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Phytochemical profile of *Allmania nodiflora* through *in vitro* antioxidant assays and GC-MS analysis

K. Kokila, S. Deepika Priyadharshini, N. Elavarasan and V. Sujatha*

Department of Chemistry, Periyar University, Salem-636 011, Tamilnadu, India

E-mail : chemsujatha@gmail.com

Abstract : Green leafy vegetables attain an important place among the food crops as they possess intense amounts of essential proteins, β-carotene and carbohydrates for trade. *Allmania nodiflora* [F : Amaranthaceae] is chosen to investigate their phenolic, flavonoid, carbohydrate, protein, DPPH-radical scavenging activity, reducing power accompanied with GC-MS, for their various solvent extracts [hexane, ethyl acetate, acetone, methanol]. Results indicate that methanol extract of *Allmania nodiflora* have marked amount of total phenolic and total flavonoid which could be responsible for antioxidant activity. Among the extracts assayed, methanol extract exhibited significant activity, thereby presenting its antioxidant activity potential by scavenging free radicals. This was evident from the SC_{50} values against standard ascorbic acid. In competence, better significance was reported by acetone fractions, which has articulated in identifying numerous constituents through GC-MS. Hence, this present study would endow the scientific validation for their medicinal, therapeutic and dietary applications.

Keywords : *Allmania nodiflora*, phenolics, flavonoid, DPPH.

Introduction

Plant foods are sources of energy, micronutrients and nutrients essential to health, in addition to phytochemicals with further health benefits including glycemic control, immuno-stimulation or antioxidant activity[1]. Green leafy vegetables are used since old periods as source of food as they contain many nutrients and minerals which are helpful in maintaining human health. The chemical constituents in them are of great pharmacological or medicinal importance, particularly for common health benefits like protection from eye problems, oxidative stress, iron deficiency diabetes, cancer and hepatotoxicity etc. Natural antioxidants such as flavonoids, tocopherols, vitamin C and polyphenols are identified as good sources of greens which are responsible to maintain good health and protect human against adverse diseases. Such greens as food combat free radicals generated via normal cellular metabolism due to oxidation of bio-molecules for the sake of producing energy to support biological processes. The natural drugs derived from them supplies safer effects and effectively replace synthetics, which has attracted massive population to rely the true effects.

The present study is set to assess edible leafy vegetables available in South India and their pharmacological benefits, essential in this modern world to support the benefits of their consumption. Hence, *Allmania nodiflora* (commonly-Node flower '*Allmania*') has been selected to investigate its antioxidant activities. They are reported to possess anti-diabetic, hypolipidemic, nutritive, appetizer activities[2], but study on antioxidant activity is scanty. Hence, *in vitro* biochemical assays accompanied with GC-MS analyses has been targeted to reveal its vital role so that it may play an active role in several therapeutic and dietary formulations.

Results and discussion

A detailed and systematic chemical analysis for the presence of essential phytoconstituents was conducted using one or more tests for individual constituent. Compounds like alkaloids, flavonoids, phenolics, carbohydrates, tannins, proteins, glycosides were found to be ubiquitously present in almost all the extracts. Due to the complicated constituents, some non-enzymatic assays were estimated. The results of the antioxidant assay clearly outlined the richest source of flavonoids in ethyl acetate (45.29%) and equal amount of phenolics in both ethyl acetate and methanol extract as (21.91%) present in their

whole respective extracts. From Table 1, it can be noted that the ethyl acetate extract were shown to possess significant antioxidant content, evidencing its ability of having extracted a considerable amount of phenols and flavonoids with specific distribution in the particular representatives. Rest three extracts cannot be considered to be insignificant because satisfactory contents were obtained enough to show their antioxidant activity.

Table 1. Antioxidant contents

Contents (μg/ml)	Hexane	Ethyl acetate	Acetone	Methanol
Total phenol	12.47 ± 0.02	21.91 ± 0.24	15.61 ± 0.08	21.71 ± 0.23
Total flavonoid	24.94 ± 0.05	45.29 ± 0.07	43.09 ± 0.04	41.67 ± 0.11
Carbohydrate	15.50 ± 0.7	89.50 ± 0.27	85.34 ± 0.09	82.14 ± 0.21
Protein	6.94 ± 0.08	18.29 ± 0.08	12.03 ± 0.30	17.99 ± 0.17

The statistical significance ($p^* < 0.05$), $n = 3$, for all the extracts is calculated from gallic acid, catechin, glucose and bovine serum albumin equivalence. The results are expressed in terms of mean ±S.D.

In carbohydrate and protein contents too, ethyl acetate extracts of *Allmania nodiflora* have show cased its significance. Whereas, hexane extracts have produced its potential in extracting immense amounts of carbohydrates and proteins compared to other extracts. Phenolics and flavonoids, being high polar solvents like methanol and acetone and vice-versa in case of carbohydrate and proteins.

In vitro antioxidant assays have served as a pillar support for the present investigation. In DPPH scavenging assay, ethyl acetate extract exhibited an excellent scavenging activity (0.198 mg/ml). *Allmania nodiflora* extracts with DPPH solution lead to the quick disappearance of purple color of the free radical accompanied by decrease in absorption with increasing concentration. A gradual increase in %RSA with the increase in concentration can be made clear with Fig. 1A, all the tested extracts. SC_{50} values of hexane (1.584 mg/ml), acetone (0.230 mg/ml) and methanol extracts (0.225 mg/ml) were higher than the ethyl acetate extract indicating their lower efficiency comparatively. Fig. 1B shows the dose response curves for the reducing power of *Allmania nodiflora* and its derived extracts (0.2–1.4 mg/ml). Furthermore, the absorption range plays a vital role in the observation of reducing power. It is noteworthy that a gradual increase in concentration is based on its electron donating activity that serve as an important action. This action was found to be intense in the ethyl acetate extract with 50% efficient concentration (EC_{50}) of about 0.167 mg/ml. Next stood the methanol extract ranging about 0.289 mg/ml. Reports[4,5] describe the relationship between the phenolic content and the reducing power, which shall be applied in this present work because an appreciable phenolics content has been recorded, for the reducing activity.

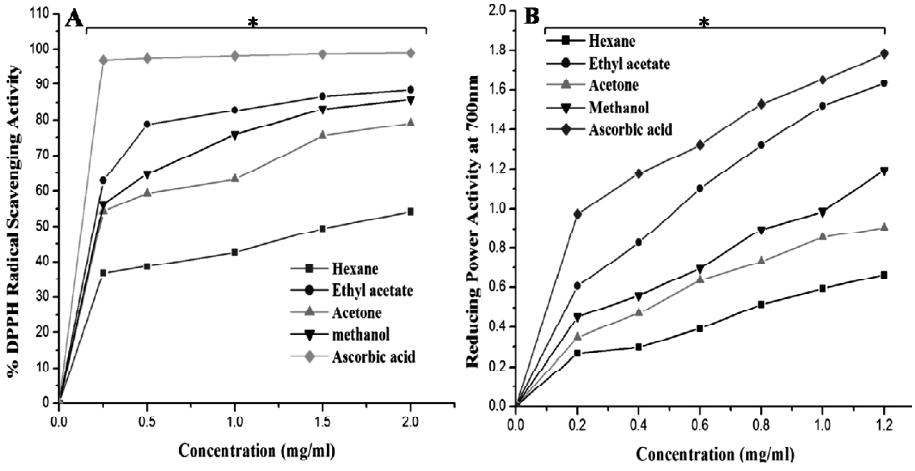


Fig. 1. DPPH-radical scavenging assay (A) and reducing power assay (B) of *Allmania nodiflora* extracts. The statistical significance ($p^* < 0.05$) of each concentration of each extracts is calculated from the standard, expressed in mean ±S.D.

With the above implications of the antioxidant activities, it is worth to analyze the constituents present in the plant through GC-MS analysis. The compounds present in *Allmania nodiflora* were identified by GC-MS analysis and chromatogram obtained is shown in Fig. 2. The major components present in whole plant were 9-dodecenoic acid-methyl ester (RT-14.02), 9,12,15-octadecaantrienoic acid (RT-14.92), 1,*E*-11,*Z*-13-octadecatriene (RT-14.77), penta decanoic acid (RT-15.19), 3-hexadecyloxycarbonyl-

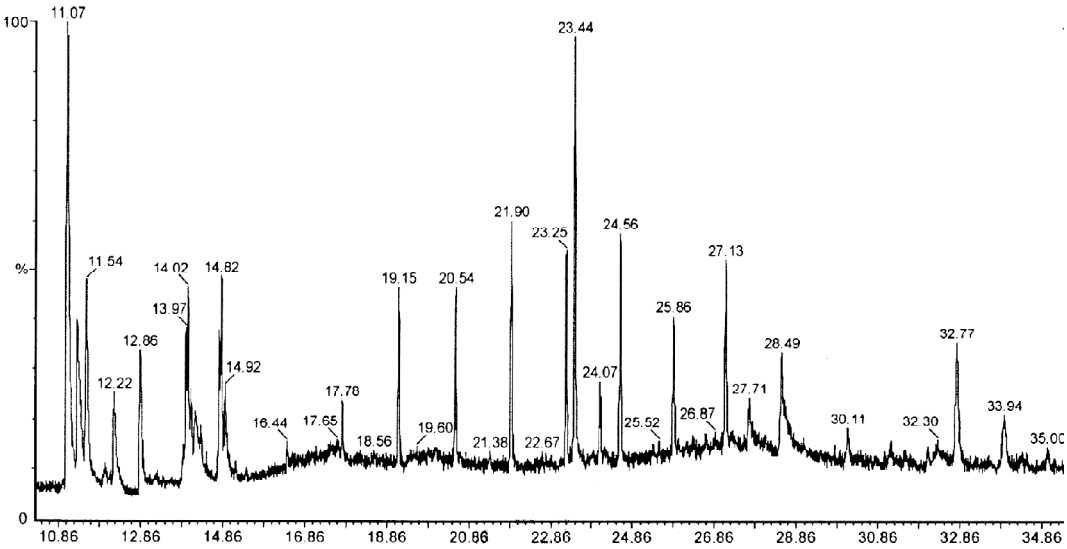


Fig. 2. GC-MS chromatogram of *Allmania nodiflora*.

5-(-2-hydroxyethyl)-4-methylimidazolium ion (RT-17.78), squalene (RT-23.44), valeric acid (RT-28.49), 1-napthalenepropanol (RT-32.77). These constituents are extensively used as anti-inflammatory, anti-cancer, anti-histaminic, anti-androgenic, anti-arthritic, anti-coronary, anti-microbial and antioxidant properties[6]. For instance, squalene detected in *Allmania nodiflora* has been found to be effective at different stages of the tumor, as well as preventive and a safe therapeutic agent.

Experimental

Authentification and extraction :

Plant : *Allmania nodiflora* whole plant samples; Collected area : Mallur, Salem Dt, Tamilnadu; Authentication : BSI/SRC/5/23/2012-13/Tech-703 by TNAU; Extraction : Sequential soxhelation using various solvents [*n*-hexane, ethyl acetate, acetone, methanol]; Storage : 4–5 °C.

Phytochemical screening :

Using standard methods[7], the preliminary qualitative screening was analyzed for the presence of the secondary metabolites.

Estimation of phenolic content :

Estimated method : Standard procedures[8] using Folin and Ciocalteu's phenol reagent; Absorbance range : 725 nm (Analytik Jena 200-2004 spectrophotometer); Standard : Gallic acid; Calculation : Standard curve at the range 0.01–0.4 mM, $Y = 2.94848x - 0.09211$, $R^2 = 0.99914$; Results expressed : µg of gallic acid equivalents (GAEs) per mg of extract.

Estimation of flavonoid content :

Estimated method : Determined[8] using aluminium chloride; Absorbance range : 510 nm (Analytik Jena 200-2004 spectrophotometer); Standard : (+)-Catechin; Calculation : Standard curve at the range 0.250–2.500 mM, $Y = 0.2903$, $R^2 = 1.0000$; Results expressed : µg of (+)-catechin equivalents (CEs) per mg of extract.

Estimation of carbohydrate content :

Estimated method : Determined using anthrone method[9]; Standard : Glucose; Calculation : Standard curve the range 20–120 µg/ml, $Y = 0.0263x+0.0532$, $R^2 = 0.9992$; Results expressed : µg of glucose equivalents (GEs) per mg of extract.

Estimation of protein content :

Estimated method : Estimated using Lowry's method[10]; Standard: Bovine serum albumin; Calculation : Standard curve the range 20–160 µg/ ml, $Y = 0.0159x+0.0319$, $R^2 = 0.9569$; Results expressed : µg of bovine serum albumin equivalents (BSAEs) per mg of extract.

DPPH-radical scavenging assay :

Concentration : 0.5, 1.0, 1.5, 2.0 mg/ml concentrations of *Allmania nodiflora* extracts; Procedure : Spectrophotometrically assayed using methanol solution containing DPPH radicals; Absorbance range : 517 nm; Calculation : The radical scavenging activity – $\%RSA = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the sample extract and A_{DPPH} is the absorbance of the DPPH solution[8]; Standard : Ascorbic acid.

Reducing power assay :

Concentration : 0.2, 0.4, 0.6, 0.8, 2.0 mg/ml concentrations of *Allmania nodiflora* extracts; Procedure : Spectrophotometrically assayed[11] by measuring the reduction system of Fe^{3+} to Fe^{2+}; Absorbance range : 700 nm; Standard : Ascorbic acid.

GC-MS analysis :

GC Clarus 500 Perkin-Elmer equipment[12]. Column : Compounds separated on a 30 m × 0.25 mm capillary column coated with a 0.2 µm film of HP-5-MS. Samples were injected with a split ratio of 50 : 1; helium was used as carrier gas at 1 ml/min at 100 °C for 1 min. Time : 10 °C/ min to 275 °C sustained for 20 min. The time required for chromatography of one sample was 36 min.

Statistical analysis :

All the above *in vitro* studies were experimented in triplets and the results calculated using one way are expressed as mean ±SD using one way analysis (ANOVA).

Conclusion

Allmania nodiflora has proved that it serves as a reservoir of phenolics, flavonoid, carbohydrate and protein contents. The overall antioxidant activity of its extracts may be attributed to the presence of enormous phytochemical constituents. Contrastingly, *Allmania nodiflora* ethyl acetate extract has showed its excellence for almost all the enzymatic and non-enzymatic *in vitro* assays and carbohydrate content. The present work if established with supportive *in vivo* and extrapolated *in vitro* studies, *Allmania nodiflora* plant shall fuel the present pharmacological profile of Indian medicinal plants.

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