PHYTOCHEMICAL ANALYSIS OF NUTRACEUTICAL PLANTS SELECTED FROM HILLY AREA OF PAKISTAN

Sanam Zarif Satti[1], Tanvir Ahmad Quereshi[2], Mehwish Zarif[3] and Iftikhar Ahmad[4]

ABSTRACT

Five wild medicinal plants viz*: Bacopa monnieri, Bauhinia variegata, Berberis aristata, Caltha alba, Cordia oblique* were collected from different areas of Abbotabad, Murree, Swat and Kashmir in the month of May-June analyzed for their nutraceutical potential. Results of phytochemical screening reveals that among all the plant species promising total phenolics total flavonoids and antioxidant activity was observed. Highest value of total phenolic content was observed for *B.aristata* (80.25 mg GAE g^{-1}) while lowest values was recorded for *C.obliqua* (10.25 mg GAE g^{-1}), highest value of flavonoid content was recorded as (38.5 mg QE g^{-1}) and comparatively highest value of antioxidant activity was recorded for *B.aristata* (57.80%) It was concluded from the present results that antioxidant of the plant extract might be due to the presence of total phenols and total flavonoids content.

INTRODUCTION

Plants are the fundamental module of selected recipes of folk medicines in remote areas of Pakistan. They are the key reserves for diets, medicines, pharmaceutical intermediates, food additives and biological entities for various synthetic drugs (Ncube *et al.,* 2008). According to WHO about 80% of world population used natural resources for their primary health care needs. These plants are found as weeds across Pakistan and research literatures reveal that it is the key source of diversified bioactive molecules. Likewise, in other parts of Asia the population particularly the rural people of India, Iran, Afghanistan and China etc. mostly depend on these wild plant. The research conducted in the last few decades reveal that the most common drugs are obtained from plants or other natural resources (Sukanya *et al.,* 2009).

Each medicinal plant species has its own nutrients composition besides having pharmacologically important phytochemicals. These nutrients are essential for the physiological functions of human body. Medicinal plants come into preparation of various drugs singly or in combination or used as the principal source of raw materials for the other medicines (Mohanta *et al.,* 2003).

1 Biochemist, Pakistan Forest Institute, Peshawar
2 Forest Chemist/DBSRD, Pakistan Forest Institute, Peshawar
3 M. Phil Scholar, Quaid-e-Azam University, Islamabad
4 Ph.D. Scholar, University of Agriculture, Peshawar

Berberis aristata belonging to family *Berberidaceae* is native to mountainous parts of North India and Nepal. In Pakistan many species of *Berberis* are found in hilly areas like Chitral, Gilgit, Kurram, Swat, Murree and Ziarat.

Ethnobotanical studies indicate that the decoction of *B. aristata* leaves is commonly used to treat skin diseases, menorrhagia, diarrhea, cholera, jaundice, eye and ear infections, as well as urinary tract infections. The decoction of the root is used as a wash for infected wounds and ulcers, and is said to help healing and promote cicatrisation. *B. aristata* extracts have also been reported to cure hepatotoxicity (Khory and Kartak, 1985; Kirtikar and Basu, 1995).

Bacopa monnieri belongs to the family *Scrophulariaceae*, is a creeping, glabrous, succulent herb grows in marshy areas throughout the country. It has been traditionally used to treat anxiety, anger, nerve pain, insomnia, learning problems and concentration difficulties. It is used as a laxative and curative for ulcers, inflammation, anemia, scabies, leucoderma, epilepsy and asthma. As well as possess various biological activities such as anticancer and cytotoxic activity, antidepressant activity.

Bauhinia variegata belonging to family *Leguminosae* is locally known as Kachanar. The various parts of the plants viz., leaves, flower buds, flower, stem, stem bark, seeds and roots are used in fever, as tonic, astringent, diarrhoea, dysentery, hemorrhoids, piles, laxative, in skin diseases, wound healing, and as carminative. It was native to Southeast Asia and grows in tropical and subtropical climate. It is also distributed in most tropical countries, including Africa, Burma, China and South America. *B. variegata* consist of about 300 species. It is medium sized, deciduous tree which remain leafless in the month of January-April and in that period flowering of the tree starts. (Arvind *et al*., 2012).

Caltha alba belonging to family *Ranunculaceae* is widely distributed in wet lands in temperate regions of the Northern Hemisphere. In Pakistan the plant is found in Kashmir and other surrounding areas. Stem-leaves are smaller, irregularly sharply toothed, stalkless or with a short stalk. The plant is used as an antispasmodic and sedative and is a rich source of phenols and alkaloids (Hazrat *et al*., 2011).

Cordia obliqua locally known as Lasora belongs to family *Boraginaceae*. It is a deciduous tree with medium height and found throughout the mid Himalayas up to an elevation of 1470 meters. It is also found in other parts of the world like Tropical Australia and Philippines. The

fruit is very important medicinal source and is sweet in taste, slightly cooling, anthelmintic, purgative, diuretic, expectorant, and useful in diseases of the chest, urethra, dry cough, biliousness and chronic fever. Seeds are utilized as an anti-inflammatory agent

MATERIAL AND METHODS

Sampling of plant materials

Five wild medicinal plants samples, leaves of *Bacopa monnieri, flowers of Bauhinia variegata, roots of Berberis aristata, leaves of Caltha alba, fruit of Cordia obliqua* were collected from different areas of Abbotabad, Murree, Swat and Kashmir in the month of May-June in the year 2014 to analyze their nutraceutical potential. All the plants were identified by eminent Taxonomist and were deposited at the Herbarium of Botany Department at the University of Peshawar. The fresh parts of the selected plants species were washed to remove debris, dust and other adhering materials. The plant samples were kept in shade for several days till complete dryness was achieved. Afterward the plant parts were crushed, grind and chopped with the help of electrical grinder. Clean plastic bags were properly labeled to pack the powder samples and stored at low temperature for further investigation.

Extraction of plant sample

The air-dried plants were exhaustively extracted with methanol using cold maceration techniques. About 1.5 kg of powder samples were dissolved in approximately 2000 ml of methanol in separating funnel for several days and then filtered. The respective methanol extracts of each plant species was concentrated under reduced pressure at 40°C in the rotary evaporator. The crude methanol extract were kept in vials and stored in refrigerator at 4°C until analyzed. (AOAC, 2012).

Determination of total phenolic content

The total phenolic content in the crude methanolic extract was determined according to a well-cited protocol (Singleton and Rossi, 1965). A known amount of an extract was dissolved in 5 mL of methanol from which 40 µL was taken and dissolved in 3.16 mL of distilled water. To this 200 µL of Folin-Ciocalteu reagent was mixed and, after an interval of 8 min, 20% of 600 µL sodium carbonate solution was added. The mixture was incubated at 40°C for 30 min and absorbance was measured at 765 nm on UV/Visible spectrophotometer. The standard calibration curve was prepared with Gallic acid standard solution (25 to 250 mg/L) and the results were expressed as

mg of Gallic Acid Equivalent (GAE) per g of dried mass of plant extract.

Determination of total flavonoid content

The total flavonoid contents in the samples were determined following the method reported by Park *et al.* (2008). The crude methanolic extract was dissolved in 5 mL of methanol, from which 300 µL were transferred into 3.4 mL of 30% aqueous methanol. To this mixture, 150 µL each of 0.5M $NaNO_2$ and 0.3 M $AlCl_3.6H_2O$ were added and mixed thoroughly. After the interval of 5 min, 1 mL of 1 M NaOH was also added. The absorbance was observed immediately at 506 nm against a blank on UV visible spectrophotometer. Quercetin was used as standard (25 to 250 mg/L) and the results were expressed as mg Quercetin Equivalent (QE) per g of dried mass of the extract.

Determination of antioxidant activity

Antioxidant activity was determined using the 2, 2-diphenyl-1-pycrihydrazyl (DPPH) radical scavenging assay reported by the standard procedure. (AOAC, 2012) In the procedure it is advised that a known amount of plant sample was extracted with known amount of methanol for 3 to 4 days. The excess solvent was removed by using rotary vacuum evaporator at 50°C under reduce pressure. Added 0.394 g DPPH in 1000 ml distilled water to prepare 0.001 molar solution of DPPH. Afterwards, 500 ppm stock solution of each plant sample was prepared. About 25 mg of each plant fraction was dissolved in 50 ml methanol. Mix 5 ml of stock solution with 25 ml methanol to make 100 ppm working solution. Afterward, added 3 ml of 0.001 molar DPPH solutions in one minute with 100 ppm solutions of sample and 1 ml of acetate buffer (pH 6.5). The solution was vigorously shake and allowed to stand for 30 minutes. For control solution, add 3 ml DPPH with l ml acetate buffer solution. The % radical scavenging activity (RSA) was determined using the following formula.

$$RSA\,(\%) = \frac{Control\,Absorbance - Sample\,Absorbance}{Control\,Absorbance} \times 100$$

Statistical Analysis

All the experimental data obtained were subjected to Analysis of Variance (ANOV) by Completely Randomized Design (CRD) using Statistics 8.1 computer software. Each value is the mean of three replications (n=3r) along with Standard Error (SE) of mean. The similar means were separated by Least Significance Difference (LSD) test at P≤0.05.

RESULTS AND DISCUSSION

The study was conducted to determine the nutraceutical potential of selected wild plants. Under investigation plants were analyzed for proximate composition, phytochemical analysis. The results obtained are described below in detail:

Phytochemical analysis

The results depicted in Table 1 divulged that among all the plant species highest phenolic content was recorded for *B.aristata* (80.25 mg GAEg^{-1}) whereas lowest value was observed for *C. obliqua* (10.25 mg GAE g^{-1}). In case of flavonoid moiety promising amount was found for *B.aristata* followed by *B.monnerie* (61.33 mg QE g^{-1}).In case of antioxidant activity highest value was found for *B.varigata* (74.5 %) while lowest value was observed for *B.aristata* (57.80). Plants presents to have a sufficient amount of free radical scavenger in the form of secondary metabolites like phenol and flavonoids. They are present in each and every part of the plants. Flavonoids is an important plant secondary metabolite exhibit different biological property including anticancer, anti-allergic, antimicrobial and anti-inflammatory. They are also known as nature's biological response modifier because of their inherent ability to modify the body's reaction to allergies and virus. The phenolic compounds and antioxidant activity have a considerable nutrition value in providing flavor, taste, color and aroma. The results of present study also determined various concentration of total phenols, total flavonoid and antioxidants activity in the examined plant species.

Table 1. Total phenolics and total flavonoids content of methanol extract of different plant species

Plant species	Total Phenolic Content (mg GAE g^{-1})	Total Flavonoids Content (mg QE g^{-1})	Antioxidant activity (%)
B. aristata	80.25	38.50	57.80
B. monneria	42.75	28.17	66.31
B. varigata	35.25	24.66	74.5
C. alba	20.25	18.00	68.8
C. obliqua	10.25	11.33	71.3
SE of Mean	3.94	2.66	1.18
LSD ($p<0.05$)	Sig	Sig	Sig

SE = Standard Error; LSD = Least Significance Difference; Sig = Significant

The data showed that plants have the ability to eradicate free radicals which drastically effect the human body by interference and disrupting the physiological functions of the body.

CONCLUSIONS

From the present study it was concluded that:

1. The studied plants are the potent source of medicinally important compounds like phenols, flavonoids and natural antioxidant. Due to the presence of biological active constituents the examined plants can be utilized in the treatment of many ailments and also be exploited for use in the pharmaceutical and cosmetic industries.

2. The crude methanol extract of all plants species have appreciable antioxidant activity so may exhibit bactericidal and fungicidal activities, indicating that the active ingredients are broad spectrum compounds. The studied plants were effective against a variety of human diseases therefore they can be exploited as antimicrobial agent in the development of new drugs.

RECOMMENDATIONS

On the basis of results of present study, following recommendations are made:

1. The results reveal that medicinal values of studied plants are very promising therefore it is recommended that all the plants especially endanger species should be cultivated in different parts of the province to avoid their extinction.

2. Phytochemical constituent are mostly depended on geographical location so it is advised that a comprehensive study should be designed to investigate the phytochemical constituents of all the investigated plants and then the different concentration of compounds for its biological potential.

3. Future work on the identification, isolation and structural characterization of the active components should be the goals of further investigations as there compounds will serve as good therapeutic agents.

REFERENCES

Arvind, N., N. Sharma and M. F. Singh, 2012. Spectrum of Pharmacological Activities from *Bauhinia variegata*: A Review. J. Pharm Res. 5: 792-797.

AOAC, 2012. Association of Official Analytical Chemists. Official Methods of Analysis 19th Edition (George Latimer.ed.). AOAC Intl, Arlington, Virginia.

Hazrat, A., Nisar, M., Shah, J. and S. Ahmad. 2011. Ethnobotanical study of some elite plants belonging to Dir, Kohistan Valley, Khyber Pukhtunkhwa, Pakistan. Pak. J. Bot. 43: 787-795.

Kirtikar, K. R. and B. D. Basu. 1995. Indian Medicinal Plants. International Book Publications: Dehradun, India, pp. 102-103.

Khory, R. N. and N. N. Kartak. 1985. Materia Medica of India and Their Therapeutics. Neeraj Publications: N. Delhi, India, pp. 32-34.

Mohanta, B., Chakraborty, A., Sudarshan, M., Dutta, R. K. and M. Baruah, 2003. Elemental profile in some common medicinal plants of India. Its correlation with traditional therapeutic usage. J. Radioanalytical, Nuclear. Chem. 258: 175-179.

Ncube, N. S., Afolayan, A. J. and A. I Okoh. 2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. Afr. J. Biotech. 7: 1797-1806.

Park, Y. S., Jung, S.T., Kang, S. G. Heo, B. K., Arancibia-Avila, P. and F. Toledo. 2008. Antioxidants and proteins in ethylene treated kiwifruits. Food Chem. 107: 640-648.

Singleton, V. L. and J. A. Rossi. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16: 144-158.

Sukanya, S. L., Sudisha, J., Hariprasad, P., Niranjana, S. R., Prakash, H. S. and S. K. Fathima. 2009. Antimicrobial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria. Afr. J. Biotech. 8: 6677-6682.