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Qualitative and quantitative phytochemical analysis of *Artemisia indica* Willd.

Pushpa Ruwali[1]*, Tanuj Kumar Ambwani[2], Pankaj Gautam[1] and Ashish Thapliyal[1]

[1]Dept. of Biotechnology, Graphic Era University, Dehradun
[2]Animal Biotechnology Lab., V. P. B., College of Veterinary & Animal Sciences, G. B. Pant University of Agriculture & Technology, Pantnagar; Uttarakhand, India

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ABSTRACT

Plants have been used for food and also for medicinal purposes since antiquity. Medicinal plants are Nature's gift to help pursue a disease-free healthy life, and thus can play an important role in preserving health. In India, Artemisia indica Willd. (Asteraceae) vernacularly known as ''Titepati'' is a perennial herb found in the western Himalayas and is one of the most utilized locally as a traditional medicinal plants, especially in the Kumaun hills, though, surprisingly, is also one of the lesser studied one, with meager information available on its phytochemical analysis. The powdered aerial parts were extracted with methanol (AME), ethanol (AEE) and hydro-methanol (AHME).The AHME gave the highest yield (20.29%), followed by AME (13.27%) and AEE (7.08%).Out of the total of 11 phytocomponents targeted, carbohydrates, reducing sugars, flavonoids, sterols, tri-terpenoids, phenolics and glycosides were detected in all three extracts. Saponins and tannins were absent in AEE, while AME and AHME contained both these phytoconstituent groups. Amino acids and alkaloids were present only in the methanolic extract. The AME (255.5±6.71)exhibited the highest total phenolic contents followed by AEE (139.4±7.49) and AHME (22.7±2.7) as Gallic acid equivalent. Flavonoid content exhibited similar pattern as in the case of total phenolic, i.e. an increased magnitude in total flavonoids in the order of AME (161.2±4.95) > AEE (71.9±3.53) > AHME (11.15±0.80) as Quercetin equivalents.

Key words: *Artemesia indica* Willd.; Phytochemicals; Medicinal plants; Plant extracts; Western Himalayas.

INTRODUCTION

Plants have been used for food and also for medicinal purposes since antiquity [1]. Medicinal plants are Nature's gift to human beings to help them pursue a disease-free healthy life, and thus can play an important role in preserving health [2]. Since ancient times, natural products obtained from plant sources remains as a major source of preventive and curative preparations. Traditional medical systems throughout the world have been relied on to support, promote, retain, and regain human health for millennia [3]. In recent times, there has been growing interest in exploiting the biological activities of different ayurvedic medicinal herbs, owing to their natural origin, cost effectiveness and lesser side effects [4,5]

A large number of population is still dependent on the ethnomedicinal practices and medicinal plants for their preventive and curative properties. According to the World Health Organization, traditional medicines, including herbal medicine, have been, and continue to be, used in almost every country around the world in some capacity [6]. In much of the developing world, 70-95% of the population relies on these traditional medicines for primary care, owing to better cultural acceptability, better compatibility with human body and lesser side effects [7,6].However, in the last few years there has been a considerable increase in their use in the developed world. It is now recognized that about half the population of industrialized countries regularly use complementary medicine [8].

Medicinal plants are an integral part of the diverse traditional medical practices in the Himalayan region and are highly valued both in folk medicine and in codified traditional medical systems, such as Chinese traditional medicine and Ayurveda [9].The Himalayas represents one of the most important mega centers of biodiversity, sharing over fifty percent of the vegetational wealth of the Indian subcontinent. A large percentage of crude drugs in the Indian market come from this region [10].

The state of Uttarakhand is a part of north-western Himalaya, located between $28^o43'–31^o27'$ N latitudes and $77^o34'–81^o02'$ E longitudes [11] and has a dense vegetation cover harboring a vast range of medicinal plants, earning the frontrunner status in this regard, in India [12,13,14].A medicinal plant genus *Artemisia*, named in honor of 'Artemis' the Greek goddess of chastity, has been used extensively in folk medicine and as food by many cultures since times immemorial. Genus *Artemisia* (Asteraceae), popularly known as 'Sage Brush' or 'Worm wood', is bitter aromatics and is distributed worldwide, mainly across the temperate zones of the Northern Hemisphere, some species reaching the Arctic, but a few species can also be found on the Southern Hemisphere [15]. *Artemisia* is a diverse and economically important genus and it has more than 500 species reported in the world and out of which about 45 species, are found in India [16,17].

In India, *Artemisia indica* Willd. vernacularly known as ''Titepati'' is a perennial herb found in the western Himalayas and is one of the most utilized locally as a traditional medicinal plants, especially in the Kumaun hills, though, surprisingly, is also one of the lesser studied one with meager information available on its phytochemical analysis. Ethnomedicinally, It has been employed by local people to alleviate chronic fever, dyspepsia and hepatobiliary ailments [18]. The leaves and flowering stems are said to be anthelmintic, antiseptic, antispasmodic, emmenagogue, expectorant and stomachic [19]. A good number of reports confirm the use of *A. indica* as a culinary herb and a food plant all over the world, including India [20], Pakistan [21,22], Nepal [23] and Japan [24].

Regarding phytochemical analysis of *A. indica*, literature survey revealed that some efforts have been directed towards chemical analysis of essential oils [25,26,18]. In spite of sincere efforts, authors could not find any reports on preliminary phytochemical analysis of *A. indica*. The present communication reports the preliminary qualitative and quantitative phytochemical analysis of various extracts of *A. indica* Willd.

EXPERIMENTAL SECTION

Collection and authentication of plant materials

Fresh aerial parts of *Artemisia indica* Willd. Specimens were collected at an altitude of 1560 meter, strictly abiding by the standard precautions in the month of June, from the Kumaun hills of Okhalkanda block, near Bhimtal, Nainital district, Uttarakhand state, India. The plant specimen were authenticated in the Botanical Survey of India (BSI), Northern Circle, Dehradun (Uttarakhand, India). A voucher specimen (Acc. no. 114879) was deposited at the herbarium of BSI.

Preparation of Extracts

Fresh aerial parts of *A. indica* were rinsed 2-3 times in metal deionized water and then subjected to shade drying at room temperature. The dried plant materials were powdered using a clean grinder and stored in air-tight container in a cool place until analysis. The powder was extracted with methanol, ethanol and hydro-methanol. For Soxhlet extraction, the sample was taken in the ratio of 1:10 (w/v) with each of the solvent, viz. methanol, ethanol and hydromethanol (50 %). 50 gram (gm) sample powder was extracted with 500 milliliter (ml) of solvents for 10hours (hrs) at temperatures not exceeding the boiling point of the respective solvent. Firstly, the extracts were filtrated with muslin cloth (2 times) and then through filter paper (Whatman® Grade 1). The extracts were concentrated using a rotary evaporator at a maximum temperature of 45^0C and dried extracts were stored (air-tight) in refrigerator at 4^0C till further analysis [27,28,29]. The percent yield of extracts were calculated, and subjected to the relevant phytochemical analysis.

Yield calculation

The extracts obtained with different solvents were weighed and their percentage were calculated as compared to the initial weight of the plant material to get the extractive values.

Percentage Extraction Yield = $W_E / W_S \times 100$

Where, (W_E = Weight of the plant extract; W_S = Weight of the initial sample)

Qualitative and quantitative phytochemical analysis of various *Artemisia* extracts

Qualitative phytochemical tests for the identification of carbohydrates, reducing sugars, amino acids, saponins, flavonoids, alkaloids, tannins, sterols, triterpenoids, phenolics and glycosides were carried out for all *Artemisa* (*A. indica* Willd.) extracts (AEs) viz. *Artemisia* ethanolic extract (AEE), *Artemisia* methanolic extract (AME) and *Artemisia* hydro-methanolic extract (AHME) as per the methods described by [30,31,32,33].

Molisch's test for Carbohydrates:

The extract was treated with Molisch's reagent and concentrated $H_2SO_4$was added from the sides of the test tube to form a layer. A red or dull violet ring indicated the presence of carbohydrates.

Fehling's test for reducing sugars:

Extract was heated with equal amount of Fehling's A and B solutions. Formation of brick red colour confirmed the presence of reducing sugars.

Ninhydrintest for Amino acids:

1 ml of extract and 3 drops of Ninhydrin solution were heated in a boiling water bath for 10 minutes (min). Appearance of purple color was indicative of amino acids.

Foam test for Saponins:

Small amount of extract was shaken with little quantity of triple glass distilled water (TGDW), foam produced persisting for 10 min was indicative of the presence of saponins.

Ferric chloride test for Flavonoids:

Few drops of neutral $FeCl_3$ solution was added to little quantity of extract. Formation of blackish green color indicated the presence of flavonoids.

Wagner's test (Iodine in Potassium iodide)for Alkaloids:

50 mg of extract was stirred with few ml of dilute HCl and filtered. To a few ml of filtrate, few drops of Wagner's reagent were added very carefully by the side of the test tube. A reddish-brown precipitate confirmed presence of alkaloids.

Ferric chloride test for Tannins:

To the extracts a few drops of 1% neutral $FeCl_3$solution were added, formation of blackish blue color indicated the presence of tannins.

Liebermann-Burchard test for Sterols:

Extracts were dissolved in chloroform and filtered. To the filtrates few drops of acetic anhydride was added and mixed well. 1 ml of concentrated H_2SO_4 was added from the sides of the test tube, appearance of reddish brown ring was positive for sterols.

Salkowski test for Tri-terpenes:

Extracts were dissolved in chloroform and filtered. Few drops of concentrated H_2SO_4 was added to the filtrates, shaken and allowed to stand, appearance of golden yellow colour indicated the presence of triterpenes.

Ferric chloride test for Phenols:

1ml of extract was mixed with 2ml of 2% solution of $FeCl_3$. A blue-green coloration indicated the presence of phenols.

Salkowski's test for Glycosides:

Extract was mixed with 2 ml of $CHCL_3$. Then 2 ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown color indicated the presence of glycoside.

Estimation of total flavonoid content (TFC):

TFC was measured by the aluminium chloride colorimetric assay described by Zhishen *et al*. [34].For this, 1ml of plant extract (60µg/ml) or standard solution of quercetin [2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-chromen-4-one] (10, 20, 40, 60, 80 and 100 µg/ml) was added to 10 ml volumetric flask containing 4 ml of TGDW, followed by addition of 0.3 ml 5% $NaNO_2$. After 5 min, 0.3 ml of 10% $AlCl_3$ was added. At 6^{th} min, 2 ml of 1 M NaOH was added and the total volume was made up to 10 ml with TGDW. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. TFC was expressed as mg quercitin equivalent (QE)/gm of plant dried extract.

Estimation of total phenolic content (TPC):
TPC of the extracts were determined using the methods described by Singleton and Rossi [35] and Demiray *et al*. [36]with slight modifications. Calibration curve was prepared by mixing different solutions of gallic acid [3,4,5-trihydroxybenzoic acid](1ml; 20-120µg/ml) with 5 ml of Folin-Ciocalteu reagent (tenfold diluted) and $Na_2CO_3$7.5%. Absorbance values were measured at 765 nm and the standard curve was plotted. 1 ml of each of the extract (60µg/ml) was also mixed with the reagents as above and after 30 min the absorbance was measured to determine the TPC which was expressed as mg of quercertin equivalents (QE) per gm of dried extract

Statistical analysis
All measurements were performed in triplicates and the results are expressed as mean±SD (standard deviation).

RESULTS AND DISCUSSION

Artemisia indica Willd. specimens were collected, authenticated, aerial parts dried, powdered, and were extracted with methanol (AME), ethanol (AEE) and hydro-methanol (AHME).Table-1 depicts the percentage yield of various extracts viz. AME, AEE and AHME of *A. indica* Willd. aerial parts. The AHME gave the highest yield (20.29%), followed by AME (13.27%), while the ethanol extract gave the least yield (7.08%). Thus extraction with more polar solvent(50% hydro-alcoholic)resulted in the higher amount of total extractable compounds, whereas the extraction yield with pure alcohols viz. methanol and ethanol extracted less material in comparison.

Table-1: Percentage yield of various *A.indica* Willd. extracts

Extract	Percentage Yield
AME	13.27%
AEE	7.08%
AHME	20.29%

Phytochemical screening of various extracts i.e. AME, AEE and AHME was carried out following the methods reported in literature and the results are presented in Table-2.Out of the total of 11 Phytocomponents targeted, carbohydrates, reducing sugars, flavonoids, sterols, tri-terpenoids, phenolics and glycosides were detected in all three extracts. Saponins and tannins were absent in AEE, while AME and AHME contained both these phytoconstituent groups. Amino acids and alkaloids were present only in the methanolic extract (AME).

Table-2: Phytochemical analysis of various *A.indica* Willd. extracts

Phytochemicals	AME	AEE	AHME
Carbohydrate	+	+	+
Reducing sugars	+	+	+
Amino Acids	+	-	-
Saponins	+	-	+
Flavonoids	+	+	+
Alkaloids	+	-	-
Tannins	+	-	+
Sterols	+	+	+
Triterpenoids	+	+	+
Phenolics	+	+	+
Glycosides	+	+	+

Different concentrations of gallic acid and quercetin were used for preparing standard curves for the determination of total phenolics and flavonoids, respectively. Table-3 shows the TPC and TFC of *A. indica* in three different solvents used. The AME (255.5±6.71)exhibited the highest TPC followed by AEE (139.4±7.49) and least in the AHME (22.7±2.7) as GAE. Results depicted a similar pattern as in the case of TPC, i.e. an increased magnitude in TFC in the order of AME (161.2±4.95) > AEE (71.9±3.53) > AHME (11.15±0.80) as QE.

Table-3: Total phenolic and flavonoid contents of *A.indica* Willd. extracts

Extracts	TPC (mg of GAE/gm of dried extract)	TFC (mg of QE/gm of dried extract)
AME	255.5±6.71	161.2±4.95
AEE	139.4±7.49	71.9±3.53
AHME	22.7±2.7	11.15±0.80

The knowledge of the chemical constituents of plants is not only desirable to understand its pharmacological and medicinal values, but also a crucial exercise needed to isolate and characterize the chemical constituents present. In addition, the knowledge of the chemical constituents of plants would further be valuable in realizing and validating

the actual value of folkloric remedies. The preliminary phytochemical screening tests are undoubtedly useful in the detection of the bioactive principles, which subsequently may lead to the drug discovery and development. Such analysis of various phytoconstituents make the plant amenable to treating different ailments and affirming the potential of providing useful drugs for human and animal use.

The present preliminary qualitative and quantitative analysis of phytochemical constituents of various extracts of *A. indica* revealed the presence of carbohydrates, reducing sugars, amino acids, saponins, flavonoids, alkaloids, tannins, sterols, triterpenoids, phenolics and glycosides. All these phytoconstituents are known to exhibit medicinal as well as physiological activities[30].

Analyzing the percentage yield of various extracts viz. AME, AEE and AHME, it was observed that the AHME gave the highest yield (20.29%), followed by AME (13.27%), while the ethanol extract gave the least yield (7.08%). It was obvious to note that extraction with more polar solvent(50% hydro-alcoholic)resulted in the higher amount of total extractable compounds, whereas the extraction yield with pure alcohols viz. methanol and ethanol extracted less material in comparison.

A good amount of literature supports that, variation in the yields of various extracts is attributed to polarities of different compounds present in the plant [37] and hence, as a major factor, the extraction yield is dependent on the nature of the solvent used [38]. Based on our results reported here, as just mentioned, the highest extraction yield was found with hydro-alcoholic solvent. This indicates that probably a larger fraction of phytoconstituents in *A. indica*is hydrophilic or water-soluble. It is an established fact that the extract yield increases with the solvent polarity. The addition of water into methanol tremendously increases the extract yield. These yields are higher than those seen using pure solvents [39].

Methanolic fractions have been reported as the most suitable and chosen solvent in plant extraction method for plant compound analysis than many other solvents as it extracts out most of the plant compounds[40,41,42,43,44]. This was corroborated well with results obtained with the methanolic extract in the present study, as the AME was positive for all the 11 phytochemical groups analyzed/ studied (Table-2). Saponins and tannins were absent in AEE, while AME and AHME contained both these phytoconstituent groups. Amino acids and alkaloids were present only in the methanolic extract (AME). It is worthwhile to mention here, that in spite of sincere efforts, the authors could not come across any similar previous report of such preliminary phytochemical analysis of *A. indica* Willd.

Plants synthesize a wide range of chemical compounds which are classified based on their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites. Primary metabolites are directly involved in growth and development while secondary metabolites are not involved directly and they have been worked as biocatalysts [45].Secondary metabolites are biosynthesized in plants for different purposes including growth regulation, inter and intra-specific interactions and defense against predators and infections [46,47].

Many of such secondary metabolites found to be present in the *A. indica* extracts in the present study, like sponins, alkaloids, tannins, sterols, triterpenoids, phenolics, flavonoids, glycosides etc. have been reported to exhibit interesting biological and pharmacological activities and are used as chemotherapeutic agents or serve as the starting point in the development of modern medicines.

Saponins have been reported to have antifungal activity[48], produce inhibitory effect on inflammation[49], property of precipitating and coagulating red blood cells, hemolytic activity, cholesterol binding properties[50], traditionally have been extensively used as detergents, as piscicides and molluscicides, as foaming and surface active agents[51], etc. Also, steroidal compounds are of importance and of interest in pharmacy due to their relationship with sex hormones[52].Alkaloids have been associated with medicinal uses for centuries for their varied biological properties such as cytotoxicity[53], analgesic [54] antispasmodic and antibacterial[55,56]and antiviral[57].Glycosides are known to lower the blood pressure according to many reports[58]. Terpenoids and tannins are attributed for analgesic and anti-inflammatory activities. Apart from this tannins contribute property of astringency and antimicrobial activity i.e. faster the healing of wounds and inflamed mucous membrane[59,60].

There is a considerable renewed interest in the role of the phenolics and flavonoids as natural antioxidants and free radical scavengers[61,38]. This fact assumes a greater importance on account of realization of potential toxic and carcinogenic effects of many widely used synthetic antioxidants[44].

One of the most important and probably the largest group of secondary metabolites, are phenolic compounds. Phenolics are characterized by at least one aromatic ring (C6) bearing one or more hydroxyl groups[62,63].They possess varied biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation,

antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities[64]. Numerous reports have described the antioxidant properties of medicinal plants which are rich in phenolic compounds[65,44,62]. Natural antioxidant mainly comes from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc.[31,66]. The largest and best studied natural phenols are the flavonoids [67]. They also are very effective antioxidant and show strong anticancer activities[68].

Quantitative phytochemical analysis of all extracts of *A. indica*, in the present study, revealed variable amounts of TPC and TFC. The AME (255.5±6.71) exhibited the highest TPC followed by AEE (139.4±7.49) and least in the AHME (22.7±2.7) as GAE. Here again methanol proved to be a better solvent system in comparison to ethanol and even hydro-methanol (50%). A similar pattern was observed in the case of TFC too, i.e. an increased magnitude in TFCin the order of AME (161.2±4.95) > AEE (71.9±3.53) > AHME (11.15±0.80) as QE.

Though no previous report on the total phenolics and flavonoids of *A. indica* Willd. could be found by the authors, in spite of exhaustive literature search, albeit several reports on various species of *Artemesia* supports the utility of methanol over other solvents. Ahameethunisa and Hopper [28]reported that in comparison to different extracts *A. parviflora* methanolic extract had significantly higher concentration of phenolic compounds. Methanolic extract of *A. annua* leaves showed the highest phenolics and flavonoids concentration confirming the ability of methanol to solubilize a larger fraction of the phenolic components present in *A. annua* leaves [43].

One of the most plausible explanation of pure methanol extracts (AME) exhibiting considerably higher phenolic and flavonoid contents in comparison to the hydromethanolic one (AHME), is that methanol is said to be the most suitable solvent in the extraction of phenolic compounds due to its ability to inhibit the reaction of polyphenol oxidase that causes the oxidation of phenolics and its ease of evaporation compared to water [41].

It is relevant to mention here that, going through the exhaustive literature available regarding phytochemicals, their estimation and evaluation, it was observed that there is lot of variations in the chemical constitution and bioactive molecules of a plant and its extracts by various workers. This is quite obvious, as the final outcome of such analysis would depend on many variables, such as the natural habitat of the plant, its age, climatic and geographic conditions, seasonal variations, method of harvesting, physiochemical nature of the solvent system selected and most important, the experimental procedures used. However, what is of scientific relevance is the qualitative and preferably, the quantitative confirmation of the in-question phytochemicals before being evaluated for a specific biological action of the plant being studied [69].

In conclusion, in the present study, the preliminary qualitative phytochemical analysis of various extracts of *A. Indica* Willd. revealed the presence of major phytochemicals, albeit with little differences owing to the nature of the solvents used. Quantitative estimation also revealed a good amount of total phenolics and total flavonoids, especially in case of methanolic extract. These major bioactive molecules and chemicals, undoubtedly are fundamentally responsible for the various biological actions/ effects of the plant. It would be worthwhile to study and further evaluate *A. indica* Willd. both *in vitro* and *in vivo* (in a suitable model system) not only to validate its ethnomedicinal claims, but to develop it into a natural and potential holistic preparation, with special reference to as an antioxidant, immunomodulatory and antiviral agent both for humans and animals.

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