Qualitative Analysis for Phytochemicals of Selected
Medicinal Plants from Gilgit-Baltistan, Pakistan

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Three medicinal plants from Gilgit-Baltistan region of Pakistan were selected for detailed qualitative analyses to identify different phytochemicals. The leaves and stems of these medicinal plants were used during this study. For each plant sample the methanolic extracts were initially obtained which were further fractionated into *n*-hexane, dichloromethane and water fractions by solvent-solvent extraction method. All these fractions were qualitatively analyzed for alkaloids, flavonoids, steroids, tannins, saponins, sterols, phenols, quinones and coumarins by using standard methods for analysis. Among three selected plant species, alkaloids were present in all extracts with equal positive results except hexanes extract of *A. brevifolia,* while flavonoids were present in all extracts of *A. brevifolia* and absent in all other extracts of *C. botrys* and *S. surrattense* (except water extract). The results also indicated that the steroids were present in only water extracts and tannins were present in both dichloromethane and water fractions of all three-plant species in this study. The results showed that the phytochemicals such as saponins, quinones and coumarins were present in one or the other fractions of *A. brevifolia, C. botrys and S. surrattense* while sterols were present in *A. brevifolia* and *C. botrys* and phenols in only dichloromethane fraction of *S. surrattense*. In present study, we observed that the water extract in comparison to other types of extracts gave the highest positive results with different reagents for the qualitative determination of phytochemicals, similarly *n*-hexane extract gave mostly negative results and dichloromethane extract shows moderate results. The qualitative analysis showed that the alkaloids were the major phytochemical constituents present in highest concentration in all plant extracts with positive results while flavonoids, sterols and phenols were absent in majority of different fractions.

Keywords: Phytochemical, *Artemisia brevifolia, Chenopodium botrys, Solanum surrattense*.

INTRODUCTION

Medicinal plants have vital role in life of human beings on the globe. The medicinal value of any plant is due to presence of some chemicals that can interact with the enzymes working in human physiology. Plants produce a wide range of natural products belonging to different molecular families with different physiological functions. These molecules possess different biological activities, which fascinated several researchers that eventually lead to the development of marketable products [1]. Naturally occurring compounds can be isolated from animals, minerals and plants but mostly their main sources are plants. These organic molecules can be obtained from both secondary and primary metabolic process. The plants are widely used in the world's pharmaceuticals. The well-studied bioactive constituents of plant kingdom include alkaloids, tannins, steroids, terpenoids, flavonoids, carotenoids and glycosides [2]. Researches have shown that the compounds isolated from natural sources show antiinflammatory, antitumor, antibac-

terial, antiviral, anticarcinogenic, antiatherosclerotic and antimutagenic activities. The risks of cancer, cardiovascular disease, diabetes and other diseases related with ageing can be reduced by using bioactive ingredients as food supplement [3]. In modern research, there is an international trend towards the use of the natural phytochemicals present in teas, herbs, beans, fruits, berry crops and vegetables as a remedy for most of the common ailments [4]. The traditional usage of active natural products come from ancient cultures without any understanding of basic chemistry. The Inca society initially used coca, because of its strong stimulatory effects. Similarly, opium was used by many ancient cultures as an efficient pain killer. The systematic use of natural products started during nineteenth century (1783-1841) when morphine was obtained from Opium. The formal progress in natural products resulted in isolation of first antibiotic, penicillin from the moulds of *Penicillium notatum* in 1928. Therefore, Alexander Fleming was awarded Nobel prize in 1945 for his ground-breaking contribution in drug discovery [5]. The preliminary records

indicate that human beings used natural products for over 3,000 years. The Egyptian pharmaceutical record known as The Ebers Papyrus (2900 B.C.) essays over 700 plant based drugs ranging from swallowing pills, infusions, to ointments. The ancient anti-inflammatory drug aspirin is derived from the bark of the willow tree, similarly morphine was first reported in 1803 from *Papaver somniferous* L. [6].

Recently thousands of plant secondary metabolites have been discovered, which include alkaloids, terpenoids, flavonoids, steroids, *etc*. These natural products take an important role in the communication of plants with their biotic and abiotic environment. Plant resultant secondary metabolites function as self-protective compounds or a poisonous substance that protect it from pathogens, UV radiations and herbivores. In the past two decades, almost two thirds of accepted new drugs were obtained from natural plant products [7]. The prime aim of current study was to evaluate qualitatively phytochemicals from three medicinal plants *i.e. A. brevifolia, C. botrys* and *S. surrattense*.

EXPERIMENTAL

Analytical or laboratory grade solvents and chemicals were purchased from E. Merck, Fisher Scientific or Sigma Aldrich.

The aerial parts of all plant species *i.e. Artemisia brevifolia* (Wall. Ex DC.)*, Solanum surrattense* Brum F. and *Chenopodium botrys* L., were collected from the Hunza-Nagar region of Gilgit-Baltistan during August-September 2014. The samples were dried in the laboratory at ambient temperate (28-30 °C) and were ground to powder. The plant species were identified by Dr. Sujjad Haydar, a resident botanist at the Department of Biological Sciences, Karakoram International University.

Extraction method: Dried and ground aerial parts of each plant *i.e. A. brevifolia* (31.2753 g), *S. surrattense* (35.9533 g) and *C. botrys* (43.8180 g) were poured into reagent bottles and filled it with MeOH for soaking and left for 2 weeks at room temperature. Each mixture was filtered and added 250 mL hexane to filtrate. This mixture was transferred into the separating funnel, shaked well and placed on stand for 20 min to get clear layers. Methanolic layer was transferred into 500 mL conical flask. The process was repeated three times to get *n*-hexane fraction. The methanolic extract was further fractionated into dichloromethane and aqueous fractions by using solvent-solvent extraction method. The qualitative tests by using different reagents were performed on crude extracts of *A. brevifoli* (110 g), *S. surttense* (103 g) and *C. botrys* (120 g).

Phytochemical analysis

Test for alkaloids: The powdered leaves (2 mg) of each plant sample were separately boiled by using water bath with hydrochloric acid (5 mL). The pH of mixture was maintained between 6-7 with ammonia during cooling process. The stock solution (0.5 mL) was added separately to each plant extract against the standard tests outlined below:

Kraut's reagent: Bismuth nitrate (4.0 g), potassium iodide (14.0 g), nitric acid (10.0 mL) and distilled water (50 mL). The formation of precipitate (reddish brown) indicated presence of alkaloids after mixing of sample with reagent.

Marquis reagent: Two to three drops of 40 % HCHO, conc. H_2SO_4 (3 mL) and a small amount of plant extract was added in a test tube. The time and change in colour was carefully observed.

Hager's reagent: Appearance of a yellow precipitate indicates the presence of alkaloids after mixing saturated solution of picric acid with sample plant extract.

Mrame's reagent: Turbidity in the filtrate indicates positive result for alkaloids by addition of cadmium iodide (5 g), potassium iodide (10 g) and distilled water (5 mL).

Scheiber's reagent: The formation of coloured precipitate by addition of plant extract to the solution of sodium tungstate (10 g), disodium hydrogen phosphate (35 g) and distilled water (50 mL) indicates the presence of alkaloids.

Erdmann reagent: Few drops of the reagent comprising conc. HNO_3 (5 drops), distilled water (50 mL) and conc. H_2SO_4 (10 mL) was added to each sample in test tube. The persistence of turbidity indicated the presence of alkaloids.

Mandalian's reagent: A solution comprising ammonium vandate (0.5 g) and conc. H_2SO_4 (100 g) was added to few drops of plant sample extract. The change in filtrate colour indicated the existence of alkaloids.

Test for flavonoids: Lead acetate test: A small amount of extract filtrate was treated with lead acetate and observed the formation of white precipitate as positive result.

Hydroxide test: The sample plant extract was mixed with aqueous solution of sodium hydroxide and hydrochloric acid to detect yellow orange colour as positive result.

H_2SO_4 test: A fraction of extract was treated with concentrated sulphuric acid and observed the formation of orange colour for flavonoids.

Test for tannins: KOH test: A fresh solution of 10 % KOH was prepared and 1 mL was added to the small amount of plant extract. The positive results were associated with dirty white precipitate.

Braymer's test: Few drops of sample extract was treated with 10 % alcoholic ferric chloride solution. Blue or greenish colour appeared in solutions containing flavonoids.

Test for saponins: Frothing test: Few milligrams of powdered leaves were placed in a test tube and 10 mL of distilled water was added. Continuous sshaking for 30 s and stain for 30 min lead to the formation of honey comb froth for saponins.

Foam test: 1 mL of the sample plant extract was placed in a test tube followed by addition of 1 % lead acetate solution. White precipitates appeared for saponin containing plant extracts.

Test for steroids: Salkowski test: Few drops (3-5) of concentrated H_2SO_4 was added to 1mL of the plant extract in a test tube. Presence of steroids was associated with red colouration.

Liebermann's reaction: 3 mL of acetic anhydride was added to 3 mL of ethanolic plant extract. The test solution was then heated and cooled followed by addition of a few drops of conc. H_2SO_4 appearance of blue colour showed the presence of sterols.

H_2SO_4 test: The ethanolic extract of plant was treated with few drops of conc. H_2SO_4 to observe violet blue or green colour in a solution for sterols.

Test for quinones: A yellow colour precipitate appeared for quinones by addition of sample plant extract with concentrated HCl.

Test for phenols: Deep blue or black colour appeared by mixing 5 % ferric chloride solution to the small amount of plant extract.

Test for coumarins: 3 mL of 10 % NaOH was added to 2 mL aqueous plant extract and yellow colour was observed in positive results.

RESULTS AND DISCUSSION

Medicinal plants have great importance in modern health-care system. Phytochemical analysis on different plant extracts showed the presence of different constituents of medicinal species as well as physiological importance. Most of the secondary metabolites isolated from natural sources are used in pharmaceuticals, food additives, fragrances and pesticides and herbicides [8-10]. Several researchers in subcontinent have carried out similar analysis on different plants [11,12].

In current study, determination of phytochemicals in different plant extracts showed the presence of phytochemicals such as alkaloids, tannins, flavonoids, saponins, phenols, coumarins, steroids, *etc*. In some plant extracts, certain phytochemicals were absent and at the same time in other plant extracts these phytochemicals were present. Phytochemical constituents from different plant extracts were analyzed qualitatively and presented in Table-1. The results of this study showed that some plant extracts which contain certain phytochemical constituents are nutritionally important and some extracts, which have phyto-chemicals are beneficial by having antibacterial, antiviral, antioxidant and antitumor activities. Seven tests were performed to determine the presence of alkaloids. *C. botrys* has shown positive results. *S. surrattense* has shown the lowest concentration of alkaloids. Among three extracts types, dichloromethane was found as alkaloid rich fraction whereas *n*-hexane extract has shown mostly negative results. Similarly,

for the qualitative determination of flavonoids we applied three tests and revealed that flavonoids were present in all plant extracts where water and dichloromethane extracts showed positive results (Table-1). Similarly, for three selected plant species we performed different tests for the determination of different phytochemical constituents such as tannins, saponins, steroids, sterols, quinones, phenols and coumarins where in some extracts these phytochemicals were present and in other extracts they were absent (Table-1).

Different classes of bioactive compounds obtained from plants have been reported to possess different activities. Saponins are known to have anti-inflammatory, hemolytic and cholesterol binding properties [13,14]. Many glycosides have been reported as blood pressure lowering agents [15]. Several tanins with potent antioxidant, antiviral and antimicrobial effects have been reported in literature [16]. The steroids isolated from plant extracts are known to be active against inflammation [17]. Alkaloids can be used as analgesic, anti-spasmodic and antibacterial natural compounds [14]. The presence of alkaloids, coumarin, phenolics, flavonoids, saponnins, tannins and terpenoids are the most commonly occurring natural products that have been already isolated and characterized from different plant species [18-20].

Conclusion

Present study provides basic information about phyto-chemical constituents of different plants collected from Gilgit-Baltistan, Pakistan. Most of the plants found in Gilgit-Baltistan are rich and diverse in bioactive metabolites, which have great importance in medicines and for human health. Qualitative analysis is very essential for identifying the compounds present in the medicinal plants. During the qualitative analysis water was found to be the best solvent with highest positive results with different reagents for different class of phytochemical

TABLE-1
PHYTOCHEMICAL PROFILE OF *A. brevifolia, C. botrys* AND *S. surrattense*

Class of phyto-chemicals	Test/reagent	Extract type/plant species								
		Hexane			Dichloromethane			Aqueous		
		Ab	*Ss*	*Cb*	*Ab*	*Ss*	*Cb*	*Ab*	*Ss*	*Cb*
Alkaloids	Kraut's	–	–	–	–	++	–	–	–	–
	Marqui's	–	++	++	++	++	++	++	++	++
	Hager's	–	–	–	++	–	–	–	–	++
	Marme's	–	–	–	++	++	–	++	–	–
	Scheiber's	–	–	–	++	++	–	++	–	–
	Erdmann's	–	++	–	–	++	–	++	–	–
	Mandalian	–	–	++	–	–	–	++	++	++
Flavonoids	Pb(OAc)$_4$	–	–	++	++	–	–	–	++	–
	NaOH	–	–	–	–	–	–	++	–	–
	H$_2$SO$_4$	++	–	–	++	–	–	–	–	++
Steroids	Salkowski	–	–	–	++	–	–	–	–	–
	Liebermann reaction	–	–	–	–	–	–	++	++	++
Tannins	KOH	–	–	–	–	–	–	–	++	–
	Braymer's	–	–	–	++	++	++	++	++	++
Saponins	Frothing	–	++	–	–	–	–	++	–	–
	Foam	–	++	++	–	–	++	–	++	–
Sterols	H$_2$SO$_4$	++	–	–	–	++	–	++	++	–
Phenols	FeCl$_3$	NA	–	–	NA	–	++	NA	–	–
Quinones	HCl	–	++	–	++	–	++	++	–	++
Coumarins	NaOH	–	NA	++	++	–	++	–	–	++

++ = Present, – = Absent; *Ab*: *Artemisia brevifolia*; *Ss*: *Solanum surrattense*; *Cb*: *Chenopodium botrys*

constituents. The hexane extract mostly showed the negative results whereas dichloromethane extract showed 50 % positivity against different reagents. In current study among three plants, *A. brevifolia* showed highest amount of phytochemicals and *S. surrattense* showed lowest concentration of phytochemicals; whereas *C. botrys* indicated moderate results. Among all phytochemicals, which was qualitatively analyzed, alkaloids and flavonoids were the major phytochemicals in all plant extracts and quinones and phenols were present in lower concentration with negative results with different reagents.

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