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# Assessment of bioactive compounds of some plants of South Kashmir

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**Abstract**
Bioactive compounds are phytochemical found in food that have potential to regulate modulating metabolic processes and resulting in the promotion of better health. Analysis and distribution of medicinal plants indicate that high consumption of foods rich in bioactive compounds with antioxidant activity, including vitamins, phytochemical and mainly phenolic compounds, such as flavonoids and carotenoids, has a positive effect on human health and could diminish the risk of various diseases, such as cancer, heart disease, diabetes, cataracts and age-related problems. Bioactive compounds are mainly present in fruits, whole grains and vegetables. Bioactive compounds are different from nutrients because bioactive compounds are not essential and currently there are no recommended daily intake values. However, it is well established that a range of compounds from plants and animal sources has a positive influence on human health.

**Keywords:** bioactive compounds, analysis, antioxidant activity, therapeutic potential

## 1. Introduction
Food and agricultural organization (FAO) jointly launched The State of Food Security and Nutrition in the World, marking the beginning of a new era in monitoring progress towards achieving a world without hunger and malnutrition, within the framework of the Sustainable Development Goals (SDGs) (Bone *et al*., 2012). Collecting, analyzing, interpreting and disseminating high-quality food, agriculture and knowledge of natural resources has been a central activity of FAO (Davies *et al*., 2013) [23]. With the increase in global population and decrease in food production, the world is struggling with the challenges of food and nutrition (FAO, 2016). In addition the challenges of food and nutritional security which are highlighted objectives recognized by United Nations for future generation can be met through diversifying the food container and inclusion of several ignored and underutilized nutritious plants in food system.

Bioactive compounds are phytochemicals found in plants that are able to regulate metabolic processes and related to promotion of better health. They show beneficial effects such as antioxidant activity, inhibition of receptor activities, and induction and inhibition of gene expression (Correia *et al.*, 2012) [19]. Based on recent research, bioactive compounds have the potential to cure heart disease, cancer and other diseases (Borges *et al.*, 2012) [12]. Many bioactive compounds are antioxidants that destroy free radicals in the body. This activity prevents cellular and protein destruction. The bioactive properties of several plant foods have been calculated extensively and are believed to have important health benefits (Carbonell *et al*., 2014) [15].

Plants are serving the human beings in number of ways, therefore they are considered as gift from the nature for them. Their effective use as medicine has been reported since written human history (Samiullah *et al.*, 2017) [41]. From immemorial times, these are known as constituents of phytomedicine. Wonderful collection of industrial chemicals has also been obtained from plants. These plant based natural chemicals can be extracted from different parts of the plant such as leaves, fruits, flowers, seeds, roots, stems, barks, rhizomes etc. which reveal that each and every part of the plant contain these biologically active components (Baqi *et al*., 2018) [3]. The Kashmir Himalayas often referred to as terrestrial paradise on earth, is situated at the north western zone of the Himalayan biodiversity hot spot. The zone supports a rich and spectacular biodiversity of great scientific curiosity and

promising economic benefits owing to its topographic variations spanning from valley floor through the terraced table lands (karewas) and dense forests elevating up to the snow caped alpine peaks. Since ancient times, people in the region have practiced the usage of various medicinal plants growing in their locality which are used to cure various ailments. Kashmir is popular for its economically valued plants and their products such as medicine, food, fodder, fiber etc. The herbal wealth of wild medicinal plants of high mountains has not only been a potential source of revenue to the state but also the only relied indigenous health care system of people in the past".

The territory of Kashmir represents a wide range of altitude and climate conditions. The height varies from 5000 m to 15000m or more above the sea level, the maximum temperature (40 ºC) goes as low as -2º under such a wide range of climatic conditions, it is natural to have a wide range of medicinal plants growing in the state (Seidemann *et al.*, 2005). The unani pharmacopoeia Sub-committee on identification of drugs, constituted by the Govt of India, feels that the Himalayan ranges especially J&K retains a variety of plant species till now unexplored and unexploited. More than 50% of the plants used in the British Pharmacopoeia are reported to be growing in this region. Five hundred seventy-two (572) plant species have been reported to be of medicinal value by various survey units (Garcia *et al.*, 2006) [27]. They have been identified and classified by Botanical survey of India as belonging to 109 different families of plants.

The introduction of plant derived drugs in modern medicine has 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) [35].

In our present study, we have selected three wild plants that are locally used for the medicinal purposes. *Berberis aristata, Cydnia oblonga* and *Malus domestica* are the locally used medicinal plants mostly grown in South Kashmir. These plants are widely grown in Jammu and Kashmir, Uttarakhand and Himachal Pradesh. *Berberis aristata,* traditionally used in inflammation, wound healing, skin disease, diarrhea, jaundice and affection of eyes and *Cydonia oblonga* is a medicinal plant of family *Rosaceae* which is used to prevent or treat several ailments such as cancer, diabetes, hepatitis, ulcer, respiratory, and urinary infections, etc (Galini *et al.*, 2005). It has been reported that *Berberis aristata* contains barberine, oxyberberine, berbamine, aromoline, karachine, palmatine, oxyacanthine and taxilamine. *Berberis aristata* contains protoberberine and bis isoquinoline type of alkaloid (Bhatt *et al.*, 2017) [9]. *Malus* domestica contains several phytoconstituents and different vitamins reportedly vitamin A, B and C (Anshika *et al.*, 2011). *Malus domestica* has various pharmacological activities like anti-oxidant, antiproliferative, antidepressant, anti-inflammatory, antimicrobial, inhibition of lipid oxidation and cholesterol-lowering effect (Vaibhav *et al.*, 2012).

## 2. Materials and Methods

### 2.1 Collection of plant materials

In this study, three indigenous plants (*Berberis aristata, cydonia oblonga* and *Malus domestica) are* collected from district Kulgam of South Kashmir (latitude 32º17´ and 37º05´North and Longitude 72º31´ and 80º20´East). These medicinal plants were collected during February to September.


**Fig 1:** Images of study material: (a) *Berberis aristata* (b) *Cydonia oblonga* (c) *Malus domestica*

### 2.2 Qualitative phytochemical screening

Phytochemical analysis of aqueous and ethanol extract of different medicinal plant species collected from South Kashmir was carried out qualitatively according to the standard procedures described by Harborn 1998 to detect the presence of Alkaloids, Flavonoids, Tannins, Amino acids and phenols.

**a) Detection of alkaloids**

The extracts obtained from the three selected plants were 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). Fomation of yellow colour precipitate indicates the presence of alkaloids (Nalini *et al.*, 2018).

**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 yellow colour, which becomes colourless after the addition of dilute acid, indicates the presence of flavonoids (Kokate *et al.*, 2008).

**c) Detection of tannins**

The detection of tannins was carried out by Van Buren test by dissolving the plant extracts in few drops of ferric chloride. Formation of brownish green or blue-black colour indicates the presence of tannins (Nalini *et al.*, 2018).

**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 by adding ferric chloride solution to the plant extract. Formation of bluish-black color indicates the presence of phenols (Dahiru *et al.*, 2006).

### 2.3 Quantitative determination of bioactive compounds

Phytochemical analysis of three selected medicinal plants collected from South Kashmir was carried out quantitatively for the presence of Alkaloids, Flavonoids, Tannins, Amino acids and phenols.

**a) Estimation of alkaloid**

Quantification of alkaloids is performed by using the method of Harborn, 1984. 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 measured at 415nm on UV spectrometer.

**b) Total flavonoids content**

The Total flavonoids content was measured spectrophotometrically bt Chang *et al*., 2002. 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. 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 415nm on UV spectrophotometer.

**c) Estimation of tannins**

Quantification of tannins was carried out by following the method of Van Buren, 1981.

About 5 mg of each extract was diluted in 10 ml of distilled water, shaken for 1 hour in a mechanical shaker and filtered. About 0.5 ml of each plant extract was added with 0.2 ml ferric chloride in 0.1N hydrochloric acid and 0.008M potassium ferro-cyanide. Optical density reading was taken at 120 nm within 10 minutes.

**d) Total free amino acids**

The amount of total free amino acids in the samples was determined after Lee and Takahashi (1966) 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.

**e) Total phenols**

The total phenolic content in the sample was determined by using the Folin-Ciocalteu method or equivalence method (Singleton and Rossi, 1965).

1g 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 reagent A (Folin-Ciocalteu reagent) was added to above. After 5 to 8 minutes, 3.5 mL of reagent B (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 UV spectrophotometer.

## 3. Results and Discussion

In recent years, the use of traditional medicinal plants for primary health care has regularly increased worldwide. According to the World health organization (WHO), herbal medicines are being used by about 60% of world population primarily in developing countries for primary health care. The chemical constituents present in them are a part of the physiological function of living flora and hence better compatibility. Herbal raw materials are renewable and eco-friendly and these are much more affordable than synthetic drugs. During the last few decades, it was observed that use of modern drugs have resulted in a variety of drug induced resistance against microbes. The synthetic drugs being pure synthetic chemicals, induce cellular changes, act as foreign substance to the body system and produce several side or toxic effects. Plants have been particularly valuable sources for new or better drugs and new lead molecules for drug development programmers.

### 3.1 Qualitative analysis of phytochemicals

Qualitative analysis was done to determine the chemical constituents present in leaf extract of *Berberis aristata*, seeds of *Cydonia oblonga*, and fruit of *Malus domestica* and the results are framed in table 1. It was investigated that alkaloids and flavanoids are present in all the selected flora in both ethanolic and aqueous extract. Similar results were obtained in *H. halimifolium* in ethanolic extract (Badria *et al*., 2014) Tannins are present in *Berberis aristata Cydonia oblonga* and *Malus domestica* in aqueous extract after phytochemical screening. Similar results were found in *Terminalia arjuna* (Ashraf *et al.,* 2016). Phenolic content and total free amino acids are present in *Berberis aristata, Cydonia oblonga and Malus domestica* only in ethanolic extract. Similar results were found in *Morinda citrifolia* which contain amino acids after phytochemical screening (Bindu *et al*., 2014).

**Table 1:** Phytochemical analysis of aqueous and ethanolic extract of selected medicinal flora

| S. No. | Phytochemical | *Berberis aristate* (Leaves) | | *Cydonia oblonga* (Seeds) | | *Malus domestica* (fruit) | |
|---|---|---|---|---|---|---|---|
| | | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol |
| 1. | Alkaloid | + | + | + | + | + | + |
| 2. | Flavonoids | + | + | + | + | + | + |
| 3 | Tannins | + | - | + | + | + | - |
| 4. | Aminoacid | + | + | + | + | _ | + |
| 5 | Phenol | - | + | - | + | - | + |

### 3.2 Quantitative analysis of phytochemicals

All 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 five different medicinal plants were analyzed for total free amino acids, total phenols, proteins, flavonoids and alkaloids.

The phytochemical quantitative composition of different secondary metabolites in *Berberis aristata, Cydonia oblonga, Malus domestica* are shown in table 2 and fig 2. Alkaloids are observed in higher quantity in *Cydonia oblonga* than the other two selected medicinal plants, while *Malus domestica* was found to contain the least amount of alkaloids. Due to presence of higher range of alkaloids in *Cydonia oblonga*, it exhibits wide range of pharmacological properties like antimalarial, antiasthma, anticancer, vasodilatory, antiarrhythmic, antihyperglycemic, analgesic and antibacterial properties (Kittakoop *et al*., 2014). Amino acid were found to be highest in *Berberis aristata* than the other two selected medicinal plants, while *Malus domestica* was found to contain least amount of amino acids. Due to presence of amino acid in higher range in *Berberis aristata*, it can be used for the treatment of gastrointestinal problems, and genetical disease like Alkaptonuria (Akram *et al*., 2011). Flavonoids were observed in higher quantity in *Malus domestica* than the other two selected medicinal plants, while *Berberis aristata* contains least amount of flavonoids. *Malus domestica*, can be used in the health promoting effects like antioxidant, antibacterial, anticancer and anti-inflammatory due to the presence of higher levels of flavanoids (Tungmunnithum *et al.*, 2018) [44]. Tannin were observed in higher quantity in *Malus domestica* than the other two selected medicinal plants, while *Berberis aristata* was found to contain least amount of tannins. Due to presence of tannins in higher range in *Malus domestica*, it has astringent and haemostatic properties, which hasten wound healing and ameliorated inflamed mucus membrane (Cushnie *et al*., 2014) [21]. Phenolic contents were observed in higher quantity in *Cydonia oblonga* than the other two selected medicinal plants, while Malus *domestica* contains least amount of phenols. Due to presence of phenolic content in higher amount in *Cydonia oblonga*, it shows various medicinal properties like cardiovascular disease, certain type of cancers, neurodegenerative disease, and diabetes (Iweala *et al*. 2015) [32].

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

| S. No. | Medicinal plant | Total Free phenol | Total Amino acid | Total tannin Content | Flavanoids | Alkaloids |
|---|---|---|---|---|---|---|
| 1. | *Berberis aristata* | 0.141± 0.002 | 0.06± 0.002 | 0.167± 0.001 | 0.245± 0.002 | 0.521± 0.001 |
| 2. | *Cydonia oblonga* | 0.24±0.010 | 0.019±0.002 | 0.321±0.01 | 0.421±0.002 | 0.531±0.009 |
| 3. | *Malus domestica* | 0.126±0.002 | 0.05±0.008 | 0.412±0.002 | 0.509±0.008 | 0.217±0.015 |

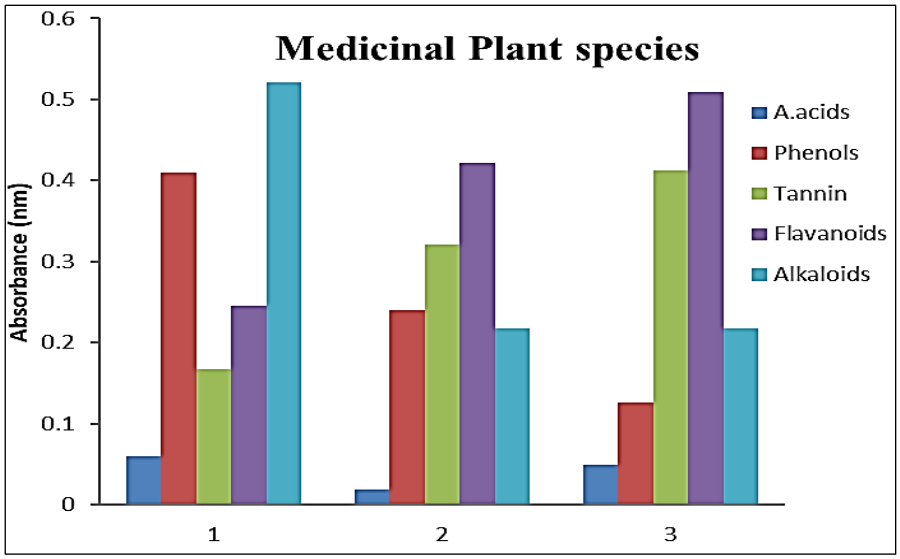


**Fig 2:** Comparision of phytochemicals of three selected medicinal plants 1: Berberis aristata 2 Cydonia oblonga 3 Malus domestica

### 4. Conclusion

The present investigation shows that the presence of various secondary metabolites like alkaloids, flavonoids, tannins, Amino acids, and phenols present phytochemical analysis of *Berberis aristata, Cydonia oblonga, and Malus domestica*. The presence of secondary metabolites in these plants may be the responsible for its therapeutic activities like antiulcerogenic, antidiabetic, antimicrobial and antioxidant activities. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties which are considered to be beneficial to human health. The present study should be performed for the activity of the bioactive compounds to use these selected plant species as potent drug.

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