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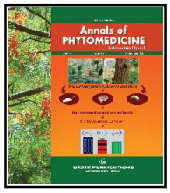

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Biochemical estimation of *Artemesia absinthium* L. powder and qualitative phytochemical screening of its hexanic and ethanolic extracts for assessment of purity

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Abstract

Medicinal plants have been used since times eons against a number of ailments. These natural sources of nutraceuticals are having incalculable benefits with negligible or no side-effects. *Artemesia absinthium* L. is an indispensable medicinal plant which plays a significant role in alleviating diseases owing to its anti-inflammatory, antioxidative, antimicrobial and wound healing effects. This plant is a depot of large number of bioactive compounds that can find a potential role in drug formulations against various diseases. In the present study, analysis of important biochemical proximate principles for the presence of cell and cell wall constituents and phytochemical constituents of ethanolic and hexanic extract of *A. absinthium* for qualitative detection of carbohydrates, proteins, steroids, alkaloids, phytosterols, tannins, phenols, *etc*., using different methods. Proximate analysis of plant for crude protein (CP), crude fibre (CF), total ash and nitrogen free extract (E.E.) and components of cell wall like neutral detergent fibre (NDF), acid detergent fibre (ADF), cellulose, hemicellulose and mineral components like calcium and phosphorus determine the usefulness of the plant as an essential feed supplement. The phytochemical screening established certain phytoconstituents having well known pharmacological properties, hence assuring purity of these extracts. As per the available literature, this is first study where in all these nutrients have been evaluated in the *A. absinthium* of Kashmiri origin. This depicts the purity of the species that can help in using these extracts for further pharmacological experimentations at *in vitro* and *in vivo* levels, a preliminary step towards drug discovery.

1. Introduction

Herbal medicines are critical to the health and prosperity of human race **(**Shukla *et al*., 2015). The World Health Organization (WHO) claims that derived from medicinal plants which are used by about 80% of people in developed nations as a source of potentially effective medications (Ekor, 2014).Traditional herbal medicine offers a fascinating and mostly untapped source for the creation and development of possibly novel chemotherapeutic medications that could help to address the growing problem of drug resistance to currently available commercial antibiotics as well as the drug associated toxicities (Sawant and Godghate, 2013). Plant extracts have been produced and are being considered for use as antimicrobials, due to the presence of their secondary metabolites like alkaloids, flavonoids, tannins, and other compounds (Chauhan *et al*., 2017), secondary metabolites in plants have a high capacity for scavenging free radicals and reactive oxygen species (Singh *et al*., 2012). The plant derived medications that boosts our defense mechanism and provide protection against a variety of ailments (Wadood *et al*., 2013).Wormwood is the common name of *A. absinthium* and is locally known as Tethwan in Kashmir. *A. absinthium* is a herbaceous plant with fibrous roots that is used for medicinal purposes and as a herbal cure in Central Europe, Southern Siberia, North America, and Asia (Abad *et al*., 2012). Due to the inclusion of multiple active components that work in different ways, *A.absinthium* plant extracts have a wide range of bioactivity (Fernando Wendel *et al*., 2021). The essential oil and bitter chemicals contained in *A. absinthium* are its most active ingredients (Goud and Poornima, 2018). Bitter components in plant extracts such as artabsin (sesquiterpene lactone) and absinthin (dimer of sesquiterpene lactone) induce *A. absinthium* with stimulant properties (Wright, 2002). The woodworm has found its usage as antimicrobial, (Juteau *et al*., 2003), antifungal, antioxidant (Kordali *et al*., 2005), anthelmintic, (Tariq *et al*. 2009), antimalarial (Irshad *et al*., 2011) agents besides its use in orthopedic conditions (Shubeena *et al*., 2018). It has also been found to be useful in providing osmotic

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stability in human erythrocytes, cognitive improvement, antiulcer, anticancerous and hepatoprotective activities (Goud and Poornima, 2018). The ethanolic extract of *A. absinthium* has a stronger scavenging capacity for free radicals/reactive oxygen species and can provide more positive effects (Singh *et al*., 2012). Many herbal products are taken orally, and knowing the proximate and nutritional analyses of these items is critical for determining nutritional importance and health impacts (Pandey, 2006; Kochhar, 2006; Taiga, 2008). In terms of herbal medicine standardization, WHO has stressed the need for identifying nutrients in the plants in order to design drug formulations to treat various diseases (Niranjan and Kanaki, 2008; Ojokoh, 2008).

2. Materials and Methods

2.1 Collection of medicinal plants

The *A. absinthium* was obtained from the CCRUM Lab, Habak. The plant was procured from higher altitudes of Kashmir valley and was identified by taxonomists at CCRUM Lab. Plant was washed properly using distilled water, dried under shade and grinded to fine powder. The powder was sieved using hand sieve and a portion of the powder was used for proximate principle analysis and the other portion was subjected to solvent extraction.

Table 1: Nomenclature and part of plant used

Scientific Name	Common name	Kashmiri name	Part to be used
Artemesia absinthium L.	Wormwood	Tethwan	Leaves

2.2 Proximate principle analysis

The dried sample of *A. absinthium* was analysed for proximate principles like dry matter (DM), crude protien (CP), crude fibre (CF), total ash and nitrogen free extract (NFE) as per Association of Official Analytical Chemistry (AOAC), 2005 guidelines (Abdul-Hamid *et al.*, 2020) and cell wall components like neutral detergent fibre (NDF), acid detergent fibre (ADF), hemicellulose and cellulose were determined by Van Soest method (Van Soest *et al.*, 1991).

Calcium and phosphorus were estimated by Talapatra *et al*. (1940).

2.3 Extraction using various solvents

Plant powder was subjected to cold extraction with different solvents in 1 : 5 ratio of powder and solvent. Solvents were selected according to the standard polarity index in order to obtain the extract. First, the less polar solvent was used followed by more polar, that is hexane, followed by ethanol. The extraction was done in the incubator shaker that was set at 25°C for seven days. Both the extracts were filtered separately through Whatmann filter paper No. 1 and the percolate was kept in incubator set at 30°C for drying. After proper evaporation of solvent from both extracts, they were weighed and kept in clean vials at –20°C till further use.

2.4 Calculation of extractive yield

The weight of the dried crude ethanolic and hexanic extracts was recorded and the extractive yield was calculated as under:

$$\text{Extractive yield} = \frac{\text{Wt. of extract}}{\text{Wt. of sample taken}} \times 100$$

2.5 Qualitative phytochemical screening

The hexanic and ethanolic extracts of *A.absinthium* were dissolved in dimethyl suphoxide (DMSO) and mixed to form homogenous solutions. The various (Table 2) qualitative phytochemical screening tests were performed for both the extracts.

Table 2: List of the various phytochemical screening tests performed for hexanic and ethanolic extracts of *A. absinthium*

S.No	Component	Test performed
1.	Carbohydrate	Molisch test
2.	Reducing sugars	Benedict's test
3.	Non-reducing sugars	Saliwanoff's test
4.	Proteins	Biuret test
5.	Proteins	Xanthoproteic test
6.	Flavonoids	Sodium hydroxide test
7.	Test for saponins	Foam test
8.	Test for steroids	Acid anhydride-H_2SO_4 test
9.	Tannins	Acetic acid test
10.	Phytosterols	Salkowich's test
11.	Phenolic compounds	Ferric chloride test
12.	Glycosides	Keller killanis test
13.	Alkaloids	Wagner's test
14.	Triterpenoids	Acetic anhydride-chloroform test

3. Results

3.1 Biochemical proximate principles in dried *A. absinthium*

The calculated biochemical proximate constituents of *A. absinthium* are given in Table 3. It can be seen that dried powder of the said plant contains moisture (10.66%), dry matter (89.34%), organic matter (92.5%), *etc*.

Table 3: Biochemical constituents in dried powder of *A. absinthium*

S.No.	Constituent	Concentration %age
1.	Moisture	10.66
2.	Dry matter	89.34
3.	Organic matter	92.5
4.	Crude protein	18.04
5.	Ether extract	5.01
6.	Crude fiber	18.15
7.	Neutral detergent fibre	45.20
8.	Acid detergent fibre	41.50
9.	Nitrogen free extract (NFE)	40.67
10.	Cellulose	6.30
11.	Hemicellulose	3.70
12.	Total ash	7.47
13.	Calcium	1.14
14.	Phosphorus	0.84

3.2 Estimation of hexanic and ethanolic extractive yields in the *A. absinthium*

The hexanic and the ethanolic extractive yields of the *A. absinthium* are represented in Table 4.

3.3 Phytochemical analysis

3.3.1 Phytochemical screening of hexanic *A.absinthium* extract (HAE)

The presence and absence of phytochemical constituents of hexanic extract of *A. absinthium* have been given in Table 5.

3.3.2 Phytochemical screening of ethanolic *A.absinthium* extract (EAE)

Table 6 depicts the presence and absence of important phytochemical constituents in ethanolic extract of *A.absinthium*.

Table 4: Extractive yield of the hexanic and eethanolic extracts of *A. absinthium*

Plant name	Weight of plant powder	Weight of extract after drying	Extractive yield
A.absinthium for hexanic extraction	100 g	3 g	3%
A.absinthium for ethanolic extraction	90 g	4.07 g	4.5%

Table 5: Phytochemical screening of hexanic *A. absinthium* extract (HAE)

S.No.	Constituents	Tests	Observation	Result
1.	Carbohydrates	Molisch's	Purple ring formed at junction	+ve
2.	Reducing sugars	Benedict's	Red color not formed	-ve
3.	Sucrose	Saliwanoff's	Deep red color formed	+ve
4.	Proteins	Biuret	Voilet color not formed	-ve
		Xanthoproteic	Yellow color formed	+ve
5.	Alkaloids	Wagner's	No precipitate formed	-ve
6.	Flavonoids	Sodium hydroxide test	Yellow color formed	+ve
7.	Steroids	Acid anhydride-H_2SO_4	No color change	-ve
8.	Phytosterols	Salkowich's	Slight change in color to golden red	+ve
9.	Glycosides	Keller killanis	No brown ring formed at interface	-ve
10.	Triterpenoids	Acetic anhydride-chloroform	Reddish violet color appeared	+ve
11.	Saponins	Foam test	No foam layer seen	-ve
12.	Tannins	Glacial acetic acid	No change in color	-ve
13.	Phenolic compounds	Ferric chloride	No change in color	-ve

Table 6: Phytochemical screening of ethanolic *A. absinthium* extract (EAE)

S.No.	Constituents	Tests	Observation	Result
1.	Carbohydrates	Molisch's	Purple ring formed at junction	+ve
2.	Reducing sugars	Benedict's	Red color not formed	-ve
3.	Sucrose	Saliwanoff's	Deep red color formed	+ve
4.	Proteins	Biuret	Voilet color not formed	-ve
		Xanthoproteic	Yellow color not formed	-ve
5..	Alkaloids	Wagner's	Yellow precipitate formed	+ve
6.	Flavonoids	Sodium hydroxide test	Slight change in color to yellow	+ve
7.	Steroids	Acid anhydride-H_2SO_4	No color change	-ve
8.	Phytosterols	Salkowich's	Golden red color appeared	+ve
9.	Glycosides	Keller killanis	No brown ring formed at interface	-ve
10.	Triterpenoids	Acetic anhydride-chloroform	Reddish violet color appeared	+ve
11.	Saponins	Foam test	No foam layer seen	-ve
12.	Tannins	Glacial acetic acid	Reddish brown color appeared	+ve
13.	Phenolic compounds	Ferric chloride	Deep violet or black color formed	+ve

4. Discussion

The current study depicts the presence of high crude protein (CP) (18%), dry matter (DM) (89%) and ether extract (EE) (5%) in the extracts of *A. absinthium* that lies in agreement with earlier studies of Seo *et al.* (2015). The high protein in the aerial parts of plant can be of high nutritional importance as protein/aminoacid supplementation from leaves can be of great help in meeting animal's protein and energy requirements. On the other hand, *A. absinthium* showed the presence of comparatively lower fibre in the form of NDF (45%) and ADF (42%) and cellulose (6%) which is in concomitance with the study of Beigh *et al.* (2018). The high ash content (7.47%) of the plant depicts that there is a large deposit of mineral elements, the most important being the calcium (Ca) (1.18%) and phosphorus (P) (0.9%) as Ca and P are essential ions for regulation of osmotic pressure in cells and water distribution throughout the body.

Thus, the raw plant is an essential source of minerals and other readily available nutrients that may be required for the maintaining the integrity of cell membranes and can play a pivotal role in overall wellbeing of the body.

Biologically active compounds found in herbal plants are directly attributed to antioxidant, antibacterial, antifungal, antidiabetic and anticancer activities (Hussain and Nagoori, 2011; Bagheri, 2020). *A. absinthium* ethanol extracts are known to show significant antibacterial action against *Staphylococcus aureus* (ATCC29213) strain and antiulcer activity in acetylsalicylic acid treated mice (Shafi *et al.*, 2004). Ethanolic extracts of this plant species have also shown immunomodulatory effect by upregulating the dendritic cell maturation and CD40 expression hence, stimulating release of cytokines that are potent inflammatory mediators and is also known to reduced TLR4, toll like receptor (TLR) and Bax expression while increasing Bcl-2 expression and improving oxidative stress in rats.(Shahnazi *et al.*, 2015; Bagheri, 2020).

The findings of the phytochemical screening of hexanic and ethanolic extracts of *A. absinthium* were recorded as indicated in Table 5 and Table 6.

Both the extracts showed positive results for the presence of carbohydrates like sucrose as found in previous studies. The polysaccharides isolated from *Artemesia* are known to have potent immune stimulatory effect as they mediate Th1 dependent immune response in sheep RBCs (Danilets *et al.*, 2010). Furthermore, both the extracts showed negative biuret test, indicating absence of proteins or presence of free amino acids (without peptide bonds) which is in agreement with Ashok and Upadhyaya (2013). To confirm whether there is presence of free amino acids in the two extracts, both were subjected to xanthoproteic test. Both extracts gave positive xanthoproteic reaction, indicating presence of free amino acids having aromatic rings.

Hexanic extracts show absence of alkaloids and phenolic compounds while the same were present in ethanolic extract which is in agreement with other studies showing absence of alkaloids in hexanic and presence in methanolic extracts of *A. absinthium* (Ashok and Upadhyaya, 2013). This depicts the presence of the alkaloids and phenols in non-polar solvents and their absence in polar solvents of plant extracts. Chlorogenic acid, dicaffeoylquinic acid, ferulic acid, caffeic acid, gallic acid, salicylic acid, coumaric acid are some of the phenolic compounds present in *Artemesia*. In our study, flavonoids were present in both extracts that is in concomitance with previous research (Singh *et al.*, 2012; Szopa *et al.*, 2020). Flavonoids that are significantly found in *A. absinthium* are quercetin, apigenin, flavone, kaempferol, catechin, myristin, naryngenin, artemetin, *etc.* The presence of the flavonoids are attributed to anti-inflammatory property of the *Artemesia* that have high antioxidant action by free radical scavenging and preventing the generation of reactive oxygen species by blocking the prooxid-ative enzymes (Bhat *et al.*, 2018). Ivanescu discovered the flavones eupatorin and hispidulin in *A. absinthium* (Ivanescu *et al.* 2016). Hispidulin is an important bioactive flavone having anticancer and antiepileptic characteristics, whereas eupatorin exhibits wide anticancer action (Ferreira *et al.*, 2010; Atif *et al.*, 2015). According to certain investigations, the therapeutic and pharmacological effects of *A. absinthium* are attributed to the presence of polyphenols (Saiedi and Masoudi, 2017).

Hexanic extracts of *A. absinthium* showed presence of the triterpenoids and at the same time, triterpenoids were absent in ethanolic extracts. Tannins were present in only ethanolic extracts while as glycosides were absent in both extracts. Very recently, Afzal *et al.* (2021) reported that hexanic extracts of *A. absinthium* have abundance of triterpenoids only ethanolic extracts are positive for tannins and both extracts gave negative results for glycosides which is in agreement with our study. These extracts were known to possess significant antimicrobial activity. The presence of the high flavonoid, phenolic, and tannin content in ethanolic extract of *A. absinthium* were also reported by Singh *et al.* (2012) in their study.

Steroids and saponins were absent in both extracts while as both the extracts were positive for phytosterols. Phytosterols like β-sitosterol, stigmasterol, campesterol and ergosterol are significantly present in *A. absinthium* as it was found through chromatography (Ivanescu *et al.*, 2013). Phytosterols are known for their anti-inflammatory and immunomodulatory activities along with their protection against the cardiovascular diseases (Berdiel *et al.*, 2009; Bouic, 2002)

Hexanic extracts of the *A. absinthium* were found to possess antipyretic effects (Khare, 2004), suppression of phytopathogens (Bisht *et al.*, 2020), strong fascioliscidal activity (Moreno *et al.*, 2012) and high prohibitory or strong antibacterial efficacy against *Staphylococcus aureus*, *Escherichia faecalis* and *Klebsiella pnumoniae* (Ahameethunisa and Hopper, 2010).

5. Conclusion

The genus *Artemesia* has aroused great interest among scientific community due to its wide range of pharmacological properties owing to its antioxidative, anti-inflammatory, immune boosting and anti-parasitic role. The biochemical estimation of the dried plant material depicts high concentration of proteins in it, as also depicted in both the extracts. Higher protein concentration can help the body by acting as a source of energy and also boosting the immune system, and thus help fighting various diseases. The high ash content in *A. absinthium* depicts that the plant is a good source of essential minerals like Ca and P. Both these minerals are essential part of many signaling pathways and enzymes, essential for both anabolic and catabolic reactions. The ethanolic and hexanic extracts of *A.*

absinthium show the presence of alkaloids, phytosterols and flavonoids which may be responsible for its anti-inflammatory, analgesic, antibacterial and antioxidative properties. Hence, the plant is pure and can be further used to study its properties in *in vitro* and *in vivo* experimentation trials.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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