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Preliminary Phytochemical Screening and Physico-Chemical Parameters of *Artemisia absinthium* and *Artemisia annua*

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The family Asteraceae or compositae known as the ester, daisy or sunflower family is the largest family of flowering plants. *Artemisia* is a large diverse genus of plants with between 100 to 150 species belonging to the family asteraceae (compositae). It comprises hardy herbs and shrubs known for their volatile oils. They grow in temperate climate of the northern hemisphere and southern hemisphere usually in dry or, semidry habitats. The collected herbs were authenticated, dried and extracted to calculate the percentage of yield. Phytochemical studies of the Hexane and alcoholic extracts showed the presence of various phytoconstituents i.e. carbohydrate, saponins, phytosterol, proteins and amino acid, tannin, phenolic compounds and flavonoids. It was observed that all the extracts show more important chemical constituents for various pharmacological activities. The determination of these characters will aid future investigators in their Pharmacological analysis of this species.

Keyword: *Artemisia*, Phenol, Phytochemical screening, Tannin.

1. Introduction

The life cycle of *A. absinthium* has been reported[1]. Growth begins in late April, and new plants are 4-12 inches tall by mid-May. Flowering begins in late July to early August. During late fall, the aboveground portion of the plant dies. Seedlings may emerge at any time from late spring to early fall. Seedlings may be unnoticed for some time as they are low with small leaves before the upright flowering stems emerge[2]. The leaves and flowering tops are gathered when the plant is in full bloom, and dried naturally or with artificial heat. Its use has been claimed to remedy indigestion and gastric pain, it acts as an antiseptic, and as a febrifuge. For medicinal use, the herb is used to make a tea for helping pregnant women during pain of labor. A wine can be made by macerating the herb. It is also available in powder form and as a tincture. The oil of the plant can be used as a cardiac stimulant to improve blood circulation. Pure wormwood oil is very poisonous, but with proper dosage poses little or no danger. Wormwood is mostly a stomach medicine[3]. Safe in moderation, but large doses are toxic. Poisoning leads to seizures, delirium, hepatoprotective, and hallucinations and death[4]. The whole plant had shown various pharmacological activity viz. antihelmintics[5], stomachache, appetizer, diabetes, uterus cyst, tuberculosis,

antihypertensive, and leaves may also showed Antihypertensive, Wounds, Diabetes,[6] anti-microbial activity[7], antimalarial[8], and antifertility[9].

Artemisinin is a sesquiterpene lactone with an endoperoxide bridge and has been produced semisynthetically as an antimalarial drug. The efficacy of tea made from *A. annua* in the treatment of malaria is contentious. According to some authors, artemisinin is not soluble in water and the concentrations in these infusions are considered insufficient to treat malaria[10,11]. In 2004, the Ethiopian Ministry of Health changed Ethiopia's first line antimalaria drug from sulfadoxine/pyrimethamine (Fansidar), which has an average 36% treatment failure rate, to artemether/lumefantrine (Coartem), a drug therapy containing artemisinin which is 100% effective when used correctly, despite a worldwide shortage at the time of the needed derivative from *A. annua*[12]. Artemisinin has no reported toxicity if taken in recommended doses for short periods in the treatment of malaria[13]. The plant shows more pharmacological activity viz. treatment of malaria[14], immunosuppressive[15], antifungal activity[16] and antipyretic activity[17].

2. Materials and Methods:

2.1 Plant Materials:

The plant (*Artemisia absinthium* and *Artemisia annua*) used for this study was collected from around dehradun and identified at Department of Pharmacy, Kumaun University Nainital. A voucher specimen has been deposited in the herbarium of the institute for future references. All parameters were studied as per Ayurvedic Pharmacopoeia of India[18] and Fluorescence analysis; primary and secondary plant metabolites were also investigated. All the reagents used were of the analytical and highest purity grade from standard companies.

2.2 Fluorescence Analysis:

The fluorescent method is adequately sensitive and enables the precise and accurate determination of analyze over a satisfactory concentration range without several time-consuming dilution steps prior to analysis of pharmaceutical samples. To check the fluorescent property of crude drug powder is used for analysis under ultra violet light. Powder as such, Powder + Water, Powder + Conc. HCl, Powder + Conc. H_2SO_4, Powder + Conc. HNO_3, Powder + 5% NaOH, Powder + methanol, Powder + acetone, Powder + NaOH (0.1N) and Powder + HCl (0.1N) are used to perform for fluorescence analysis[19].

2.3 Extraction:

The air dried both plant parts were cleaned and reduced to powdery form with the help of mechanical grinder after which each 250 gm of powdered sample was exhaustively extracted with 2.0 lt of alcohol (analytical grade), for 3 days (by soxlet apparatus). The extracted plant materials were separated by filtration technique and the hexane and alcoholic extracts were concentrated under reduced pressure (by Rotavapour, Büchi, Switzerland) and lyophilized to preserve it. The residues was found to be 5.4%, 21.52% w/w and 6.4%, 20.46 % w/w respectively for *Artemisia absinthium* and *Artemisia annua* and stored in a refrigerator at 4^0C for further investigation.

2.4 Phytochemical screening:

The various extract of *Artemisia absinthium* and *Artemisia annua* was subjected to preliminary phytochemical screening using standard screening method. The molish's test and fehling's test were carried out for carbohydrate, foam test for saponins, salkowiski test & libermann burchard test for phytosterol, sodium hydroxide test, concentrated sulphuric acid test and shinoda's test for flavonoids, biuret test, ninhydrin test and million's test for proteins and amino acid[20].

3. Results and Discussion:

3.1 *Artemisia absinthium*

Fresh plant material (*Artemisia absinthium*) was collected and subjected to various physicochemical parameters such as moisture content and foreign matter was determined. The total ash of the plant sample, acid insoluble ash

and water-soluble ash values were also determined. Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily, estimated by any other method. Further, these values indicate the nature of the constituents present in a crude drug. The percentage of alcohol soluble extractive value and water-soluble extractive value were also determined (Table: 01).

The percentage of starch calculated in leaves was found to be 11.66%. The percentage of sugar calculated in leaves was found to be 6.38%.The percentage of tannin calculated in leaves was found to be 0.20%. The percentage of total phenol calculated in leaves was found to be 2.78% (Table: 01).

Table 1: Quantitative Physico-Chemical Analysis of *Artemisia absinthium*.

S.No.	Parameters	Range(%)	Mean(%)	S.D
1	Moisture content (w/w)	19.8-16.1	17.2	±0.8124
2	Foreign matters (w/w)	0.2-0.8	0.5	± 0.0702
3	Total ash (w/w)	2.42-2.52	2.50	±0.1714
4	Acid insoluble ash (w/w)	0.22-0.27	0.25	±0.3762
5	Water soluble ash (w/w)	0.35-0.44	0.39	±0.0327
6	Alcohol soluble extractive (w/w)	11.37-13.59	12.67	±0.2731
7	Water soluble extractive (w/w)	10.60-11.57	10.98	±0.3521
8	Starch	11.55-11.73	11.66	±0.1622
9	Sugar	6.15-6.43	6.38	±0.0023
10	Tannin	0.20-0.21	0.20	±0.0132
11	Total Phenolic	2.75-2.86	2.78	±0.0520

Fluorescence study is an essential parameter for first line standardization of crude drug. In fluorescence, the fluorescent light is always of greater wavelength than the exciting light. Light rich in short wavelengths is very active in producing fluorescence and for this reason ultraviolet light produces fluorescence in many substances which do not visibly fluoresce in daylight. (Table: 02).

Table: 02 Fluorescence analysis of *Artemisia absinthium*

S.No.	Drug	Short ultra violet (256nm)	Long ultra violet (365nm)	Visible/Day light
1.	Powder as such	Black	Black	Light green
2.	Powder+water	Brown	Black	Light green
3.	Powder+conc.HCl	Black	Black	Brown
4.	Powder+conc.H_2SO_4	Black	Dark brown	Brown
5.	Powder+conc.HNO_3	Pale green	Black	Dark green
6.	Powder+5% NaoH	Pale brown	Black	Black
7.	Powder+Methanol	Light green	Light black	Pale green
8.	Powder+Aetone	Brown	Black	Green
9.	Powder+NaoH(0.1N)	Pale yellow	Black	Light yellow
10.	Powder+HCl(0.1N)	Brown	Black	Brownish black

Table: 03 phytochemical screening of *Artemisia absinthium*

S. No.	Constituents	Tests	Hexane extract	Methanolic extract
1.	Carbohydrate	Molish's test	_	+
		Fehling's test	_	_
2.	Glycoside	Borntrager's test	_	_
		Keller killanis test	_	+
3.	Fixed oil & fats	Spot test	+	–
		Saponification test	+	–
4.	Proteins & amino acids	Million's test	_	+
		Ninhydrin test	_	_
		Biuret test	_	+
5.	Saponins	Foam test	_	+
6.	Phenolic compunds & tannins	$FeCl_3$ test	_	+
		Lead acetate test	_	+
7.	Phytosterol	Salkowiski test	_	+
		Libermann burchard test	_	+
8.	Alkaloids	Dragendroff's test	_	_
		Mayer's test	_	_
		Hager's test	_	–
10.	Resin	Resin	_	+
11.	Flavonoids	Aq. NaOH test	+	+
		Conc. H_2SO_4 test	+	+
		Shinoda's test	_	+

(+) = Presence, (−) = Absence

3.2 *Artemisia annua*

Fresh plant material (*Artemisia annua*) was collected and subjected to various physicochemical parameters such as moisture content, foreign matter, total ash, acid insoluble ash, water-soluble ash values were determined. The percentage of alcohol soluble extractive value and water-soluble extractive value were also determined (Table: 04).

Table: 04 Quantitative Physico-Chemical Analysis of *Artemisia annua*.

S.No.	Paramaters	Range(%)	Mean(%)	S.D.
1	Moisture content (w/w)	15.52-16.43	15.70	±0.6545
2	Foreign matters (w/w)	0.14-0.5	0.313	±0.1803
3	Total ash (w/w)	13.68-13.95	14.18	±0.6464
4	Acid insoluble ash (w/w)	0.21-0.26	0.25	±0.0360
5	Water soluble ash (w/w)	0.18-0.19	0.17	±0.0152
6	Alcohol soluble extractive (w/w)	7.65-7.44	7.30	±0.4315
7	Water soluble extractive (w/w)	9.36-10.56	10.2	±0.7299
8	Starch	14.26-14.21	13.95	±0.4942
9	Sugar	9.25-10.68	9.69	±0.8527
10	Tannin	0.16-0.19	0.18	±0.0167
11	Total Phenolic	1.72-1.83	1.83	±0.1100

The percentage of starch calculated in whole plants was found to be 10.52%. The percentage of sugar calculated in whole plants was found to be 7.23%. The percentage of tannin calculated in whole plants was found to be 0.21%. The percentage of total phenol calculated in whole plants was found to be 2.14% (Table: 04).

Fluorescence provided by a drug is one of the several methods used for analyzing crude drugs. Fluorescence is a type of luminescence in which the molecule emits visible radiation passing from higher to lower electronic state. The molecules absorbs light usually over a specific range of wavelength, get excited from ground state to a high energy level and many of them emit such radiations while coming back to the ground state. Such a phenomenon of re-emission of absorbed light that occurs only when the substance is receiving the exciting rays is known as "Fluorescence". For fluorescence analysis, powdered drug was sieved through 60 mesh and observations were made following (Table: 05).

Table: 05 Fluorescence analysis of *Artemisia annua*

S.No.	Drug	Short ultra violet (256nm)	Long ultra violet (365nm)	Visible/Day light
1.	Powder as such	Yellowish	Black	Pale green
2.	Powder+water	Light green	Brown	Pale green
3.	Powder+conc.HCl	Brown	Black	Black
4.	Powder+conc.H_2SO_4	Brown	Dark brown	Light brown
5.	Powder+conc.HNO_3	Dark green	Black	Dark green
6.	Powder+5% NaoH	Brown	Black	Light black
7.	Powder+Methanol	Brown	Light black	Pale brown
8.	Powder+Aetone	Light yellow	Black	Yellowish
9.	Powder+NaoH(0.1N)	Pale yellow	Black	Pale yellow
10.	Powder+HCl(0.1N)	Light brown	Black	Brownish black

3.3 Phytochemical screening:

The results (*Artemisia absinthium* and *Artemisia annua*) of the phytochemical screening carried out on two extracts was recorded as shown in Table: 03 and Table: 06 respectively. Preliminary phytochemical studies revealed the presence of saponins, phytosterols, carbohydrates, proteins, amino acid, and flavonoids in alcoholic extract of *Artemisia absinthium* and *Artemisia annua*. Phytoconstituents in the various part of the plant vary significantly.

Ascorbic acid and phenolics contains plants are showing powerful antioxidants. The presence of saponins protects plant from microbial pathogens[21].

Flavonoids act as an anti-inflammatory agent in the same way as the non-steroidal anti-inflammatory drugs, i.e. by inhibiting the enzymes that cause the synthesis of prostaglandins[22]. Further studies may reveal the extract mechanisms of action responsible for the analgesic, anti-inflammatory and hepatoprotective activity of *Artemisia absinthium and Artemisia annua.*

Results reveal that the all the extracts have large class of phytoconstituents, which may be responsible for many pharmacological activities; further work is required to investigate all extracts of plant parts of *Artemisia absinthium and Artemisia annua* for various pharmacological activities.

Table: 06 Phytochemical screening of *Artemisia annua*

S. No.	Constituents	Tests	Hexane extract	Ethanolic extract
1.	Carbohydrate	Molish's test	_	+
		Fehling's test	_	+
2.	Glycoside	Borntrager's test	_	_
		Keller killanis test	_	+
3.	Fixed oil & fats	Spot test	+	–
		Saponification test	+	–
4.	Proteins & amino acids	Million's test	_	+
		Ninhydrin test	_	+
		Biuret test	_	_
5.	Saponins	Foam test	_	+
6.	Phenolic compunds & tannins	$FeCl_3$ test	_	+
		Lead acetate test	_	+
7.	Phytosterol	Salkowiski test	_	+
		Libermann burchard test	_	+
8.	Alkaloids	Dragendroff's test	_	_
		Mayer's test	_	_
		Hager's test	_	–
10.	Resin	Resin	_	+
11.	Flavonoids	Aq. NaOH test	+	_
		Conc. H_2SO_4 test	+	+
		Shinoda's test	_	+

(+) = Presence, (−) = Absence

4. Conclusion:

Preliminary phytochemical screening of the alcoholic extracts shows the presence of various phytoconstituents i.e. flavonoids saponins etc. These bioactive agents (flavonoids and saponins) have the ability to inhibit pain perception and they can also serve as antiinflammatory agents[23]. Flavonoids act as an antiinflammatory response in the same way as the nonsteroidal antiinflammatory drugs, i.e. by inhibiting the enzymes that cause the synthesis of prostaglandins. Further studies may reveal the mechanisms of action responsible for the analgesic, antiinflammatory and hepatoprotective activity of *Artemisia absinthium and Artemisia annua.*

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