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Research Article

PHYSICOCHEMICAL AND ANTIOXIDANT ASSAY OF AYURVEDIC FORMULATIONS OF *ALTERNANTHERA PHILOXEROIDES*

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ABSTRACT

There are several scientific works has been done with alternative medicines in preventive measurement of diabetes mellitus. The present study was designed to evaluated in-vitro antioxidant activity of newly developed three ayurvedic formulations of *Alternanthera philoxeroides* and quantifies possible groups of phyto-constituents present in it. The prepared ayurvedic formulations are 'swarasa kalpana', 'hima kalpana', and 'phanta kalpana', which were mentioned in the ayurvedic classical book 'Sarangadhar Samhita'. This is first effort to explore the potentially of these formulations by in-vitro antioxidant activity as well as level of antioxidant groups (total flavonoid, total flavonoid etc.). The Graph pad prism (Version-3) software evaluates the interrelationship between the formulations as well as the analyzed parameters. It can be concluded that the antioxidant activity might be responsible for the presence of phenolic, flavonoid and other phytoconstituents.

Keywords: DPPH, flavonoid, phenolic compound, alkaloid, graph pad prism

INTRODUCTION

A. philoxeroides (Martius) Grisebach (Amaranthaceae family) is an amphibious of South Africa and invades to other countries like India, commonly known as alligator weed[1]. It is used by India as vegetables. Gives the preventive effect on diarrhea, dysentery, influenza, stomach disorders etc[2,3]. Successful laboratory experiment gives evidences against dengue virus[4], respiratory syncytial virus[5] and hemorrhagic fever virus[6].

Reported cytotoxic chemical compounds of these plants are triterpene saponins like philoxeroideside A, philoxeroideside B, philoxeroideside C and philoxeroideside D[7]. Alternanthin B and N-trans-feruloly-3, 5-dimethoxytyramine are the two antitumor chemical compounds found from the ethanol extract of the plant[8]. C-glycosylated flavonoid like alternanthin was isolated from the leaves and stem of the plant[9].

The phenolic and flovonoid compounds have the power to scavenge free radicals. They have the capability to donate proton to the free radicals and neutralize them. They can also prevent the oxidative reaction by inhibit the action of responsible enzyme or by chelating traces of metals responsible for it[10]. These can be characterized as natural antioxidant[11]. Antioxidant activity of the plant is mostly depends on the presence of these secondary metabolites. Due to some specific external and internal stress, body produces free radical due to oxidation reaction. This oxidation reaction is occurred in chain formation in the body and more and more free radicals are generated which also effect the normal cells by damaging them. Antioxidant compounds scavenge the free radicals and terminate the oxidation reaction[12].

The aim of this study was to determine the presence of secondary metabolites like phenolic compounds, flavonoid and alkaloids and free radical scavenging potential of folklore plant *A. philoxeroides* and its three-ayurvedic primary formulations.

MATERIALS AND METHODS

Chemicals

Folin-ciocalteu reagent, gallic acid, potassium acetate, aluminium chloride, quercetin, bromocresol green, atropine, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sodium phosphate, hydrogen chloride, ascorbic acid, atropine and sodium carbonate. All reagents and chemicals were obtained commercially as that of the analytical grade.

Plant

A. philoxeroides was collected from the campus of Jadavpur University, Kolkata and further, the identity of the plant was confirmed (Specimen No.-CNH/28/2014/Tech.II/SM-05) by Botanical Survey of India, Office of Scientist-'F', Central National Herbarium, Botanical Garden, Howrah, west Bengal.

Sample preparation

The shoot parts of the plant material were washed thoroughly with plenty of distilled water to remove the adhering soil and dirt. Thereafter the plants were made into pieces of with a mechanical cutter and were kept for 21 days under shade for drying. The fresh plants were needed to prepare Swarasa kalpana and the dried parts were needed for the Hima kalpana and Phanta kalpana.

Swarasa kalpana

Fresh shoot part of the plant was cut into small pieces with a mechanical cutter. Further 300gm of the plant was triturated with the mortal-pestle and the juice was collected by squeezing it. The final filtrate was taken after filtered through the muslin cloth and finally prepared the sample-A[13].

Hima kalpana

For the preparation of sample-B, 1200ml of distilled water were mixed with the 200gm of the course powder of the plant. This mixture was kept for overnight (12 hours) and the next morning the filtrate was macerated well. Then the filtrate was collected after filtering it through the muslin cloth[13].

Phanta kalpana

Sample-C was prepared by pouring 800 ml of boiled (100° C) distilled water into the 200 gm of course powdered plant material. This mixture solution was kept for 2 hours until it become at room temperature. The mixture then macerated well and filtered through the muslin cloth[13].
All three above samples were kept in cool and dry place for further use.

Phytochemical analysis of the plant extract

Qualitative study of the plant was done according to the standard procedure[14]. The phytochemical screening gives the evidence of presence of various functional groups like saponin, carbohydrates, phenolic compounds, alkaloids, flavonoids, amino acids, steroids etc.

Total phenolic content

Total phenolic content of the samples were determined by the Folin-Ciocalteu method. Phenolic content of the samples were expressed in terms of µg of gallic (GAE) acid equivalent per ml of plant extract[15].

Total flavonoid content

Total flavonoid content of the samples were estimated by aluminium chloride colorimetric method and were expressed in terms of µg of quercetin (QUA) equivalent per ml of plant extract[16].

Total alkaloid content

Total alkaloidal content was done as per the procedure described by F. Shamsa et. al. and total alkaloid content was expressed in terms of µg of atropine (ATP) equivalent per ml of plant extract[17].

Antioxidant activity

DPPH assay

Antioxidant activity of the samples was evaluated by spectroscopic method based on the scavenging activity for DPPH-free radical. It is measured by the methodology proposed by Mensor et al.[18] and Gopal et al.[19] The percentage of inhibition of the samples was calculated by the following formula.
Inhibition (%) = (Control - Test) / Control X 100

Table 1: Total alkaloids, flavonoids and phenolics content of different prepared samples of *A. philoxeroides*

Sample	Total alkaloid content (µg ATP/gm)	Total flavonoid content (µg QUA/gm)	Total phenolic content (µg GAE/gm)
Methanol extract	13.26±2.01	13.31±1.85	0.96±0.03
Sample-A	7.83 ±6.09•	11.76±0.93	0.96±0.02
Sample-B	5.41±4.71	11.77±0.76	0.93±0.04
Sample-C	14.31±9.51	11.08±0.79	0.92±0.01

Table 2: IC_{50} values different prepared samples of *A. philoxeroides*

Sample	IC_{50} value
Methanol extract	1066.85
Sample-A	1862.33
Sample-B	1818.11
Sample-C	1152.71

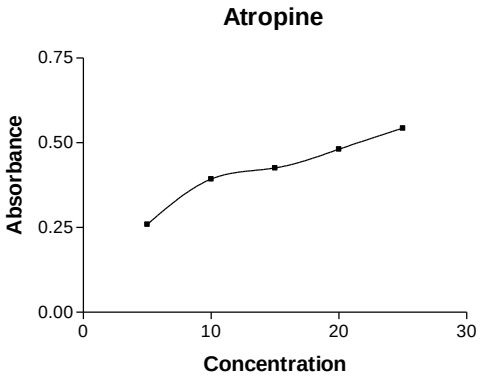

Figure 1: Atropine standard curve of total alkaloid content
Y=0.013x+0.223, R^2 = 0.945

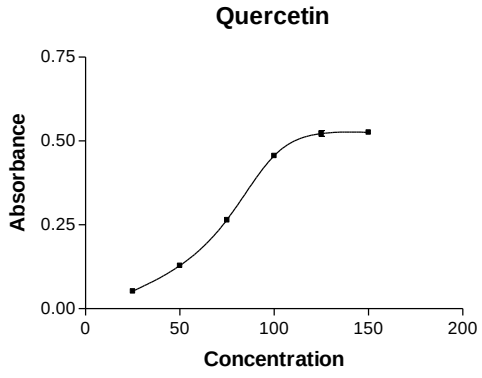

Figure 2: Quercetin standard curve of total flavonoid content
Y=0.003x, R^2 = 0.925

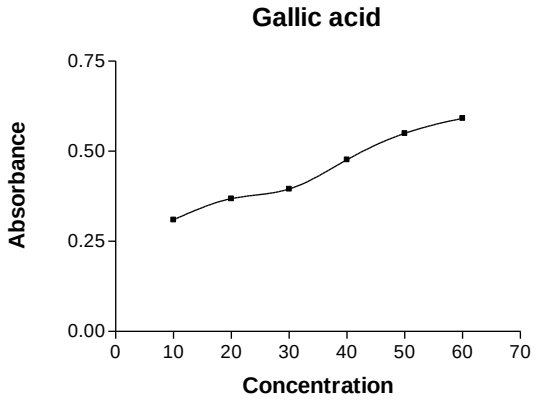

Figure 3: Gallic acid standard curve of total phenol content Y= 0.005x + 0.245, R^2 = 0.985

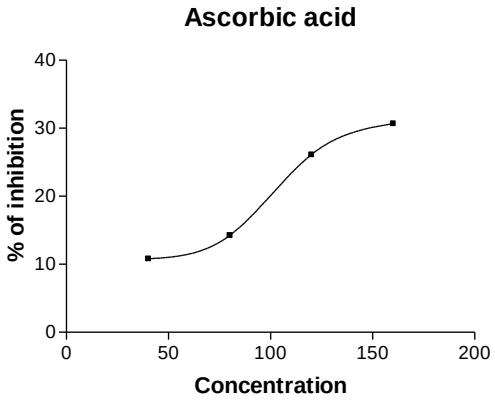

Figure 4: Ascorbic acid standard curve of DPPH activity Y=0.178x + 2.582, R^2 = 0.952

RESULT AND DISCUSSION

Phytochemical investigation of the folklore plant *A. philoxeroides* revealed the presence of the pharmacologically impotent phyto-constituents like carbohydrates, flavonoids, phenolic compounds, alkaloids, amino acids and saponins. Glycosides and tannins were found to be absent.

The data of all quantitative studies as well as the antioxidant study were analyzed by the software that is graph pad prism 3. All the experiments were performed thrice and the results were averaged and reported in the form of mean ± S.E.M.

Alkaloidal content was found to be highest in sample-A and then in sample-B, sample-C respectively. Flavonoid content was found to be almost more or less same in all samples. Presence of phenolic compound was found to be in low quantity in the samples. The results are summarized in Table 1.

Inhibition concentration of the samples was 50 µg/ml - 200 µg/ml scavenge DPPH radicals. IC_{50} value of the samples were found significant are shown in Table 2.

DISCUSSION

The profile of phytochemical screening of the plant was shown the nature of the chemical composition. The alkaloid, flavonoid and phenolic compounds are one of the major chemically responsible factors for the free radical scavenging property.

Flavonoids are chemically characterized by the bonding between two benzene ring by a linear carbon chain, which generally produced due to microbial response in the plant. It is responsible for the decreasing the risk of several chronic diseases including cancer, arthrosclerosis and also the neurodegenerative diseases[20, 21].

Phenolic compounds are primarily responsible for scavenging of free radical by donating active hydrogen ion and able to reduce the oxidative stress[22].

The presence of phytochemical like flavonoid, phenolic, alkaloid, saponin etc , gives free radical scavenging potential to the drug which is detected by the DPPH assay. In this assay, the free radicals are scavenged with changing the colour from deep violet to light yellow depending upon the reduced rate of absorption and gives absorption band at 517 nm wavelength[22].

These absorption measurements are the parameters to conclude antioxidant activity of the samples.

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