compounds using sophisticated chromatography techniques such as GC-MS, LC-MS and HPLC methods can help to find out biologically active compounds.

Andrographis producta is lesser known medicinally important plant of the genus used by tribal/native people in the Western Ghats region where it has been called with regional name 'Chevidantu'. The plant has

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been used to treat skin diseases.[7] During the present study, we found that the leaf decoction is used to relieve the constipation, to control the intestinal worms in children and also used to eliminate phlegm in women during postpartum. Though species belongs to the medicinally important genus, the investigation on the chemical composition, antioxidant properties and pharmacological activities have not been carried out till date. Hence, in the present work, an attempt has been made to explore the chemical compounds present in the different parts of the plant i.e. root, stem and leaves using spectrophotometric and GC-MS technique. Further, antioxidant potential of root, stem and leaves has been assessed by DPPH free radicals scavenging and FRAP assays to gather comprehensive information. Therefore, the present study aimed to investigate the available chemical compounds in root, stem and leaves of *A. producta* and their antioxidant properties.

MATERIALS AND METHODS

Plant materials

The plant materials of *Andrographis producta* were collected from Bababudan Hill range (Lat: 13° 25' 10.2108" Long: 75° 44' 37.0026"; MSL 1467.30 m), Chikkamagaluru district, Karnataka, India. Plants were identified by using Flora of British India and voucher specimen (DSD-06A) was deposited at Herbarium (SUK), Shivaji University, Kolhapur, India.

Chemicals and reagents

Analytical grade chemicals were used throughout the experiments. Methanol, ferric chloride ($FeCl_3$), sodium carbonate (Na_2Co_3), sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), hydrochloric acid (HCl), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2, 4, 6-tripyridyl-s-triazine (TPTZ), ascorbic acid, butylated hydroxyl anisole (BHA), and Folin-Coicalteu (FC) reagents were procured from HiMedia Laboratories Pvt. Ltd. (Mumbai, India). Gallic acid (GA), quercetin, tannic acid (TA) and Folin-Denis (FD) reagent were purchased from Sigma Aldrich Chemical Co. (USA).

Preparation of extracts

The plants were separated into different parts viz. root, stem and leaves were shade dried and ground to a coarse powder in blender followed by drying at 35 °C in hot air over 24 h. 10 g powder of each material was extracted with 100 ml methanol individually with Soxhlet extractor at 55-60 °C for 8 h. The extracts of root, stem and leaf were dried to evaporate the excess solvent using rotary evaporator under reduced pressure at 40 °C and used in experiments.

Quantification of phytochemicals

Determination of total phenolics

The amount of total phenolics in the methanolic extracts of root, stem and leaf was analyzed spectrophotometrically, as described by Folin and Ciocalteau[8] with slight modifications. The methanolic extracts (0.5 ml) were taken in test tubes containing 2.5 ml of deionized distilled water and mixed with 0.1 ml (2N) of Folin-Ciocalteau (FC) reagent. The mixture was allowed to stand for 6 min before adding 0.5 ml of 20 % sodium carbonate solution. The absorbance of the developed color after 30 min of incubation at room temperature was detected at 760 nm on UV visible spectrophotometer (UV-1601, Shimadzu). The amount of total phenolic content was calculated by comparing to the standard curve of gallic acid and expressed as the mg gallic acid equivalent (GAE) per g of dry samples.

Determination of flavonoids

The flavonoid content in the extracts of root, stem and leaf was determined spectrophotometrically[9] with some modifications. 0.5 ml of methanolic extracts of each samples were mixed with 2.5 ml of distilled water and solution of 0.15 ml sodium nitrite (5%) was added to take place reaction for 6 min. Then, 0.3 ml of 10 % aluminum chloride was added to take up the reaction for further 5 min. Finally, 2 ml of 1M sodium hydroxide was mixed and absorbance was read immediately on a spectrophotometer (UV-1601, Shimadzu) at 510 nm. The standard calibration curve of quercetin was used to determine the amount of flavonoid contents and expressed as mg of quercetin equivalent (QE) per g of dry samples.

Determination of tannins

The methanolic extracts were used in determination of tannins present in the samples by spectrophotometrically according to the method developed by Schenderi.[10] Each extracts (0.5 ml) were mixed with 2.5 ml of distilled water and 0.25 ml of Folin-Denis reagent, followed by 0.5 ml of 30 % sodium carbonate. Then all the reagents were mixed well to complete reaction and incubated for 30 min at room temperature. The absorbance of developed color was measured at 700 nm using spectrophotometer (UV-1601, Shimadzu). The known amount of tannic acid was used to draw the calibration curve and amount of tannin in samples determined and expressed as mg of tannic acid equivalent (TAE) per gram of dry samples.

Gas chromatography and mass spectrometry (GC-MS) analysis

Gas chromatography and mass spectrometry (Model: QP2010S; Shimadzu Corporation, Japan), a sophisticated analytical instrument equipped with Rxi-5Sil MS capillary column (length 30 m × 0.25 mm ID, 0.25 µm film thickness) was used to separate the chemical compounds present in the methanolic extracts of the samples. Helium (99.9995 %) was the carrier gas with a constant flow rate of 1 ml/min. the column oven temperature was held at 60 °C initially and increased to 260 °C by 5 °C/min held for 5 min. The diluted samples of1 µl were injected in the split injection mode and the solvent delay was 4 min. The total run time was of 30 min. The ion source and interface line temperatures were set at 200 °C and 280 °C, respectively.[11] The separated components were identified by comparing the retention times of authentic compounds and mass spectra from NIST 11 & WILEY 8 mass spectral libraries.

In vitro antioxidant capacities

DPPH radicals scavenging assay

The free radical scavenging activity of the extracts was measured in vitro by 2, 2-diphenyl-1-picryl hydrazyl (DPPH) assay.[12] The stock solution was prepared with 24 mg DPPH in 100 ml methanol. The working solution was obtained by diluting DPPH stock solution with methanol to attain an absorbance of about 0.99 ± 0.02 at 515 nm on spectrophotometer (UV-1601, Shimadzu). Aliquots (3 ml) of this working solution (DPPH) were mixed with 0.1 ml sample of various concentrations (0.2-1 mg/ml). The reaction mixture was shaken well and incubated in the dark for 30 min at room temperature. Then the absorbance was recorded at 515 nm. Simultaneously, the control was prepared without any sample. ascorbic acid and butylated hydroxyl anisole (BHA) were used as standards. The percentage of DPPH scavenging activity was calculated using the following equation.

$$\text{Percentage of DPPH scavenging activity} = \frac{\text{OD of control} - \text{OD of sample}}{\text{OD of control}} \times 100$$

Ferric reducing ability of plasma (FRAP) assay

The FRAP assay was carried out according to the method developed by Benzie and Strain.[13] The different concentration (0.2-1.0 mg/ml) of 0.1 ml plant extracts were added to 3 ml of freshly prepared FRAP reagent

(300 mM acetate buffer at pH 3.6, 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 20 mM $FeCl_3$ in the ratio 10:1:1) and heated to 37 °C in an hot water bath for 10 min. Final volume was adjusted to 4 ml using distilled water and incubated in the dark at room temperature for 10 min. Optical density was measured at 593 nm. Ascorbic acid and butylated hydroxyl anisole were used as standards. The increase in the optical density (OD) indicated greater the antioxidant power.

All experiments were conducted in triplicates using three different lots. Mean, percentage, EC_{50} values and standard deviations were calculated using Microsoft Office Excel 2007.

RESULTS

Phytochemical analysis

The phytochemicals such as total phenolics, flavonoids and tannins were quantified in the root, stem and leaves of *A. producta* and expressed as gallic acid equivalent (GAE), quercetin equivalent (QE) and tannic acid equivalent (TAE), respectively. Amount of total phenolics, flavonoids and tannins in the root extract were 94.60 ± 0.51 mg/g DW, 33.50 ± 0.51 mg/g DW and 70.11 ± 0.12 mg/g DW respectively. The stem extract comprised of 163.61 ± 0.45 mg/g DW of phenolics, 35.11 ± 0.53/g DW of flavonoids and 84.52 ± 0.07 mg/g DW of tannins. The spectrophotometric quantifications affirmed that 109.75 ± 0.10 mg GAE/g DW of phenolics in higher proportion followed by 74.78 ± 0.27 mg/g DW of tannins and 32.26 ± 0.96 mg/g DW of flavonoids in the leaves of *A. producta*.

Gas chromatography and mass spectrometry (GC-MS) analysis

GC-MS analysis was performed to get insight into chemical profiles of root, stem and leaves of *A. producta*. Chromatograms of root, stem and leaf extracts (Figure 1) revealed that 30 (Table 1), 28 (Table 2) and 64 (Table 3) spectral peaks, respectively.

2-monopalmitin (12.20 %), 5-hydroxy-7,8-dimethoxyflavone (11.42 %), salvigenin (11.06 %), 2,4-di-tert-butylphenol (9.74 %), trans-cinnamic acid (8.34 %), gamma-sitosterol (8.32 %), alpha-monostearin (7.44 %) and asaraldehyde (5.60 %) were the prime chemical compounds constituted 74.12 % of the methanolic extract of roots of *A. producta* (Table 1, Figure 1A).

Methanolic extract of stem sample composed of salvigenin (12.09 %), 2-monopalmitin (10.62 %), 2,4-di-tert-butylphenol (8.79 %), phytol

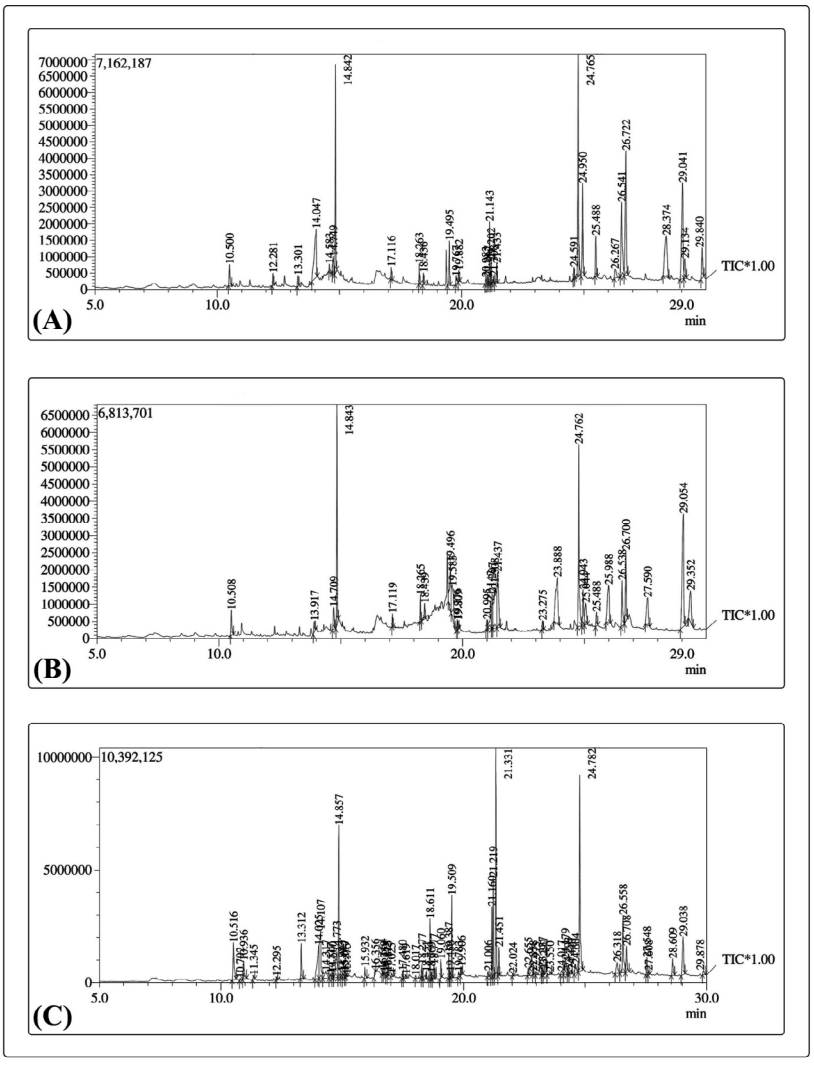

Figure 1: Chromatograms of gas chromatography and mass spectrometry (GC-MS) analysis of **(A)** root extract; **(B)** stem extract and **(C)** leaf extract.

Table 1: GC-MS analysis of *Andrographis producta* root.

Sl. No.	Chemical compounds	Retention Time	Peak Area (%)
1	Azulene	10.500	1.88
2	2-Methoxy-4-vinylphenol	12.281	0.70
3	Methyl cinnamate	13.301	0.62
4	trans-Cinnamic acid	14.047	8.34
5	Octadecane	14.583	0.38
6	1,2,3,5,6,8-Hexahydro-4,7-azulenedione	14.749	1.39
7	2,4-di-tert-butylphenol	14.842	9.74
8	Eicosane	17.116	0.65
9	3-Heptadecanol	18.263	0.95
10	Isopropyl myristate	18.436	0.52
11	Hexadecanoic acid, methyl ester	19.495	2.14
12	3-Ethyl-3-pentanol	19.767	0.70
13	Eicosanoic acid	19.882	0.60
14	1-Butylcyclohexanol	20.983	0.39
15	1-Octadecanol	21.067	0.34
16	Methyl octadeca-9,12-dienoate	21.143	2.78
17	9-Octadecenoic acid (z)-, methyl ester	21.202	1.15
18	Phytol	21.308	0.45
19	Octadecanoic acid, methyl ester	21.433	1.21
20	Hexanoic acid, pentadecyl ester	24.591	0.67
21	2-Monopalmitin	24.765	12.20
22	Asaraldehyde	24.950	5.60
23	Acetosyringone	25.488	2.41
24	6,11-Hexadecadien-1-ol	26.267	1.18
25	alpha-Monostearin	26.541	7.44
26	5-Hydroxy-7,8-dimethoxyflavone	26.722	11.42
27	gamma-Sitosterol	28.374	8.32
28	Salvigenin	29.041	11.06
29	L-Phenylalanine, n-heptafluorobutyryl-, hexadecyl ester	29.134	1.85
30	Solanesol	29.840	2.92

(7.88 %), butylphosphonic acid, butyl 4-(2-phenylprop-2-yl) phenyl ester (7.48 %), methyl 8,10-dimethyl-hexadecanoate (6.07 %) and 4-acetoxy-6',7-dimethyl-5',8'-dimethoxy-1,2'-binaphthalene-1',4',5,8-tetrone (5.19 %) as major compounds (Table 2, Figure 1B).

The GC-MS results of leaf sample are presented in Table 3, the large number of spectral peaks (i.e. 64 compounds) were detected in the leaf, compared to root and stem (Figure 1C). 2-monopalmitin (12.13 %) and phytol (10.32 %) were highest in the proportion followed by 2,4-di-tert-butylphenol (7.43 %) and trans-cinnamic acid (5.74 %). The octadecanoic acid, 2, 3-dihydroxypropyl ester (4.48 %), 9,12,15-octadecatrienoic acid, methyl ester (4.19 %) and alpha-terpinene (4.58 %) were also present in appreciable quantity.

In vitro antioxidant capacities

DPPH radicals scavenging assay

DPPH free radicals were deep purple colored due to their lone pair of electrons and the antioxidants present in the samples scavenge the lone pair of electrons that decolorize the DPPH containing solution. Thus, the decreasing in the purple color is directly linked to the antioxidant potential of methanolic extracts of the samples. The concentration dependent scavenging activity against DPPH radicals was expressed in mg/ml EC_{50} value (Figure 2A). The lower the EC_{50} value indicated higher the radical scavenging activity and vice versa. The methanolic extracts of root, stem and leaf exhibited DPPH radical scavenging capacity with EC_{50} value of 5.02 mg/ml, 3.58 mg/ml and 7.02 mg/ml respectively, which were less than ascorbic acid (2.85 mg/ml) and BHA (3.22 mg/ml) (Figure 2A).

Ferric reducing ability of plasma (FRAP) assay

FRAP assay was rapid and sensitive to assess antioxidant power with a range of concentration. Under low pH, the colorless ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex reduced to an intense blue complex, ferrous-tripyridyltriazine (Fe^{2+}-TPTZ) by the antioxidants present in the sample on dose-response relationship. The higher in the color development indicated more amount of antioxidants. Antioxidant power of standard ascorbic acid and BHA reached maximum efficacy at low concentration i.e. 1.986 ± 0.02 OD. at 0.2 mg/ml and 2.116 ± 0.04 OD at 0.4 mg/ml respectively, but dose-response of each samples tested was linear (Figure 2B) with change in concentration. The methanolic extracts of stem showed good antioxidant power i.e. 0.63 ± 0.015 OD and 1.742 ± 0.02 OD at 0.2 mg/ml and 1mg/ml of concentration respectively. The order of antioxidant power of samples was stem > root > leaf (Figure 2B).

DISCUSSION

Herbal products of *Andrographis paniculata* are in higher demands in herbal and phamceutical market and it amounts to 2197.3 tons per year (in the year 2005-2006).[14] It has been highly traded and prioritized medicinal plant in the Asian countries that has lead to decline in the availability of natural populations.[14,15] The over-exploitations of *A. paniculata* have created the necessity to search for alternatives to relieve the burden. In present study, we quantified the total phenolics, flavonoids and tannins in root, stem and leaf from *A. producta*. The total phenolics (163.61 mg GAE/g), flavonoids (35.11 ± 0.53 mg QE/g) and tannins (84.52 ± 0.07 mg TAE/g) were highest in stem compared

Table 2: GC-MS analysis of *Andrographis producta* stem.

Sl. No.	Chemical compounds	Retention Time	Peak Area (%)
1	Azulene	10.508	1.73
2	trans-Cinnamic acid	13.917	0.71
3	1,2,3,5,6,8-Hexahydro-4,7-azulenedione	14.709	1.55
4	2,4-di-tert-butylphenol	14.843	8.79
5	Eicosane	17.119	0.49
6	3-Heptadecanol	18.265	1.09
7	Isopropyl myristate	18.439	0.69
8	Hexadecanoic acid, methyl ester	19.496	4.05
9	4-Tert-Butylcatechol, Bis (Trifluoroacetate)	19.583	4.57
10	3-Ethyl-3-pentanol	19.776	0.36
11	2,6,10,14-Tetramethyl-hexadecane,	19.809	0.32
12	1-Butylcyclohexanol	20.995	0.50
13	9,12-Octadecadienoic acid (z,z)-, methyl ester	21.142	2.81
14	Hexadecadienoic acid, methyl ester	21.207	2.09
15	Phytol	21.308	7.88
16	Methyl 8,10-dimethyl-hexadecanoate or 8,10-dimethyl-16:0	21.437	6.07
17	Tetrapentacontane	23.275	0.36
18	Butylphosphonic acid, butyl 4-(2-phenylprop-2-yl) phenyl ester	23.888	7.48
19	2-Monopalmitin	24.762	10.62
20	Asaraldehyde	24.943	2.26
21	L-Phenylalanine, n-pentafluoropropionyl-, octadecyl ester	25.044	2.38
22	1-[1,1'-Biphenyl]-3-yl-ethanone	25.488	0.84
23	4-Acetoxy-6',7-dimethyl-5',8'-dimethoxy-1,2'-binaphthalene-1',4',5,8-tetrone	25.988	5.19
24	Octadecanoic acid, 2,3-dihydroxypropyl ester	26.538	3.21
25	5-Hydroxy-7,8-dimethoxyflavone	26.700	4.31
26	Stigmasterol	27.590	3.57
27	Salvigenin	29.054	12.09
28	gamma-Sitosterol	29.352	3.99

Table 3: GC-MS analysis of *Andrographis producta* leaf.

Sl. No.	Chemical compounds	Retention Time	Peak Area (%)
1	Azulene	10.516	3.44
2	2-Propyl-1-heptanol	10.792	0.06
3	2,3-Dihydro-benzofuran	10.936	1.62
4	2,4-Hexadiene, 3,4-Dimethyl-, (E,Z)-	11.345	0.29
5	2-Methoxy-4-vinylphenol	12.295	0.19
6	m-Hydroxy cinnamic acid , methyl ester	13.312	1.95
7	alpha-Terpinene	14.025	4.58
8	Trans-Cinnamic acid	14.107	5.74
9	Adenosine, 1,2-dihydro-2-oxo-	14.317	2.06
10	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl) but-3-en-2-one	14.517	0.51
11	Eicosane	14.600	0.29
12	1,2,3,5,6,8-Hexahydro-4,7-azulenedione	14.773	3.45
13	2,4-di-tert-butylphenol	14.857	7.43
14	Nerolidyl acetate	14.983	0.72
15	Benzoic acid, 4-ethoxy-, ethyl ester	15.077	0.50
16	1-(2-Ethoxyphenyl) acetone	15.117	0.30
17	Naphthalene, decahydro-1,4-dimethoxy-, (1.alpha,4.beta,4a.alpha,8a.alpha)-	15.175	0.16
18	Farnesene Epoxide, E-	15.932	0.67
19	Megastigmatrienone	16.356	0.34
20	4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene)pentan-2-ol	16.664	0.19
21	beta-Ionol	16.719	0.09
22	Endo-1,5,6,7-tetramethylbicyclo[3.2.0] hept-6-en-3-ol	16.825	0.21
23	1-Chlorohexadecane,	16.875	0.21
24	Unknown	17.025	0.21
25	2,6-Dimethyl-8-(tetrahydro-2h-pyran-2-yloxy)-2,6-octadien-1-ol	17.480	0.34
26	Octadecane, 1-chloro-	17.619	0.21

27	(-)-Loliolide	18.017	0.33
28	3-Heptadecanol	18.277	0.61
29	Isopropyl myristate	18.451	0.24
30	Neophytadiene	18.611	2.53
31	1-Dodecanol, 3,7,11-trimethyl-	18.669	0.34
32	3-Oxatricyclo[20.8.0.0(7,16)]Triaconta-1(22),7(16),9,13,23,29-Hexaene	18.777	0.69
33	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	19.060	0.76
34	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	19.387	1.16
35	Methyl palmitoleate	19.450	0.27
36	Hexadecanoic acid, methyl ester	19.509	3.47
37	3-Ethyl-3-pentanol,	19.783	0.33
38	9-Octadecenoic acid (z)-	19.906	0.54
39	1-Butylcyclohexanol	21.006	0.28
40	9,12-Octadecadienoic acid, methyl ester	21.160	3.12
41	9,12,15-Octadecatrienoic acid, methyl ester	21.219	4.19
42	Phytol	21.331	10.32
43	Methyl stearate	21.451	1.34
44	Geranyl-.alpha.-terpinene	22.024	0.12
45	6,9-Octadecadiynoic acid, methyl ester	22.655	0.40
46	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	22.858	0.13
47	2-Hexadecen-1-Ol, 3,7,11,15-tetramethyl-, [r-[r*,r*-(e)]]- (t-phytol)	22.975	0.30
48	Docosanoic acid, methyl ester	23.237	0.40
49	Tetrapentacontane	23.292	0.24
50	Card-20(22)-Enolide, 3,5,14,19-tetrahydroxy-, (3.beta.,5.beta.)-	23.550	0.13
51	Pinostrobin chalcone	24.017	0.71
52	beta-Sitosterol	24.179	2.49
53	Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha.)-	24.293	0.36
54	1h-Indole-3-acetic acid	24.440	0.32
55	2-Ethylbutyric acid, eicosyl ester	24.604	1.05
56	2-Monopalmitin	24.782	12.13
57	1-Docosanol	26.318	1.44
58	Octadecanoic acid, 2,3-dihydroxypropyl ester	26.558	4.48
59	5-Hydroxy-7,8-dimethoxyflavone	26.708	2.08
60	Squalene	27.548	1.19
61	Apigenin 7,4'-dimethyl ether	27.608	0.28
62	1-Heptacosanol	28.609	1.28
63	Salvigenin	29.038	3.76
64	3-(4-Fluoro-phenyl)-5,5-dimethyl-4,5-dihydro-3h-2,3,6,11-tetraaza-cyclopenta[a]anthracene	29.878	0.40

Table 4: List of biologically important chemical compounds indentified in *Andrographis producta* by GC-MS.

Sl. No.	Chemical compounds	Source of compounds	Biological uses	Reference
1	2,4-Di-tert-butylphenol	Root, stem and leaf	Anti-biofilm, anti-inflammatory and anticancer effects	[18,19]
2	2-Methoxy-4-vinylphenol	Root, leaf	Antimicrobial, anticancer and antiinflammatory activity	[20,21,22]
3	5-Hydroxy-7,8-dimethoxyflavone or 7-O-Methylwogonin	Root, stem and leaf	Anticancer agent	[23]
4	alpha-Terpinene	Leaf	Insecticidal, trypanocidal, oxidative stress, cytotoxic and genotoxic effects	[24 25,26]
5	Apigenin 7,4'-dimethyl ether	Leaf	Antifungal activity	[27]
6	Asaraldehyde	Root and stem	Antiobesity effects	[28]
7	Azulene	Root, stem and leaf	Anti-inflammatory activity	[29]
8	Cinnamic acid	Root, stem and leaf	Antibactearial, antioxidant, anti-inflammatory, and antidiabetic activity	[30] [31] [32]
9	gamma-Sitosterol	Root and stem	Hypolipidemic and anticancer activity	[33] [34]

10	Methyl cinnamate	Root	Larvicidal activity	35
11	Neophytadiene	Leaf	Anti-inflammatory effects	36
12	Phytol	Root, stem and leaf	Anti-inflammatory and immune-modulating properties	37 38
13	Pinostrobin chalcone	Leaf	Anti-obesity effects	39
14	Salvigenin	Root, stem and leaf	Neuroprotective effects	40
15	Solanesol	Root	Neuroprotective effects and antioxidant activity	41 42
16	Squalene	Leaf	Antitumor and antioxidant effects	43
17	Stigmasterol	Stem	Anti-diabetic activity	44
18	beta-Sitosterol	Leaf	Anti bacterial, genotoxic, antioxidant and antidiabetic effects	45

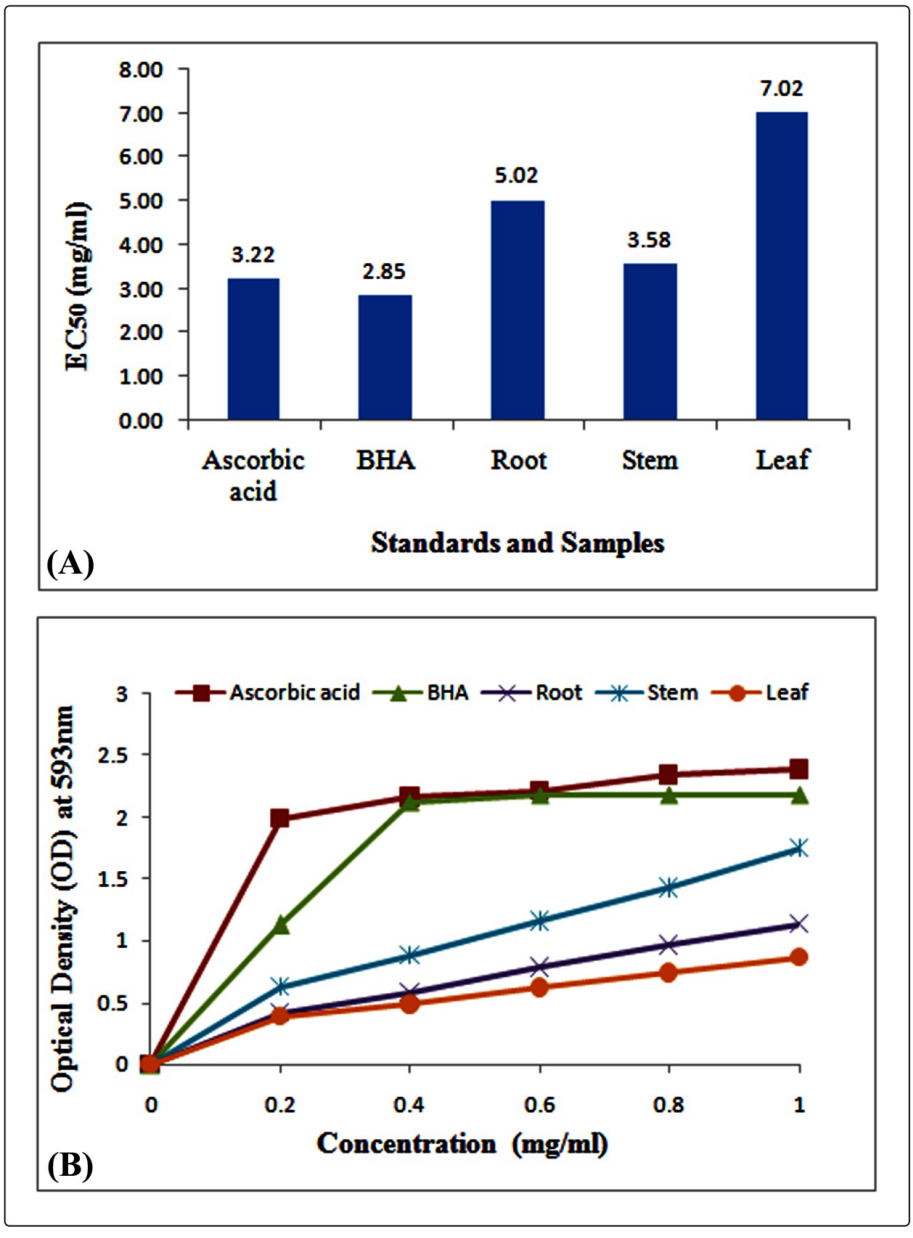

Figure 2: (A) DPPH radical scavenging activity of root, stem and leaf extracts of *A. producta* in comparison with ascorbic acid and BHA expressed in EC_{50} value (mg/ml); **(B)** FRAP antioxidant power of root, stem and leaf of *A. producta* (conc. 0.2-1.0 mg/ml concentration) in comparison with ascorbic acid and BHA expressed in Optical density (OD) at 593 nm.

to leaf and root. The total phenolic and tannins were relatively less in roots (i.e. 94.60 ± 0.51 mg GAE/g and 70.11 ± 0.12 mg TAE/g, respectively) than the leaf (i.e. 109 ± 0.10 mg GAE/g and 74.78 ± 0.27 mg TAE/g, respectively) extract. Similarly, Rafat *et al.*[16] reported the phenolic content variation in ethanolic extract of stem, leaf and fruits of *A. paniculata* which were 55.02 ± 0.35 mg of GAE/g, 75.86 ± 0.82 mg of GAE/g and 181.00 ± 1.48 mg of GAE/g respectively. However, amount of phenolic content was superior in stem (163.61 mg GAE/g) and leaf (109 ± 0.10 mg GAE/g) of *A. producta* compared to that of *A. paniculata*.

The polyphenols such as total phenolics and flavonoids derived from the plants have been more effective antioxidants.[17] The DPPH radical scavenging activity was highest in the stem samples with 3.58 mg/ml EC_{50} value (Figure 2A) which was comparable to EC_{50} values of standard ascorbic acid (3.22 mg/ml) and BHA (2.85 mg/ml). The high amount of total phenolics (163.61 mg GAE/g) and flavonoids (35.11 ± 0.53 mg QE/g) were found in stem might have contributed to antioxidant effects. Root and leaf extracts were exhibited less radical scavenging activity than stem with EC_{50} values of 5.02 mg/ml and 7.02 mg/ml of concentration respectively (Figure 2A). Further, FRAP assay affirmed the antioxidant effects of each sample as displayed by DPPH assay. The reduction of ferric-tripyridyltriazine (Fe^{3+}-TPTZ) to ferrous-tripyridyltriazine (Fe^{2+}-TPTZ) found highest in the solution of stem extract with 1.742 ± 0.02 OD (Figure 2B), whereas root (1.139 ± 0.03) and leaf (0.866 ± 0.016) showed relatively less reduction of Fe^{3+}-TPTZ (Figure 2B). The DPPH and FRAP assays have demonstrated that stem was rich reservoir of antioxidant compounds followed by root and leaf, and antioxidant capacities were attributed to total phenolics and flavonoids that are capable of donating hydrogen to free radicals to reduce oxidative stress (Figure 2).

The GC-MS analysis of *A. producta* revealed that the large number of chemical compounds present in the methanolic extracts of different parts. Total of 89 compounds were identified by GC-MS analysis, in which 14 compounds were common in all the three extracts, 2 compounds in root and stem, 2 compounds in stem and leaf and 1 compound in root and leaf extracts. However, 13, 10 and 47 compounds were specific to root, stem, and leaf extracts, respectively (Table 1, 2 and 3). The GC-MS analysis showed the presence of several biologically active compounds are listed in Table 4, including phenolics, flavonoids, terpenoids and organic acids. Azulene (3.44 %), 2-methoxy-4-vinylphenol (0.70 %), cinnamic acid (8.34 %), 2,4-di-tert-butylphenol (9.74 %), phytol (10.32 %), 5-hydroxy-7,8-dimethoxyflavone (11.42 %), gamma-sitosterol (8.32 %), salvigenin (12.09 %), solanesol, (2.92 %), stigmasterol (3.57 %), and alpha-terpinene (4.58 %) were the important bioactive compounds found in significant amount in the root, stem and leaf.

Cinnamic acid is belongs to class of aromatic organic compounds and it was present in all three parts of *A. producta* i.e. root (8.34 %), stem (0.71 %) and leaf (5.74%). The cinnamic acid was reported to exhibit in vivo anti-diabetic activity in Wistar rats.[32] The administration of cinnamic acid at 10 mg/ kg dose to non-obese type 2 diabetic rats had decreased the blood glucose level significantly on time and dose dependent mode and also, at 100 µM concentration, triggered the insulin secretion efficiently (6.06 ± 0.83 ng/islet/hour) which was comparable to insulin secretion by standard drug tolbutamide (6.56 ± 0.81 ng/islet/hour).[32] Alpha-Terpinene was detected in leaf (4.58 %) of *A. producta* belongs to group of monoterpenoids (Table 3). The investigation on the toxic effects of the alpha-terpinene in liver tissues of rats was conducted and showed that the hepatic damage caused by alpha-terpinene at a dose of 1.0 mL per kg was reported via quantitative elevation of serum ALT and AST activities.[26] Therefore, alpha-terpinene could induce oxidative stress, cytotoxic and genotoxic damages in the hepatic tissue involving the caspases activation.[26]

2-Methoxy-4-vinylphenol is used as flavoring agent found in root (0.70 %) and leaves (0.19 %) of *A. producta*. 2-methoxy-4-vinylphenol was proved to be potent anticancer agent and suppressed migratory activity of pancreatic cancer cells, Panc-1 and SNU-213 and also reduced the viability of Panc-1 cells by inhibiting the cell nuclear antigen expression.[21] 2,4-Di-tert-butylphenol belongs to phenol group was isolated from roots of *Humboldtia unijuga* showed anticancer effects through the activation of p53 gene in breast cancer cell line i.e. MCF-7 and also, anti-inflammatory effects of 2, 4-di-tert-butylphenol were significantly superior in pro-inflammatory cytokines TNFa, IL-6 and IL-1b.[19] Nair *et al.*[19] suggested for the development of 2, 4-di-tert-butylphenol as a novel anti-inflammatory and anticancer agent. Further, significant amount of 2, 4-di-tert-butylphenol was detected in root (9.74 %), stem (8.79 %) and leaf (7.43 %) of *A. producta*. Accordingly, the antioxidant activity of *A. producta* have attributed to these compounds such as 2-methoxy-4-vinylphenol and 2,4-di-tert-butylphenol due to the their capabilities of prevention of free radical mediated oxidation (Figure 2).

Solanesol is a terpene alcohol, which has absorbed the ultraviolet radiation effectively and inhibited the tyrosinae, a key enzyme in melanin synthesis and pigmentation disorder in human.[42] In the neuroprotective assessment, salvigenin at the concentration of 25 µM decreased the oxidative stress induced apoptosis by activating antioxidant factors in neuroblastoma SH-SY5Y cells.[40] Gamma-Sitosterol belongs to class of organic compound had exhibited potential anticancer activity through the growth inhibition, cell cycle arrest at G2/M phase and the apoptosis on cancer cells.[34] Solanesol, (2.92 %), salvigenin (12.09 %), and gamma-sitosterol (8.32 %) were found considerable mass in *A. producta*. However, several chemical compounds such as megastigmatrienone (0.34 %), 2-monopalmitin (12.20 %), acetosyringone (2.41 %), tetrapentacontane (0.36 %), butylphosphonic acid, butyl 4-(2-phenylprop-2-yl) phenyl ester (7.48 %), and (-)-loliolide, (0.33 %) belonging to classes of phenolics, sesquiterpenoids, isoprenoids, fatty acids, and benzofurans (Table 1. 2 and 3) are need to be scrutinized in details for their biological activities.

CONCLUSION

This is the first report on the quantification of total phenolic, flavonoid and tannin contents, identification of phytochemicals by GC-MS method and assessment of antioxidant capacities from *Andrographis producta*. The findings in the present investigation indicate that root, stem and leaf of *A. producta* can be explored as a good source of potent antioxidants due to the availability of phenolic and flavonoid contents. GC-MS studies showed the presence of important bioactive compounds such as cinnamic acid, 2,4-di-tert-butylphenol, solanesol, phytol, alpha-terpinene, 5-hydroxy-7,8-dimethoxyflavone, salvigenin, stigmasterol and gamma-sitosterol and it provides immense opportunities to isolate and validate the phytochemicals for their pharmacological activities. Further, the meticulous assessment of such bioactive compounds from the *A. producta* would be great contribution in the field of medicine.

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CONFLICTS OF INTEREST

Authors declare no conflict of interest.

ABBREVIATIONS

ALT: Alanine Aminotransferase; AST: Aspartate Transaminase; BHA: Butylated Hydroxyl Anisole; DPPH: 2,2-Diphenyl-1-Picrylhydrazyl; DW: Dry Weight; EC_{50}: Effective Concentration 50; FRAP: Ferric Reducing Ability of Plasma; GAE: Gallic Acid Equivalent; GC-MS:

Gas Chromatography–Mass Spectrometry; HPLC: High Performance Liquid Chromatography; ID: Inner Diameter; IL-1 β: Interleukin-1 β; IL-6: Interleukin-6; LC-MS: Liquid Chromatography–Mass Spectrometry; MCF-7: Michigan Cancer Foundation-7; MSL: Mean Sea Level; OD: Optical Density; Panc-1: Pancreatic-1; QA: Quercetin Equivalent; SNU-213: Seoul National University-213; TAE: Tannic Acid Equivalent; TNF-α: Tumor Necrosis Factor-α; TPTZ: 2, 4, 6-Tripyridyl-S-Triazine; UV: Ultraviolet.

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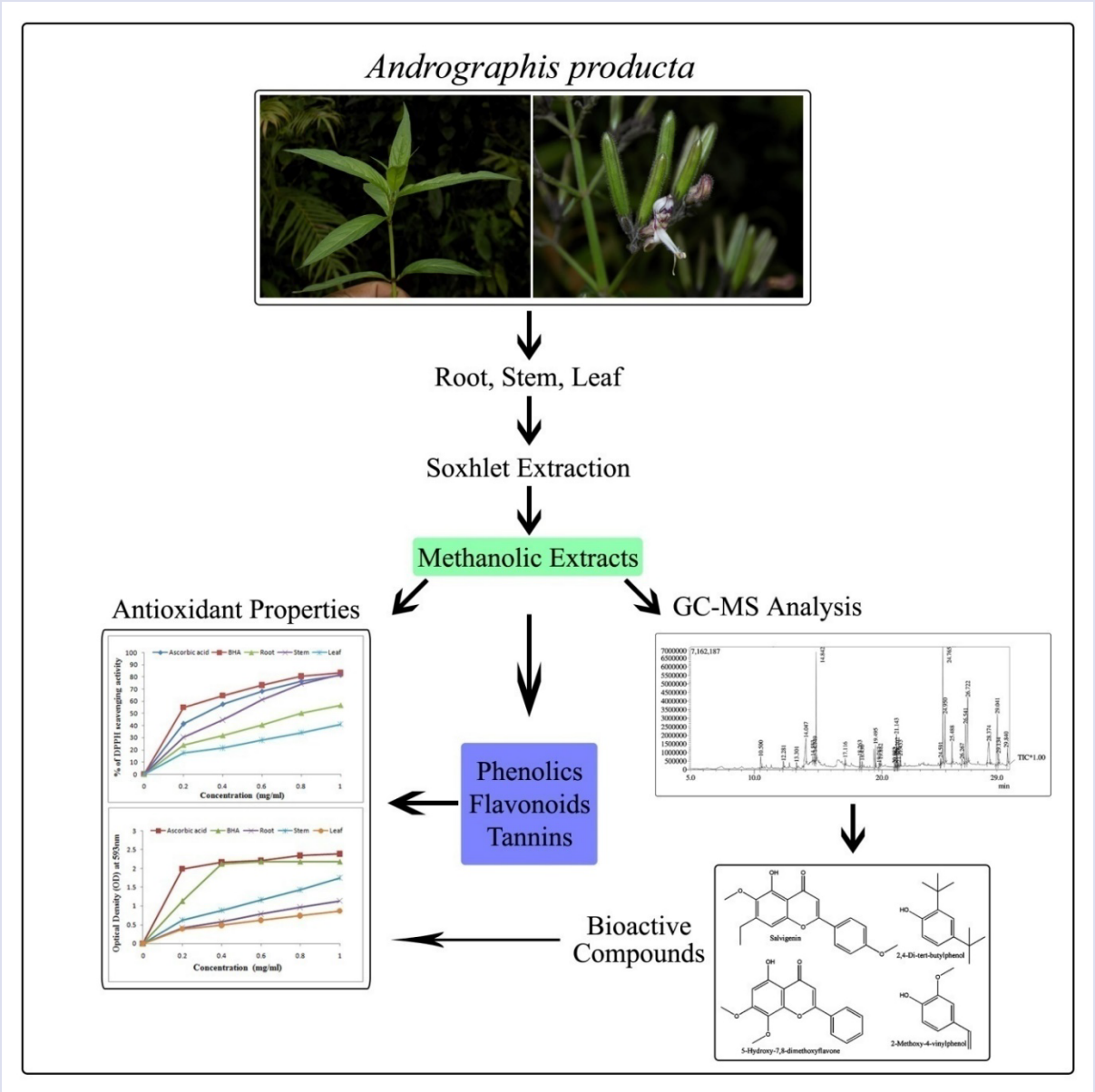
GRAPHICAL ABSTRACT



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