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Quantitative Analysis of Total Phenolic, Flavonoids and Tannin Contents in Acetone and n-hexane Extracts of Ageratum conyzoides

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Abstract: *Ageratum conyzoides* an annual herbaceous plant is found in tropical and sub-tropical regions. Present study was aimed to quantitative analysis of total phenolic, total flavonoids, tannin content present in different extracts. Total phenolic content is significantly high ($P<0.05$) in n-hexane extract 34.62 ± 0.94 mg of GAE/gm of extract as compared to acetone extract 25.70 ± 2.00 mg of GAE/gm of extract. Similarly significant high ($P<0.05$) concentrations of flavonoids (1172.55 ± 17.69 mg Quercetin/gm dried extract) and non-tannin content (12.30 ± 0.97 mg of GAE/gm of extract) have been observed in n-hexane extract as compared to acetone extract. Thus study suggested that n-hexane extract of plant have more potential in scavenging the free radicals/ROS as compared to acetone extract.
Keywords: *Ageratum conyzoides, total phenolic content, tannin, n-hexane, acetone extract*.

Introduction

North-Western Himalayas in India are well-known for their rich floral diversity, traditionally used as home remedies and form and important part of Himalayan folk medicine. *Ageratum conyzoides* (Billygoat-weed) is annual herbaceous plant native to Tropical America but now is found in several countries in tropical and sub-tropical regions, including India[1]. Phytochemical investigations on *A. conyzoides* have identified a number of secondary metabolites such us flavonoids, coumarins and alkaloidal compounds[2]. Methanolic extract of whole plant, has inhibitory action in the development of *Staphylococus aureus*, *Bacillus subtilis*, *Eschericichia coli*, and *Pseudomonas aeruginosa* [3]. Aqueous extract of the whole plant has muscle relaxing activities, reduction in pain and inflammation or improvement in articulation mobility confirming its popular use as an anti-spasmodic action[4]. Extracts is also produced significant effects against the mosquito, *Culex quinquefasciatus* and *Anopheles stephensi*, *in* India, when applied to fourth instar larvae and adult females. In female adults, a loss of fecundity, lower egg production, and production of defective eggs due to anti-juvenile potential of *A. conyzoides*[5]. Assays conducted in Colombia by Gonzalez et al[6] showed activity of this species against *Musca domestica* larvae, using whole plant n-hexane extract. The demand of medicinal plants is

increasing year by year and in many plants needs through pharmacological investigations. Some medicinal properties are still concealed with in plant which need through scientific probing. To explore the possible medicinal potential the present study has been aimed to evaluate the total phenolic total flavonoids and tannin content present in different extracts of *A. conyzoides.*

Materials and methods

Plant materials and Chemicals: The whole plants of *A. conyzoides* were collected from the different areas of R S Pura, Jammu, J&K. Sample of plant material was taxonomically identified by Department of Botany, University of Jammu. The collected whole plant material was dried at room temperature, pulverized by a mechanical grinder, sieved through 40mesh. About 100gm of powdered materials was extracted with n-hexane and acetone as a extracting medium (solvent) using soxhlet apparatus. The extract was then concentrated and dried under reduced pressure using rotatory evaporator. Different chemicals viz. Quercetin (Sigma Aldrich, USA), Gallic Acid (SD Fine Chem. Ltd, Mumbai India), Folin- Ciocalteu reagent (Central Drug House, New Delhi) and other chemicals used for the analysis were of analytical grade.

Determination of total Phenolic contents: The amount of total phenolics in extracts was determined with the Folin-Ciocalteu reagent. Gallic acid was used as a standard and the total phenolics were expressed as mg/g gallic acid equivalents (GAE). Concentration of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of gallic acid were prepared in methanol. Concentration of 0.1 and 1mg/ml of plant extract were also prepared in methanol and 0.5ml of each sample were introduced into test tubes and mixed with 2.5ml of a 10 fold dilute Folin- Ciocalteu reagent and 2ml of 7.5% sodium carbonate. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature before the absorbance was read at 760nm spectrometrically. All determinations were performed in triplicate. The Folin-Ciocalteu reagent being sensitive to reducing compounds including polyphenols is producing a blue color upon reaction which is measured spectrophotometrically[7].

Determination of total flavonoids content: The total flavonoids content of each plant extract was estimated by method described by Zhishen et al[8]. Based on this method, each sample (1.0ml) was mixed with 4ml of distilled water and subsequently with 0.30ml of a $NaNO_2$ solution (10%). After 5 min, 0.30ml $AlCl_3$ solution (10%) was added fallowed by 2.0ml of NaOH solution (1%) to the mixture. Immediately, the mixture was thoroughly mixed and absorbance was then determined at 510 nm versus the blank. Standard curve of quercetin was prepared (0-12mg/ml) and the results were expressed as quercetin equivalents (mg quercetin/gm dried extract).

Determination of tannin contents: Tannin content in each sample was determined using insoluble polyvinyl-polypirrolidone (PVPP), which binds tannins as described by Makkar et al[9]. Briefly, 1 ml of extract dissolved in methanol (1 mg/ml), in which the total phenolics were determined, was mixed with 100 mg PVPP, vortexed, kept for 15 min at 4°C and then centrifuged for 10 min at 3,000 rpm. In the clear supernatant the non-tannin phenolics were determined the same way as the total phenolics[10]. Tannin content was calculated as a difference between total and non-tannin phenolic content.

Statistical analysis: The determinations were conducted in triplicate and results were expressed as mean ± standard error. Statistical analyses were done by one-way ANOVA followed by Dunnet's test with $P < 0.05$ as a limit of significance.

Results:

Standard curve used for the determination of total phenolic content and flavonoids were prepared using different concentrations of Gallic acid equivalent (GAE) and quercetin and their respective optical density as shown in figure 1 and 2 respectively. The concentration of these compounds in different extracts is shown in table 1. Total phenolic content is significantly high in n-hexane extract 34.62 ± 0.94 mg of GAE/gm of extract as compared to acetone extract 25.70 ± 2.00 mg of GAE/gm of extract. Similarly significant high concentrations of flavonoids (1172.55 ± 17.69 mg Quercetin/gm dried extract) and non-tannin content (12.30 ± 0.97 mg of GAE/gm of extract) have been observed in n-hexane extract as compared to acetone extract.

Table 1: The total phenolic, total flavonoids, tannin and non-tannin content present in acetone and n-hexane extracts *A. conyzoides*

Parameters	**Unit**	**Acetone extract** of *A. conyzoides*	**n-hexane extract** of *A. conyzoides*
Total phenolic content	mg of GAE/gm of extract	25.70 [a] ± 2.00	34.62 [b] ± 0.94
Total flavonoids content	mg Quercetin/gm dried extract	721.98 [a] ± 72.61	1172.55 [b] ± 17.69
Non-tannin content	mg of GAE/gm of extract	4.03 [a] ± 0.35	12.30 [b] ± 0.97
Tannin content	mg of GAE/gm of extract	19.73 [a] ± 2.56	22.70 [a] ± 1.77

Values are expressed as mean ± SE of three replicates. The different superscripted have significantly differ ($P<0.05$) from the other extract.

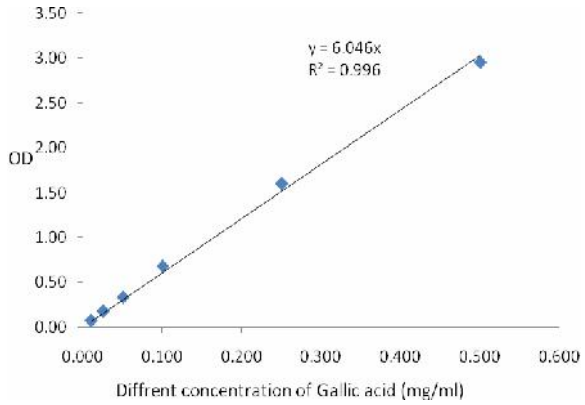

Figure-1, Standard curve of different concentrations (mg/ml) of gallic acid and their respective optical density at 760nm.

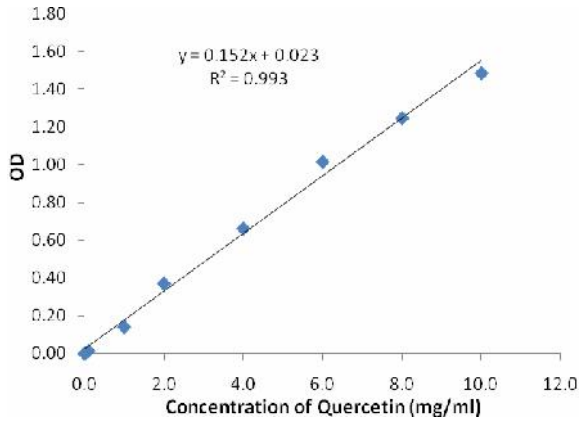

Figure-2, Standard curve of different concentrations (mg/ml) of Quercetin and their respective optical density (OD) at 510nm.

Discussion

A Word Health Organization survey indicated that about 70–80% of the world's populations rely on non-conventional medicine, mainly of herbal source, for their primary healthcare[11]. These medicinal plants are rich sources for naturally occurring antioxidants especially phenolic and flavonoids contents. Flavonoids are large family of poly-phenolic components were found to reduce blood-lipid and glucose and to enhance human immunity[12]. Tannins are generally defined as naturally occurring polyphenolic compounds of high molecular weight to form complexes with the proteins. Tannins are important source of protein in animals but unfortunately the amounts of tannins that they contain vary widely and largely unpredictably, and their effects on animals range from beneficial to toxicity and death. Dietary supplementation of these compounds reduces the oxidative damage to cell membrane lipid, protein and nucleic acid due strong quenching property of free radicals[13-14]. Thus these compounds also provide protection from cardiovascular, immune/autoimmune diseases and brain dysfunctions viz. Parkinson's, Alzheimer's, Huntington's diseases[14]. The extractive capability of phenolic components from herb material is considerably depended on the type of solvent. Observation from the present study suggested that the phenolic and flavonoids content is high in n-hexane extract as compared to acetone extract, thus the n-hexane extract is more potent in scavenging the free radicals/ROS as compared to acetone extract.

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