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Original Research

Total phenolic, flavonoids and tannin contents in different extracts of *Artemisia absinthium*

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Abstract

The *A. absinthium* is commonly known as wormwood having antipyretic, antimicrobial, antifungal, diuretic and anti-inflammatory properties. Natural bioactive compounds like phenols and flavonoids are the important secondary metabolites in plant possess high scavenging ability of free radical and reactive oxygen species produced in mammals. To maximize these agents in the extract different solvents viz. aqueous, ethanolic and chloroform are used for the extraction procedure (among these different extractions). Current study was aimed to determine the levels of total phenolic, flavonoids and tannin contents. Observations suggested that ethanolic extract has significantly high ($P < 0.05$) concentration of flavonoids, phenolic and tannin contents as compared to aqueous and chloroform extracts. Therefore, ethanolic extract of *A. absinthium* has greater potential to scavenge free radicals/ROS and can produce more beneficial effects as compared to aqueous and chloroform extracts.

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INTRODUCTION

The genus *Artemisia* L. is a member of family Asteraceae and comprises of more than 200 species, found throughout the northern half of the world. In India, it grows in Kashmir valley and is locally known as 'Tethwen'. Plant is used in indigenous traditional systems of medicine as a vermifuge, an insecticide, an antipyretic [1], antimicrobial [2], antifungal [3], diuretic and as an antispasmodic in animals [4]. It is a rich source of terpenes, antioxidant phenolics, flavonoids and other biologically-active compounds. In modern medicine these compounds have been investigated for their anthelmintic and antioxidant activities in parasitized animals by neutralizing the free radicals and toxins formed in their blood, boost their immune system, and help fighting gastrointestinal parasites [5].

Natural bioactive compounds like phenols and flavonoids are the important secondary metabolites in plants having intrinsic properties that affect appearance, taste, odor and oxidative stability of plant based foods. These compounds also possess biological properties like antioxidant, anti-aging, anti-carcinogen, protection from cardiovascular, immune/autoimmune diseases and brain dysfunctions viz. Parkinson's, Alzheimer's, Huntington's diseases, etc [6,7]. Therapeutic potential of *A. absinthium* extract is directly related to total phenolic and flavonoids contents. These active metabolites especially from herbs are the interest subject of research, but their extraction as part of phytochemical or biological investigations presents specific challenges that must be addressed throughout the solvent extraction process. Therefore, present study was aimed to investigate the

levels of phenolic, flavonoids and tannin contents in different extracts prepared using aqueous, ethanolic and chloroform solvents.

MATERIALS AND METHODS

Chemicals and plant materials: The aerial parts of *Artemisia absinthium* was purchased from Agro Food Processing Emporium, Peerbagh, Srinagar, India. All parts were cleaned of adulterants and air-dried under shade at a well ventilated place. The plant material was pulverised to powder form with a mixer grinder. The 100 g of powder was soaked in 500 mL of aqueous, 95% ethanol and chloroform solvents to exhaustion (~120 h). The extraction was carried out in a percolator by a combination of maceration and percolation at room temperature. The filtrates were collected through a piece of porous cloth. Removal of the solvents at temperatures, below 60°C for aqueous and 40°C for ethanol and chloroform, under reduced pressure and a rotation speed of 20 rpm in vacuum rotary evaporator yield the respective extract. The extracts were scrapped off, transferred to an air tight container and stored in a freezer at -20°C till subsequent uses. Different chemicals viz. Quercetin (Sigma Aldrich, USA), Gallic acid (SD Fine Chem Ltd Mumbai, India), Folin-Ciocalteu reagent (Central Drug House, New Delhi, India) and other chemicals used for the analysis are analytical grade.

Determination of Total Phenolic Contents: 1% of plant extract solution was prepared in methanol and the amount of total phenolic contents in extracts was determined by the methods of Savitree *et al* [8]. In brief, 0.5ml of each sample was taken into test tube and mixed with 2.5 ml 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. Then the absorbance was read at 760nm spectrometrically (U-1800, Hitachi, Japan) against Gallic acid as a standard (Concentration of 0.01, 0.02, 0.03, 0.04 and 0.05 mg/ml of gallic acid were prepared in methanol). The Folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols, thereby producing a blue color upon reaction. All determination was performed in triplicate and the total phenolic content was expressed as mg/g gallic acid equivalents (GAE).

Determination of total flavonoids contents: The total flavonoids content of each plant extract was estimated as per Zhishenet *al* [9]. In-brief, each sample (1.0mL) was mixed with 4ml of distilled water and subsequently with 0.30 mL of a NaNO₂ solution (10%). After 5 min, 0.30 mL of an AlCl₃ solution (10%) was added followed by 2.0 mL of NaOH solution (1%). Immediately, after thorough mixing the absorbance was

measured at 510 nm versus the blank. Standard curve of quercetin was prepared (0-12mg/mL) and the results are expressed as quercetin equivalents (mg quercetin/gm dried extract).

Determination of tannin contents: Tannin content in each sample was determined using insoluble polyvinyl-polyrrolidone (PVPP) as described by Makkaret *al* [10]. Briefly, 1.0 mL of extract dissolved in methanol (1%), was mixed with 100 mg PVPP, vortexed, left 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 in the way similar to the total phenolics content [11]. Tannin content was calculated as a difference between total phenolic and non-tannin phenolic content in the extract.

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 prepared was used for the determination of total phenolic content and flavonoids using different concentrations of Gallic acid and quercetin respectively. Tannin content was calculated as a difference between total phenolics and non-tannin phenolic content. The total phenolics, flavonoids, tannin and non-tannin content in different extracts of *A. absinthium* have been presented in table 1. Observation shows that the total phenolic content is highest in the ethanolic extract (43.04 ± 0.57mg of GAE/g of extract) followed by aqueous and significantly lower (P<0.05) in the chloroform extract (28.34 ± 2.39 mg of GAE/g of extract). Similarly concentration of flavonoids is significantly high (P<0.05) in ethanolic extract as compared to aqueous and chloroform extracts. However, the concentration of tannin content is significantly lower (P<0.05) in chloroform extract (22.62± 2.45 mg of GAE/g of extract) as compared to aqueous and ethanolic extracts.

DISCUSSION

The WHO survey indicated that about 70–80% of the world's populations rely on non-conventional medicine, mainly of herbal source, for their primary healthcare [12]. These medicinal plants are rich sources for naturally occurring antioxidants especially phenolic and flavonoids contents. These agents have ability to scavenge free radicals, super oxide and hydroxyl radicals, etc thus they enhance immunity and antioxidant defense of the body [13]. Dietary

supplementation of these compounds reduces the oxidative damage to cell membrane lipid, protein and nucleic acid due strong quenching property of free radicals [14].

For acceptance of medicinal plants into scientific medicine, it is necessary that their effectiveness and safety be evaluated and confirmed through active ingredient testing. To maximize the extractive capability of phenolic and flavonoids components from

plant material is considerably depended on the type of solvent. Highest content of phenolic, flavonoids and tannin in ethanolic extract in comparison to other solvents used, make this organic solvent (ethanol) an ideal and selective to extract a great number of bioactive phenolic compounds. Similarly, Mohammadi [15] also reported that hydro-alcoholic mixtures are suitable to extract different bioactive phenolic compounds from *Tamarixaphylla*.

Table 1. The total phenolic, flavonoids, tannin and non-tannin contents present in different extracts of *A. absinthium*

Parameters	Aqueous extract	Ethanolic extract	Chloroform extract
Total Phenolic Content (mg of GAE/g of extract)	40.00 ^a ± 2.11	43.04 ^a ± 0.57	28.34 ^b ± 2.39
Total Flavonoids Content (mg Quercetin/g extract)	550.53 ^a ± 45.93	1108.15 ^b ± 48.78	667.40 ^a ± 51.26
Non-tannin Content (mg of GAE/g of extract)	7.09 ^a ± 0.24	6.52 ^a ± 0.81	2.45 ^b ± 0.24
Tannin Content (mg of GAE/g of extract)	30.44 ^a ± 1.08	36.91 ^a ± 1.24	22.62 ^b ± 2.45

Values are expressed as mean ± SE of three replicates. The different superscripted (a, b) values have significantly differ (P<0.05) from the other extract in same row.

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 [16]. The toxic or anti-nutritional effects tend to occur in times of stress when a very large proportion of the diet having high concentration of tannins. Thus consumption of foods naturally having antioxidant activity is the most efficient way of combating such tissue injuries, undesired transformations and preventing health risks [17]. In present study the ethanolic extract have high concentration of flavonoids and phenolic concentration. Therefore, ethanolic extract of *A. absinthium* have greater potential to reduce or scavenge free radicals or produces more beneficial effects as compared to other extracts.

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