Phytochemical screening and quantitative analysis of locally used medicinal plants of Western Himalayas

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Abstract
Plants have been well known for their medicinal uses for thousands of years and traditional medicines are still a major part of habitual treatments in different parts of the world. Medicinal plants are used since ancient times for treating different ailments and providing valuable drugs such as analgesics (morphine), antihypertensives (reserpine), cardiotonics (digoxin) and antimalarials (quinine and artemisinin). The discovery of medicinal plants helps in achieving the new targets in treating various diseases like cancer, malaria and neurological disorders. Herbs proved to be the only solutions to treat a large number of health-related problems. Most of the recent drugs are obtained in one or other way from plants. These medicinal plants contain a biologically active, naturally occurring chemical compounds known as phytochemicals which provide health benefits and protect the human cell from any type of damage. Phytochemical screenings of the medicinal plants have contributed to the discovery of the new drugs. In our present investigation an attempt has been made to screen out the bioactive compounds of three selected plants and further they had been subjected to quantitative analysis for estimation of different parameters.

Keywords: medicinal plant, phytochemicals, biological activity

1. Introduction
Since prehistoric times medicinal plants are used for healing and curing a number of diseases (Mustafa et al., 2017) [38]. These plants contain essential chemical compounds which are used for the preparation of drugs that are required in modern drug producing industries. During past 2-3 decades several studies have been carried out on the plants that are rich in secondary metabolites and have a potential of antimicrobial, anti-inflammatory, anti-tumor, antioxidant and other biological activities (Karuppusamy, 2007) [28]. These medicinal plants are used as a valuable source of ingredient which can be used in the drug development (Jaberian et al., 2013) [24]. On the other hand medicinal plants have a stupendous support for human societies and consumed by people across the world (Hasan, 2012) [22]. Currently more than 80% of the population uses the traditional medicine and medicinal plants producing essential oils for the primary health needs. The 20th century revolutionized the thinking of drug use from traditional crude extract to the receptor theory of drug action. The idea of specific interaction of a drug molecule with biological macromolecules such as proteins or nucleic acids led scientists to the conclusion that chemical compounds present in the plant extracts, are the factors required for the biological activity of the drug (Swargiary et al., 2011). Most commonly used plants for the treatment of diseases like anti-inflammatory, anticancer and antioxidant are Cinnamon, Aloe vera, Crocus sativus, Withania somnifera, Gingko biloba, Adhatoda vasica, Catharanthus roseus, Ficus bengalensis, Aegle marmelos etc. (Gupta et al., 2011) [16]. Medicinal plants are an effective source of Unani, Homeopathic, and modern medicine. Traditionally medicinal plants are used for cancer treatment due to their multiple chemical compounds for discovering new materials against cancer (Pandey et al., 2019) [46]. The products derived from the plants are one of the vital sources to combat serious diseases across the world mostly in the developing countries where traditional medicinal methods play a key role to cover the basic health requirements (Das et al., 2017) [12]. In India there is a long history of using medicinal plants to treat number of diseases. It has also been believed that using fruits/herbs have better potential to heal the suffering caused by cancer diseases than synthetic medicine (Sarkar et al., 2018) [54]. The use of herbs and medicinal plants as the first medicine is a universal phenomenon.
Herbal medicines are used by 75-80% of the population in the developing countries (Konduri et al., 2010) [32]. Plant origin drugs have established much attention of the world for their efficacy and known to be safe for the human use (Karale and Karale 2017) [27]. In some cases plants based drugs are used directly (crude drug) and their constituents are separated by various methods (Nayar and Sastry 2011) [42].

Phytochemicals are chemical compounds which are biologically active naturally occurring in plants, which provide health benefits for the humans and protect the cells from damage that may lead to cancer (Savithrama, 2011) [55]. Phytochemicals are having an overlapping mechanism of action in the body, including antioxidant effects, stimulation of the immune system, modulation of the enzyme actions, antiviral anti-bacterial effects (Ngoci et al., 2015) [43]. They have an important role in plant development, being part of various physiological processes like reproduction, symbiotic association, an interaction with other organisms (Forni et al., 2019) [14]. These phytochemicals provide defense to the plants from the environmental hazards such as pollution, stress, drought and pathogenic attack and provides the colour, flavour and aroma to the plants (Shahwany et al., 2014) [58]. Phytochemicals are present in different parts of the plant such as in roots, stems, leaves, fruits or seeds (Costa et al., 2018) [10]. Phytochemicals have been used for the prevention and treatment of diabetes, high blood pressure, muscular degeneration, gastric pain, and hay fever (Mathai et al., 2014) [36]. Depending upon the role in plant metabolism phytochemicals are classified into primary and secondary constituents. Primary constituents include the common sugar, amino acids, proteins and purine. Secondary constituents or metabolites are the remaining plant chemicals such as phenols, saponins, alkaloids, terpenes, flavonoids and glycosides (Hushmendy et al., 2009) [23].

The Indian Himalayan Region is a mega hot spot of biodiversity that includes about 1748 species of medicinal plants with various traditional and modern pharmaceutical uses, 675 species of wild edible plants, and 121 rare-endangered plants. It covers about 18% of India, which is more than 2,800 km long and 220 to 300 km wide, with altitudes from 200-8000 m (Myers, 2003). The flora includes about 8,000 species of angiosperm (40% endemic), 44 species of gymnosperm (16% endemic), 600 species of pteridophyte (25% endemic), 1737 species of bryophyte (33% endemic), 1,159 species of lichen (11% endemic) and 6,900 species of fungi (27% endemic) (Singh and Hajra 1996) [65]. Medicinal plants are used in the Ayurvedic, Unani and other traditional systems of medicine and in plant-based pharmaceutical industries. Estimates indicate that at least 90% of medicinal plant species are extracted from the wild plants (Samant, 2007) [52].

The introductions of plant derived drugs in modern medicine have been linked to the use of plant derived materials as a cure in the traditional system of medicine. These plants contain the important chemical compounds that are used for the preparation of plant-based drugs. Recently scientific research on plant materials of different wild and domesticated plants is going on for their potential medicinal and health enhancing properties (Kala et al., 2004) [29]. In our present study, we have selected three wild plants that are locally used for the medicinal purposes. Artemisia absinthium, Eleusine coracana and Urtica dioica are the locally used medicinal plants mostly grown in the Western region of Indian Himalayas. These plants are widely grown in Jammu and Kashmir, Uttarakhand and Himachal Pradesh. Artemisia absinthium and Urtica dioica are used extensively for the medicinal purposes like indigestion, gastric pain, and chronic inflammation. It has been reported that the essential oil of Artemisia absinthium contains health promoting compounds and essential oils (Kim et al., 2013) [30]. The stinging nettle leaves of Urtica dioica contains wide range of nutrients like vitamins, fats, minerals and all essential amino acids (Ryan, 2018) [50]. Eleusine coracana (Finger millet) is a rich source of phytochemicals, dietary fibres and several minerals that contributes to the wide range of medicinal properties like antioxidant, antimicrobial and anti-inflammatory (Siwela et al., 2010) [64].

2. Materials and Methods

2.1 Collection of plant material

Three medicinal plants (Artemisia absinthium, Urtica dioica and Eleusine coracana) are harvested and collected from district Anantnag, Kashmir (Latitude-33.73° N & Longitude-75.15°E with Elevation-1,601meters) and Pauri Garhwal, Uttarakhand (Latitude-29.8688° N and Longitude-78.8383° E with Elevation-3100 meters). The plants were collected during February to September.

[Fig 1: Images of study material a: Artemisia absinthium b: Urtica dioica c: Eleusine coracana]

2.2 Qualitative phytochemical screening

Phytochemical analysis of aqueous and ethanolic extract of different medicinal plant species collected from Western Himalayas was carried out qualitatively to detect the presence of alkaloids, saponins, carbohydrates, flavonoids, glycosides, proteins, amino acids, and phenols

a) Detection of alkaloids

The extracts obtained from the three selected medicinal plants was dissolved separately in dilute hydrochloric acid for the presence of alkaloids by using Mayers reagent (Potassium mercuric iodide) and Hager reagent (saturated picric acid solution). Formation of yellow color precipitate indicates the presence of alkaloids (Khandelwal et al., 2015) [29].

b) Detection of flavonoids

The detection of flavonoids was carried out with alkaline reagent test by dissolving the plant extracts in few drops of sodium hydroxide solution. Formation of reddish-yellow colour, which becomes colorless after the addition of dilute acid, indicates the presence of the flavonoids (Kokate et al., 2008) [31].

c) Detection of proteins
The detection of proteins was carried out by Xanthoproteic acid test by adding concentrated nitric acid solution to the plant extract. Formation of yellow colour indicates the presence of proteins (Rajesh et al., 2016) [47].

d) Detection of amino acids
The detection of amino acids was carried out by Ninhydrin test, by adding Ninhydrin reagent to the plant extract and boiled for few minutes. Formation of blue colour indicates the presence of amino acids (Nalini et al., 2018).

e) Detection of phenols
The detection of Phenols was carried out by using Ferric chloride test. Extracts of three individual plants were treated with few drops of ferric chloride solution. Formation of bluish-green color indicates the presence of phenols (Dahiru et al., 2006) [11].

2.3 Quantitative determination of phytochemicals
Phytochemical analysis of three selected medicinal plants collected from Western Himalayas was carried out quantitatively for the presence of protein, amino acid, carbohydrate, phenol, flavonoid, alkaloid, saponins, tannin and minerals.

a) Estimation of protein content
Protein estimation was done by using the standard method by Lowry et al. (1951). 0.1ml of the sample and standard were pipette out into a series of test tubes. Distilled water was added to make it 1 ml. A tube with 1ml of distilled water was put as blank. 5ml of 2% sodium carbonate in 0.1% sodium hydroxide was added to each tube. It was mixed well and was allowed to stand for 10 minutes. Then 0.5ml of folin reagent was added, mixed well and incubated at room temperature in dark for 30 minutes. Blue color was developed. The final readings were taken at 660nm on UV spectrophotometer.

b) Total free amino acids
The amount of total free amino acids in the samples was determined after Lee and Takahashi (1966) [34] by placing glycine as standard. 1 gram of shoots was grounded in 5 mL of 80% ethanol. The homogenate was centrifuged at 10,000 R.P.M for 15 minutes. Centrifugation was repeated till we get the supernatant for estimation. To 0.1 mL of amino acid extract, 5 mL of Ninhydrin reagent was added. The mixture was vortexed vigorously and then placed in hot water bath for 12 minutes. The mixture was cooled under running tap water to room temperature and a blue purple colour developed in the solution. The optical density was measured at 570 nm on UV spectrophotometer against blank which was prepared by adding 0.1 ml of 80% ethanol instead of extract.

c) Total phenols
The total phenolic content in the samples will be determined by using the Folin-Ciocalteu method (Singleton and Rossi, 1965) [63]. 1 g of sample was extracted with 5 mL of 80% ethanol and put in water bath for 30 min at 30 °C. Centrifugation was done for 10 minutes at 4500 R.P.M. Supernatants were put in a test tube. 0.1 mL of supernatant was transferred to a test tube. 5 mL of folin-ciocalteu reagent was added to above. After 5 to 8 minutes, 3.5 mL of sodium carbonate dissolved in water; was added and vortexed. The tubes were incubated for 1 hour at 40 °C in a digital water bath. The absorbance was measured at 765 nm on a UV spectrophotometer.

d) Total flavonoid content
The total flavonoid content was measured spectrophotometrically by following the method of Chang et al., 2002 [8]. In this method, flavonoids in the plant extract reacts with aluminium chloride and potassium acetate present in the reagent giving coloured product and can be measured at 415 nm using UV spectrophotometer. The method in brief; about one ml of 2% aluminium chloride in methanol was mixed with one ml of leaf extract in the concentration of one mg per ml. The mixture was incubated at room temperature for an hour and absorbance was measured at 415 nm on UV spectrophotometer.

e) Alkaloids
Quantification of alkaloids was performed by using the method of Harborn, 1984 [20]. About 5 mg of plant extract was mixed with 20 ml of 10% acetic acid in methanol, covered the beaker and allowed to stand at room temperature for 4 hours. The mixture was concentrated to one-third of its volume by addition of ammonium hydroxide solution drop by drop in the mixture until complete precipitation occurs and filtered. The absorbance was taken at 415 nm on UV spectrophotometer.

3. Results and Discussion
Nature has blessed the humanity with countless favours and herbal medicines are one of them. In developed and developing countries, herbal medicines are in great demand due to their safe status as they have no apparent side effects like synthetic drugs (Harshberger 1986) [21]. From the past few years herbal medicines have regained a special status due to the immense faith of the people in using these medicines for curing of diseases without any side effect as compared to the allopathic medicines. Medicinal plants are used by the 70-80% of the population for major health care. (Lone et al., 2012) [35] Herbal medicines are currently in demand and their popularity is increasing day by day (Shivhare et al., 2011) [61]. In the 21st century, the most significant challenges faced by the developing countries are the emerging and reemerging of diseases which leads to the 50% of the deaths (Settu and Arunachalam 2019) [57]. India has the special status in terms of floristic diversity due to which most of Indian population uses traditional medicines for the treatment of diseases like cancer, diabetes, alzheimer's disease (Bansode et al., 2015) [5]. Plants have the ability to synthesize a wide range of bioactive compound that performs important biological functions and provides defence from the attack of herbivores, insects, fungi and mammals (Rej et al., 2014) [69]. These secondary metabolites are actually the chemical compounds derived from plants and are widely used in the traditional herbal medicines. These are also known as plant secondary metabolites or bioactive compounds (Bansode et al., 2015) [5]. These bioactive compounds are synthesized from almost all parts of plant like bark, leaves, stem, root, flower, fruits and seeds. Secondary metabolites are particularly used in the field of agriculture, help in the synthesis of drugs and provide flavour and color to the food (Shankar, 2015) [60]. Phytochemicals have the potential to prevent and cure
number of diseases like diabetes, high blood pressure, muscular degeneration, gastric pain, and hay fever (Mathai et al., 2014) [36].

3.1 Qualitative analysis of phytochemicals
Qualitative analysis was done to determine the chemical constituents present in leaf extracts of Artemisia absinthium, Urtica dioica and seeds of Eleusine coracana and the results are framed in table 1. It was investigated that alkaloids and flavonoids are present in all the selected flora in both aqueous and ethanolic extract. Similar results were obtained in Punica granatum after phytochemical screening (Wadoo et al., 2013) [68]. It was also investigated that amino acids and phenols were present in Eleusine coracana and Urtica dioica in the ethanolic extract. However the amino acids and phenols were absent in both plant species in the aqueous extract. Similar results were obtained in aqueous extract of Psidium guajava (Ghufran et al., 2013) [15].

Table 1: Phytochemical analysis of aqueous and ethanolic extracts of selected medicinal plants

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Phytochemical</th>
<th>Artemisia absinthium (Leaves)</th>
<th>Urtica dioica (Leaves)</th>
<th>Eleusine coracana (Seeds)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Aqueous</td>
<td>Ethanol</td>
<td>Aqueous</td>
</tr>
<tr>
<td>01.</td>
<td>Alkaloid</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>02.</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>03.</td>
<td>Proteins</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>04.</td>
<td>Amino acids</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>05.</td>
<td>Phenols</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

3.2 Quantitative analysis of phytochemicals
The three selected medicinal plants were subjected to quantitative analysis by standard methods. All the extracts which were prepared by using various solvents from selected parts of the three different medicinal plants were analyzed for total free amino acids, total phenols, proteins, flavonoids and alkaloids. The quantitative composition of different secondary metabolites in Artemisia absinthium, Urtica dioica, and Eleusine coracana are shown in table 2 and fig. 2. Total free amino acid content was observed in highest in Urtica dioica as compared to the two other selected medicinal plants, while as Eleusine coracana was found to contain the least amount of amino acids. Due to the presence of higher content of total free amino acids in the leaves of stinging nettle, it can be used for defence against microbes, acts as an antioxidant, provides preventive role against infectious and neurodegenerative diseases (Kumar et al., 2016) [33]. Total protein content was found to be higher in the seeds of Eleusine coracana than other two selected flora. Due to the presence of higher range of protein in finger millet, it provides wide range of health benefits like building block of bones, muscles, cartilage, skin and blood (Sood et al., 2016) [65]. Alkaloids are found to be highest in Artemisia absinthium followed by Eleusine coracana and Urtica dioica). Due to the presence of higher content of alkaloids in Artemisia absinthium it possesses wide range of medicinal properties like anticancer, antioxidant and anti-inflammatory. Higher quantity of flavonoids was detected in Urtica dioica, while Eleusine coracana was found to have the least flavonoid content. Due to the higher flavonoid content in the leaves of Urtica dioica, it possesses various medicinal properties like potential of anti-inflammatory, anti-microbial, anti-carcinogenic, anti-HIV and neuroprotective properties (Asif et al., 2013) [2].

Table 2: Quantitative Estimation of important secondary metabolites in three selected medicinal plants

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Medicinal plant</th>
<th>Total Free Amino acids</th>
<th>Total Phenolic content</th>
<th>Total Protein Content</th>
<th>Flavonoids</th>
<th>Alkaloids</th>
</tr>
</thead>
<tbody>
<tr>
<td>01.</td>
<td>Artemisia absinthium</td>
<td>0.36±0.003</td>
<td>0.23±0.001</td>
<td>0.17±0.002</td>
<td>0.36±0.003</td>
<td>0.41±0.001</td>
</tr>
<tr>
<td>02.</td>
<td>Urtica dioica</td>
<td>0.41±0.002</td>
<td>0.26±0.001</td>
<td>0.22±0.003</td>
<td>0.41±0.002</td>
<td>0.24±0.003</td>
</tr>
<tr>
<td>03.</td>
<td>Eleusine coracana</td>
<td>0.25±0.004</td>
<td>0.27±0.015</td>
<td>0.30±0.002</td>
<td>0.26±0.004</td>
<td>0.32±0.002</td>
</tr>
</tbody>
</table>

Fig 2: Comparison of phytochemicals of three selected medicinal plants: 1: Artemisia absinthium 2: Urtica dioica 3: Eleusine coracana
Conclusion
Medicinal plants play a vital role in preventing various diseases. The diuretic, anti-inflammatory, anti-analgesic, anti-cancer, anti-viral, anti-malarial, anti-bacterial and antifungal activities of the medicinal plants are due to the presence of the above-mentioned secondary metabolites. The three selected medicinal plants are the source of the important secondary metabolites i.e., alkaloids, flavonoids, amino acids, phenols and proteins. The phytochemical analysis i.e. qualitative and quantitative analysis of the three selected medicinal plants are also important and have commercial interest in both research institutes and pharmaceuticals companies for the manufacturing of the new drugs. Reported aspects which are known to affect the production of secondary metabolites in plants are physiological differences, climatic changes, topographical features, and hereditary elements, amount of plant material, space and labor needs.

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