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PHYTOCHEMICAL AND ANTIOXIDANT STUDY OF HYDROALCOHOLIC EXTRACT FROM THE DRIED ROOTS OF AGERATINA ADENOPHORA

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Abstract

We investigated the antioxidant activity and total phenolics content of the hydroalcoholic extract of *Ageratina adenophora* (Spreng) roots using standard *in vitro* methods. The total phenol and total flavonoid were determined spectrophotometrically. Quercetin and Gallic acid were used as reference standards for these parameters. The total antioxidant activity of the extract was determined by phosphomolybdenum method. Results clearly demonstrate that the extract have antioxidant activity. A correlation between the antioxidant activity and the total phenolic content of the extract indicated that the phenolic compounds were the dominant contributors to the antioxidant activity of the extract.

Key words*: Ageratina adenophora, Total flavonoid; Total phenol, Antioxidant capacity*

Introduction

Ageratina adenophora (Spreng) is a perennial herb which belongs to family Asteraceae is native to Mexico, found throughout India, America, Africa and China. The base of the plant is woody and densely clothed with stalked glandular hairs. Leaves are dark green, opposite, deltoid-ovate, serrate, purple underneath and each grows to about 10 cm in length. Flowers are borne terminally in compound clusters during spring and summer. The seed is an achene, varying from elliptic to oblanceolate, often gibbous, 1.5–2 mm long, 0.3–0.5 mm wide. Each flower head is upto 0.5 cm in the diameter and creamy white in colour [1]. Extensive work on the chemical constituents as well as on the composition of the essential oil of *Ageratina* is reported in literature. A hepatotoxic compound (9-oxo-10-11-dehydroagerophonrone) was identified from the crude extract of this plant [2]. b-farnesene, germacrene D, bisabolene, caryophyllene and four more cadinene derivatives were reported [3]. Other compounds identified from the stem and leaves of this species were found to be 9-oxo-ageraphorone, 9-β-hydroxy-ageraphorone, epifriefdelinol, stigmasterol, octacosanoic acid, b-daucosterol, o-hydroxycinnamic acis, ferulic acid, cafeic acid, and 2 isopropenyl-5-acetyl-6-hydroxy benzo furan acetate. It contains sesquiterpene compounds, together with seven known compounds such as coumarin, (−)-5-exo-hydroxy-borneol,*O*-hydroxylcinnamic acid, 9*β*-hydroxy-ageraphorone, 10*Hα*-9-oxo-ageraphorone,10*Hβ*-9-oxo-ageraphoron e and 9-oxo-10,11-dehydroageraphorone , was isolated from the leaves of *Ageratina adenophora* [4].

The aim of our study was to investigate the probable antioxidant effects of hydroalcoholic extract from the dried roots of *Ageratina adenophora.* The second aim was to expose the total phenolics of the plant.

Methods

Drugs and chemicals

Chemicals used were as follows; methanol, sodium carbonate, potassium acetate, aluminium chloride, quercetin, gallic acid and Folin-Ciocalteau reagent. All other chemicals used were of analytical grades and purchased from Sigma Life Sciences (Mumbai, India).

Plant material collection

The roots of *Ageratina adenophora* were collected from the road sides from Udhagamandalam, The Nilgiris. The plant was authenticated by Dr. B. Duraiswamy, Professor and Head, Department of Pharmacognosy and Phytopharmacy, JSS College of Pharmacy, Udhagamandalam. The collected roots were cleaned and dried in shade. The dried roots are coarsely powdered and subjected to extraction. Voucher specimen (accession no. Pharmacog./2132) was deposited for future reference in the Herbarium of Pharmacognosy Department, J.S.S College of Pharmacy, Udhagamandalam

Extraction procedure

Shade dried and coarsely pulverized roots were extracted with mixture of ethanol : water in the ratio of 7 : 3 by a Soxhlet apparatus at 45 C. The evaporation was done using a rotary evaporator at 45 C under reduced pressure and the extract obtained was used for further investigation for the potential antioxidant activities [5].

Phytochemical screening

The hydroalcoholic extract of *Ageratina adenophora* root was subjected to phytosc hemical screening for the identification of its constituents [6]. Thin Layer Chromatography (TLC) [7] was performed on Silica gel (s.d.fiNE-CHEM, Mumbai) developed by solvent system: Benzene : Benzene, diethyl ether (1:1). Camag twin trough development tank (10 × 10 cm) covered one side of the chamber with Whatman filter paper was used. 0.1g of extract was weighed and 10 ml of methanol was added. The content were sonicated in ultrasonicator for 10 minutes and used.

Estimation of total phenol content (TPC)

Total phenol content of the extract was determined colorimetrically using Folin – Ciocalteau method [8]. Gallic acid was used as a reference standard for plotting calibration curve. A volume of 1ml of the plant extract (1mg/ml) was mixed with 2ml of Folin - Ciocalteau and neutralized with 4ml of sodium carbonate solution (7.5% w/v). The mixture was allowed to stand for 2 h with intermittent shaking for colour development. The absorbance of resulting blue colour was measured at 765 nm using double beam UV-spectrometer. Using gallic acid, standard curve was prepared and linearity was

obtained in the range of 10-50 µg/ml. Total phenolics was calculated using the standard curve and the concentrations are expressed as µg/ml gallic acid equivalent. The calibration equation for gallic acid was Y = 0.07400X + 0.0562 (R^2 = 0.9968).

Estimation of total flavonoid content (TFC)

Aluminium chloride colorimetric technique [9] which is based on aluminium complex formation when flavanoids combine with metal ions to act as antioxidant was used for total flavonoid determination. Quercetin was used as a reference standard for plotting calibration curve. A volume (1.0 ml) of the plant extract was mixed with 3ml of methanol, 0.2 ml of 10% aluminium chloride, 0.2 ml of 1M potassium acetate and 5.6 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with UV Spectrophotometer. Total flavonoid content was expressed as µg/ml quercetin equivalent. The calibration equation for quercetin was Y = 0.04165 X + 0.01471 (R^2 = 0.9988)

***In-vitro* antioxidant assay**

Determination of total antioxidant capacity (TAC)

The total antioxidant capacity was determined by phosphomolybdenum method using ascorbic acid as a standard [10]. An aliquot of 0.3 ml of the extract sample was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tube containing the reaction mixture was incubated in water bath at 95°C for 90 min. Once after the sample has been cooled down to room temperature, absorbance of the mixture was measured at 695 nm against a blank on a UV spectrophotometer. A typical blank contained 1 ml of the reagent solution along with an appropriate volume of the solvent and incubated under similar conditions. The total antioxidant capacity was expressed as mg equivalent of ascorbic acid (standard).

Results

Phytochemical analysis of the extract showed the presence of alkaloids, flavonoids, coumarin glycosides, saponin glycosides and triterpenoids (Table 1). The TLC fingerprint of the extract was carried out and observed under 366 nm (Fig.1). The Rf values was found to be 0.65, 0.53, 0.33, 0.21 (Table 2). The total phenolic content (TPC) of the hydralcoholic extract of the *Ageratina adenophora* calculated from the calibration curve (R^2 = 0.998), was 60.4 ± 1.70 µg/ml gallic acid equivalent and the total flavonoid content, TFC (R^2 = 0.999) was 72 ± 2.20 µg/ml quercetin equivalent. The hydroalcoholic extract of *Ageratina adenophora* was screened for its total anti-oxidant capacity (TAC). The plant exhibited moderate total antioxidant capacity when compared to the standard ascorbic acid. The IC_{50} value of the extract of the plant is 1.24 ± 0.02 µg/ml. The IC_{50} value for the standard, ascorbic acid is 1.03 ± 0.44 µg/ml. Figure 2 and 3 shows the total flavonoid phenol contents of the plant extract. The total phenol content and total flavonoid content showed strong correlation with total antioxidant activity, with the correlation coefficient R^2 = 0.999 for *Ageratina adenophora.* This indicates that the antioxidant activity of the extract is due to its phenolic constituents. These results are in accordance with other reports in the literature, which showed strong correlation between antioxidant activities and total phenolics. [11]

Discussion

Phenolic compounds have redox properties, which allow them to act as antioxidants. As their free radical scavenging ability was facilitated by their hydroxyl groups, the total phenolic concentration could be used as a basis for rapid screening of antioxidant activity. Flavonoids, including flavones, flavanols and condensed tannins were plant secondary metabolites and the antioxidant activity of which depends on the presence of free OH groups, especially 3-OH [12]. Plant flavonoids have antioxidant activity *in vitro* and also act as antioxidants in *in vivo screening.*

The antioxidant activity of the plant extract may be due to the presence of Phenolic hydroxyl or methoxyl groups, flavones hydroxyl groups, free carboxylic groups, quinines, coumaric acid. *Ageratina adenophora* (spreng) roots showed that this plant can be one of the potential sources of safer natural antioxidants. Thus, replacement of synthetic antioxidants with secondary metabolites exhibiting safe and effective antioxidant activities from abundantly available plant sources such as *Ageratina adenophora* (spreng) may be advantageous.

The present study confirmed the *in-vitro* antioxidant potential of *Ageratina adenophora*. The results

obtained further support the view that the roots of the plant are promising sources of natural antioxidants, and could be seen as potential sources of useful drugs. Nonetheless, further *in-vivo* studies and purification of the compounds responsible for antioxidant activity are needed.

Conflict of interest statement

We declare that we have no conflict of interests

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Table 1: Showing the observation of phytochemical screening

S.No	Phytochemical tests	Results
1	Alkaloids	+
2	Flavonoids	+
3	Carbohydrates	+
4	Glycosides	+
5	Coumarin glycosides	+
6	Saponins	+
7	Saponin glycosides	+
8	Steroids	+
9	Triterpenoids	+
10	Proteins and Amino acids	-

(+) represents positive , (-) represents negative

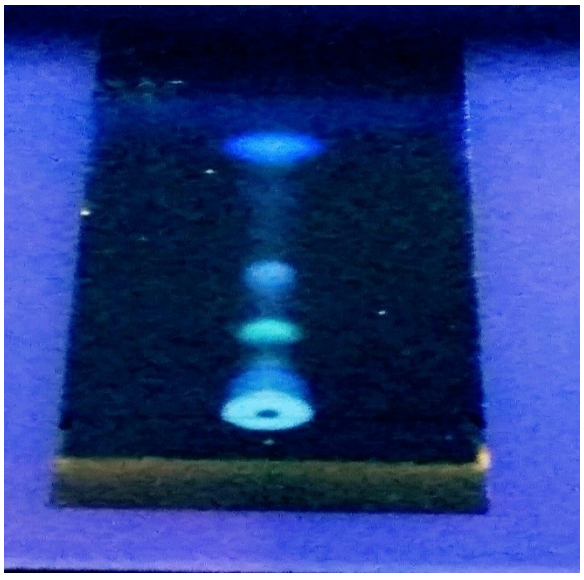

Fig 1 : TLC fingerprint of the root extract

Table 2 : Showing TLC finger print of extract at 366nm

Retardation factor	Values
Rf_1	0.65
Rf_2	0.53
Rf_3	0.33
Rf_4	0.21

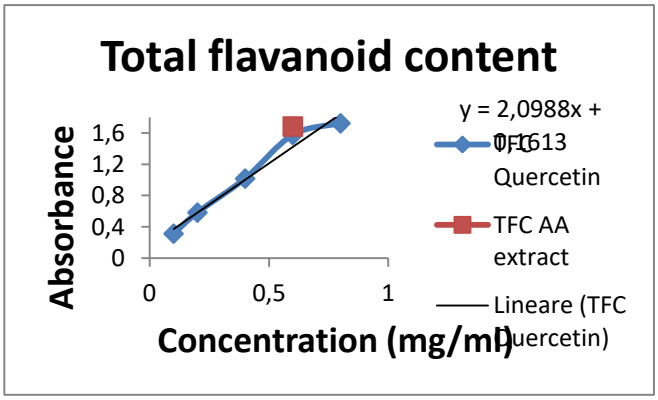

Fig 2 : Showing Graphical representation of total flavanoid content

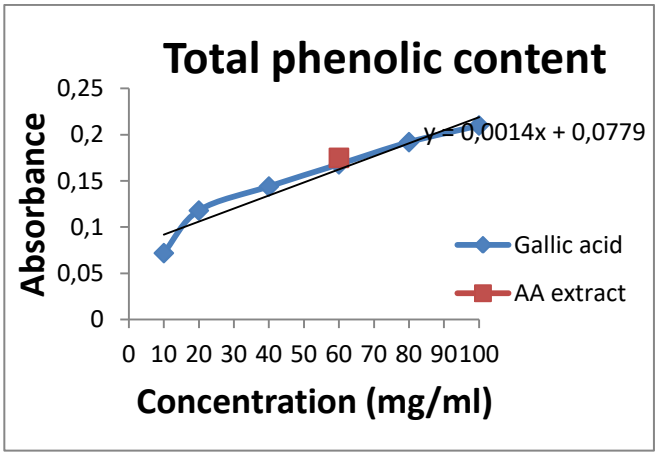

Fig 3 : Showing Graphical representation of total phenolic content