International Journal of Applied Research 2021; 7(5): 288-294

ISSN Print: 2394-7500
ISSN Online: 2394-5869
Impact Factor: 8.4
IJAR 2021; 7(5): 288-294
www.allresearchjournal.com
Received: 01-03-2021
Accepted: 03-04-2021

Aiman Aziz
Research Scholar, Department of Botany, RIMT University, Mandi Gobindgarh, Punjab, India

Dr. Manju
Assistant Professor, Department of Botany, RIMT University, Mandi Gobindgarh, Punjab, India

Corresponding Author:
Aiman Aziz
Research Scholar, Department of Botany, RIMT University, Mandi Gobindgarh, Punjab, India

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

Aiman Aziz and Dr. Manju

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 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 defence 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, mascular 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 biological diversity 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) [62]. 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) [26].

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-33.73º N & Longitude-75.15ºE with Elevation-1,601meters) and Pauri Garhwal, Uttarakhand (Lattitude-.29.8688º N and Longitude-78.8383º 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 Hagers 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) [49]. 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, mascular 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 (Wadood *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

S. No.	Phytochemical	*Artemisia absinthium* (Leaves)		*Urtica dioica* (Leaves)		*Eleusine coracana* (Seeds)	
		Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol
01.	Alkaloid	+	+	+	+	+	+
02.	Flavonoids	+	+	+	_	+	_
03.	Proteins	+	+	+	+	+	+
04.	Amino acids	+	+	+	+	_	+
05.	Phenols	+	+	-	+	-	+

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 the treatment of gastrointestinal problems, and genetical disease called McArdle disease (Joshi *et al*., 2014) [25]. Total phenolic content was found to be higher in seeds of *Eleusine coracana*, followed by leaves of *Urtica dioica* and *Artemisia absinthium*. Presence of higher content of phenols in the seeds of finger millet, 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

S. No.	Medicinal plant	Total Free Amino acids	Total Phenolic content	Total Protein Content	Flavonoids	Alkaloids
01.	*Artemisia absinthium*	0.364± 0.003	0.234±0.001	0.178±0.002	0.364±0.003	0.41±0.001
02.	*Urtica dioica*	0.414± 0.002	0.261±0.001	0.224±0.003	0.414±0.002	0.245±0.003
03.	*Eleusine coracana*	0.256± 0.004	0.279±0.015	0.303±0.0028	0.256± 0.004	0.324±0.002

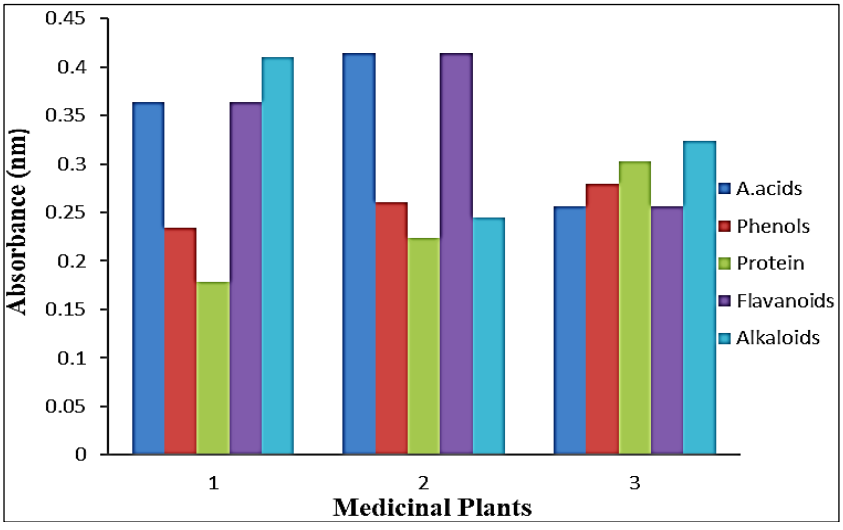

Fig 2: Comparison of phytochemicals of three selected medicinal plants: 1: *Artemisia absinthium* 2: *Urtica dioica* 3: *Eleusine coracana*

Conclusion

Medicinal plants play a vital role in preventing various diseases. The antidiuretic, anti-inflammatory, anti-analgesic, anti- cancer, anti-viral, anti-malarial, anti-bacterial and anti-fungal 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 effect 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.

Acknowledgement

The authors express deep sense of gratitude to the management of RIMT University for all the support, assistance and constant encouragement to carry out this work.

References

1. Ashok PK, Upadhyaya K. Preliminary Phytochemical Screening and Physico-Chemical Parameters of *Artemisia absinthium* and *Artemisia annua*. Journal of Pharmacognosy and Phytochemistry 2013;1:229-235.
2. Asif M, Searcvy C, Zutshi A, Fischer O. An integrated management systems approach to cooperate social responsibility. Journal of Cleaner Production 2013;56:7-17.
3. Baker H, Frank O, De Angelis B, Feingold S. Plasma tocopherol in man at various time intervals after ingesting free or acetylated tocopherol. Nutrition Report International 1980;21:531-536.
4. Bal LM, Karb A, Satya S, Naik SN. Kinetics of colour change of bamboo shoot slices during microwave drying. International Journal of Food Science and Technology 2011;46(4):827-833.
5. Bansode T, Salalkar BK. Phytochemical analysis of some selected Indian medicinal plants. International Journal of Pharmacy and Biosciences 2015;6(1):550-556.
6. Bora KS, Sharma A. Evaluation of antioxidant and free-radical scavenging potential of *Artemisia absinthium*. Pharmaceutical Biology 2011;49:1216-1223.
7. Chan E, Tan M, Xin J, Sudarsanam S, Johnson DE. Interactions between traditional Chinese medicines and Western therapeutics. Current Opinion in Drug Discovery and Development 2010;13:50-65.
8. Chang C, Yang M. Estimation of total flavonoids content in propolis by two complementary colorimetric methods. Food Drug Analysis. 2002; 10:178-182.
9. Chesher GB. Identification of 5-hydroxytryptamine in the stinging nettle (*Urtica dioica*). British Journal of Pharmacology 1956;11:186.
10. Costa O, Raaijmakers J, Kuramae E. Microbial Extracellular Polymeric Substances: Ecological Function and Impact on Soil Aggregation. Frontiers in Microbiology 2018;9:1636-1642.
11. Dahiru D, Onubiyi JA, Umar HA. Phytochemical screening and antiulcerogenic effect of *Moringa oleifera* aqueous extract. African Journal of Traditional, Complementary and Alternative medicines 2006;3(3):70-75.
12. Das K, Krishna P, Sarkar A, Iiangovan S, Sen S. A review on pharmacological properties of Solanum tuberosum. Research Journal of Pharmacy and Technology 2017;10(5):1517-1522.
13. Ferreira VLP, Yotsuyanagi K, Carvalho CRL. Elimination of cyanogenic compounds from bamboo shoots *Dendrocalamus giganteus* Munro. Tropical Science 1995;35:242-346.
14. Forni C, Facchiano F, Manuela B, Pieretti S, Antonio F, Norelli S *et al*. Beneficial Role of Phytochemicals on Oxidative Stress and Age-Related Diseases. BioMed Research International 2019;10(6):652-664.
15. Ghufran M, Jamal S, Naeem M. Phytochemical Analysis of Medicinal Plants Occurring in Local Area of Mardan. Biochemistry and Analytical Biochemistry 2013;2(4):1-4.
16. Gupta N, Jain U, Jain A, Lovanshi G, Mathan N, Tiwari V. Review of some important medicinal plants possesses anti-inflammatory activity. Research journal of Pharmacy and Technology. 2011; 4(10):1506-1512.
17. Gupta VK, Kumria R, Garg M, Gupta M. Recent updates on free radicals scavenging flavonoids: An overview. Asian Journal of Plant Sciences 2010;9:108-117.
18. Haque MR, Bradbury JH. Total cyanide determination of plants and foods using the picrate and acid hydrolysis methods. Food Chemistry 2002;77(1):107-114.
19. Harbers LH. Ash analysis In: Introduction to Chemical Analysis of Foods (ed. Nielsen SS). Jones and Bertlett Publishers, Boston, London. 1994; 3:113-121.
20. Harborne JB. Phytochemical Methods. Analytical Biochemistry 1984;21:100-101.
21. Harshberger N. People and plants in Ancient Eastern North America. Botanical Gazette 1986;21(3):146-154.
22. Hasan B. Medicinal Plants (Importance and Uses). Pharmaceutica Analytica Acta 2012;3:10.
23. Hushmendy S, Jayakumar L, Hahn A, Bhiowala D. Select phytochemicals suppress human T-lymphocytes and mouse splenocytes suggesting their use in autoimmunity and transplantation. Nutrition Research 2009;29(8):568-578.
24. Jaberian H, Piri K, Nazari J. Phytochemical composition and *in vitro* antimicrobial and antioxidant activities of some medicinal plants. Food Chemistry 2013;136(1):237-244.
25. Joshi B, Patel H. *In vitro* Phytochemical Analysis and Anti-microbial Activity of crude extract of Bacopamonniera. International Journal of Pharmaceutical and Medical Sciences. 2014;1(2):128-131.
26. Kala CP, Farooquee N, Dhar U. Prioritization of medicinal plants on the basis of available, existing practices and use value status in Uttaranchal, India. Biodiversity and Conservation. 2004; 13(2):453-469.
27. Karale PA, Karale MA. A Review on Phytochemistry and Pharmacological Properties of Milk weed family herbs (Asclepiasdaceae). Asian Journal of Pharmaceutical and Clinical Research. 2017; 10(11):27-34.

28. Karuppusamy S. A review on trends in production of secondary metabolites from higher plants by *in vitro* tissue, organ and cell cultures. Journal of Medicinal Plant Research. 2007; 3(13):1222-1239.
29. Khandelwal N, Kross EK, Engelberg RA, Coe NB, Long AC *et al*. Estimating the effect of palliative care interventions and advance care planning on ICU utilization: a systematic review. Critical care medicine. 2015; 43(5):11-02.
30. Kim Y, Lee J, Kim S. Cultivation characteristics and flavonoids contents of worm wood (*Artemisia montana*). 2013; 2(4):117-122.
31. Kokate A, Li X, Jasti B. HPLC detection of marker compounds during buccal permeation enhancement studies. Journal of Pharmaceutical and biomedical analysis 2008;47(1):190-194.
32. Konduri M, Uppuluri K, Chintha R, Mulla S, Peruri R. *In vitro* Antimicrobial Activity of Four Indigenous Medicinal Plants Belonging to Bapatla, A.P. Research journal of Pharmacy and Technology 2010;3(2):461-465.
33. Kumar A, Metwal M, Kaur S, Gupta K, Yadav R. Nutraceutical value of finger millet (*Eleusine coracana*) and their improvement using Omics Approaches. Front Plant Sciences 2016;7:934.
34. Lee Y, Takashi T. An improved colorometric determination of amino acids with the use of ninhydrin. Analytical Biochemistry 1966;14:71-77.
35. Lone FA, Aziz S, Malla FA. Study of some medicinal plants of the Shopian district, Kashmir (India) with emphasis on their traditional use by Gujjar and Bakerwal tribes. Asian Journal of Pharmaceutical and Clinical Research 2012;5:94-98.
36. Mathai K. Nutrition in the Adult Years, in Krauses Food Nutrition and diet therapy. International Journal of Current Research in Chemistry and Pharmaceutical Sciences 2014;271:274-275.
37. Molyneux RJ, Lee ST, Gardner DR, Panter KE, James LF. Phytochemicals: the good, the bad and the ugly? Phytochemistry 2007;68(22-24):2973-2985.
38. Mustafa G, Arif R, Atta A, Sharif S, Jamil A. Bioactive Compounds from Medicinal Plants and their importance in drug discovery in Pakistan. Matrix Science Pharma 2017;1(1):17-26.
39. Myers N. Biodiversity hotspots Revisited. Journal of Bioscience 2003;53:916-917.
40. Nath AJ, Das G, Das AK. Above ground standing biomass and carbon storage in village bamboos in North East India. Biomass & Bioenergy 2009;33:1188-1196.
41. National Mission on Bamboo Technology and Trade Development. Planning Commission, Government of India, New Delhi 2003.
42. Nayar MP, Sastry AR. Smilax wightii A. DC. In: Red Data Book of Indian Plants. Botanical Survey of India 2011;1:352.
43. Ngoci S, Matasyohb J, Mwanikic C, Maina M. A Review of some Phytochemicals commonly found in Medicinal Plants. International Journal of Medicinal Plants 2015;105:135-140.
44. Nirmala C, Bisht MS, Sheena H. Nutritional Properties of Bamboo Shoots: Potential and Prospects for Utilization as a Health Food. Comprehensive Reviews Food Science Food Safety. 2011; 10:153-165.
45. Pandey AK, Ojha V. Precooking processing of bamboo shoots for removal of antinutrients. Journal f Food Science and Technology 2011. DOI: 10.1007/s13197-011-0463-4.
46. Pandey K, Shrestha B, Khanal S, Adhikari D, Kunwar C. Advances in Maize-Based Bioethanol Production and Its Prospects in Nepal. International Journal of Graduate Research and Review 2019;5(2):122-130.
47. Rajesh P, Latha S, Selvamani P, Kanan R. Phytochemical analysis, *in vitro* antioxidant potential and GCMS of *Dicranopteris linearis*. Asian Journal of Pharmacological Clinical Research 2016;9(2):1-6.
48. Rao US, Mohd K, Halim S, Khamis M. Screening of phytochemicals and comperative antioxidant activity of leaf and fruit of Malaysian Mengkud using aqueous and organic solvent extract. Research journal of Pharmacy and Technology 2013;6(9):1064-1072.
49. Rej S, Dutta M, Jamal S, Das S, Chatterjee S. Study of Phytochemical Constituents and Antibacterial Activity of Clerodendrum infortunatum. Asian Journal of Research in Pharmaceutical Sciences 2014;4(4):187-195.
50. Ryan C, Roschek B, Mcmicheal M, Alberte RS. Neetle Extract (*Urtica diocia*). Affects Key Receptors and Enzymes Associated with Allergic Rhinitis. Phytotherapy Research 2018;23(7):920-926.
51. Samant S, Shreekar P, Singh M, Lal M, Singh A, Sharma A *et al*. Medicinal plants in Himachal Pradesh, north western Himalaya, India. International Journal of Biodiversity Science and Management 2010;3(4):234-251.
52. Samant SS, Butola SJ, Sharma A. Assessment of diversity, distribution, conservation status and preparation of management plan for medicinal plants in the catchment area of Parbati Hydroelectric Project Stage in North Western Himalayas 2007;4(1):34-56.
53. Saranraj P, Sivasakthi S, Deepa M. Phytochemistry of pharmacologically important medicinal plants. International Journal of current research in chemistry and pharmaceutical sciences 2016;3(11):56-66.
54. Sarkar N, Saha B, Ghosal S. *Ropidia curculioides*: Secondary metabolites and derivatives with antimycobacterial and leishmanicidal activity. Pharmacognosy Magazine 2018;14(59):535-538.
55. Savithrama N, Rao M, Ankanna S. Screening of Medicinal Plants for Secondary Metabolites. Middle East Journal of Scientific Research. 2011; 8(3):643-647.
56. Scurlock JMO, Dayton DC, Hames B. Bamboo: an overlooked biomass resource? Biomass and Bioenergy 2000;19:229-44.
57. Settu S, Arunachalam S. Comparison of Phytochemical analysis and *In vitro* Pharmacological Activities of most commonly available medicinal plants belonging to the Cucurbitaceae family. Research Journal of Pharmacy and Technology 2019;12(4):1541-1546.
58. Shahwany A, Shamma U. Effect of Phytochemicals, Mineral and Biofertizer on growth and yield of *Triticum aestivum*. 2014; 55(4):1484-1495.
59. Shakir Ullah, Gul J, Farzana G, Khan S, Husna H, Jan S *et al*. Phytochemistry and antibacterial activities of some selected plants of war affected area of bajaur agency, Pakistan. Journal of Pharmacognosy and Phytochemistry 2018;7(3):415-422.

60. Shankar LL. Sequential Extraction of Plant Metabolites. International Journal of Current Microbiology Appliation Sciences 2015;4(2):33-38.
61. Shivhare Y, Singh P, Singh S, Bharti P, Tiwari R. Ethanomedicinal Plants for the Prevention and Treatment of Gonorrhoea. Research journal of Pharmacy and Technology 2011;4(2):182-183.
62. Singh DK, Hajra PK. Changing perspectives of Biodiversity status in the Himalaya. Floristic diversity 1996, 23-38.
63. Singleton L, Rossi A. Colorimetry of total phenolics with phosphomolybedic-phosphotungstic acid reagents. Journal of Enology and Viticulture 1965;16:144-158.
64. Siwela M. Occurence and Location of Tannins in Finger millet grain and antioxidant activity of different grain types 2010;84(2):169-174.
65. Sood S, Kumar A, Babu B, Pandey D, Kant L. Advances in Finger millet Genomics-An important Nutri-Cereal of Future. Frontiers in Plant Sciences, Plant Genetics and Genomics 2016;3:1-11.
66. Swargiary A. Recent trends in traditionally used medicinal plants and drug discovery. Phytochemistry. Asian Journal of Pharmacy and Pharmacology 2017;3(4):1-11.
67. Tamang B, Tamang JP. Lactic acid bacteria isolated from indigenous fermented bamboo products of Arunachal Pradesh in India and their functionality. Food Biotechnology 2009;23:133-147.
68. Wadood A, Ghufran M, Jamal S, Naeem M. Phytochemical Analysis of Medicinal Plants Occurring in Local Area of Mardan. Biochemistry and Analytical Biochemistry 2013;2(4):1-4.
69. Waterborg JH. The Lowry Method for Protein Quantitation. Methods in Molecular Biology 1984;1:1-3.
70. Yuming Y, Kanglin W, Shengji P, Jiming H. Bamboo Diversity and Traditional uses in Yunnan, China. Mountain research and development 2004;24:2.
71. Zhang J, Ji R, Hu Y, Chen J, Ye X. Effect of three cooking methods on nutrient components and antioxidant capacities of bamboo shoot (*Phyllostachys praecox* C.D. Chu et C.S. Chao). Journal of Zhejiang University. Science. B 2011;12(9):752-759.