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Original article

Comparative phytochemical profile of some medicinal plants from Gilgit-Baltistan

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Summary. The aim of this study was to evaluate some medicinal plants for the presence of phytochemicals like alkaloids, flavonoid, tannins, saponnins, phenols, quinones, steroids, and sterols in five different medicinal plants collected from Gilgit region of Pakistan. The plants were *Daphne mucronata (Dm)*, *Punica granatum (Pg), Kochia prostrata (Kp), Isodon rugosus (Ir),* and *Pistacia khinjuk (Pk).* The plants were first dried at room temperature and grounded to powder form. The powder was placed for soaking into methanol for ten days. The methanolic extract was further fractionated by using *n*-hexanes, dichloromethane, and water through solvent-solvent extraction. These extracts were further treated with different reagents to check the presence of various phytochemicals in these plant species. The results showed that all the five plants contain phytochemical constituents like alkaloid, flavonoid, steroids, and tannins. Phenols were absent in hexane and dichloromethane (DCM) extracts of *Dm, Pg, Ir*, and *Pk*. Similarly, steroids and sterols were not present in hexane and DCM extracts of *Dm*, quinones was absent in *Pg* and *Pk*, and saponins in *Ir*. The water extract of all plants showed good results, followed by DCM extracts in terms of more verities of secondary metabolites. The hexanes extracts showed poor results for the diversity of phytochemicals with different reagents.

Keywords: Quantitative analysis, phytochemicals, *Daphne mucronata*, *Punica granatum, Kochia prostrata, Isodon rugosus,* and *Pistacia khinjuk*

Introduction

Plants are important elements of the universe. They are good sources of natural bioactive secondary metabolites and work as reservoirs of phytochemicals. In a process of photosynthesis plants absorb the sun light and produce high levels of oxygen and secondary metabolites of medicinal importance that are stored in different parts of the plants (1). Human beings have used plants as medicine, flavouring and recreational drugs for treatment of different ailments with reputation as effective remedies from very beginning of time form thousand years back in history (2). Large numbers of these plants are important in present pharmaceutical industry, because they act as therapeutic agents and raw materials to produce traditional and modern medicines (3). Phytochemicals are defined as the substances found in edible fruits and vegetables that exhibit a potential for modulating human metabolism in a manner beneficial for the prevention of chronic and degenerative diseases (4). Phytochemicals start on a broad series of activities, which helps to give immunity against long term disease. The phytochemicals like, flavonoids, saponins, tannins alkaloids terpenoids, etc. are known to show medicinal activity as well as exhibit physiological activities (5).

Alkaloids play an essential role in biological activities such as antitumor, emetic, anti-cholinergic, sym-

pathomimetic, antiviral, diuretic, antihypertensive, hypnoanalgesic, antidepressant, antitussigen, anti-inflammatory, antihyperglycemic and pharmacological effects (6,7). They have also the properties of antimicrobials and anti-parasitics and used as inhibitors, growth regulators and stimulators. Tannins were first described in 1985 (8). Tannins are used as astringents, diuretic, wound healer, stimulant, antiseptics, anti-ulcer, diarrhea, and in tanning industry (9). They are also used in dyestuff industry and in food industry. However, tannins are also used in textile industry as dyes and as coagulants in rubber production (8). Szent-Gyorgyi was the man who isolated a new substance from oranges and named as vitamin P in 1930. But later, it was cleared that the substance was a flavonoid and about 8,000 varieties of flavonoids were identified (10). It is well established fact that flavonoids are biologically active secondary metabolites that helps to prevent various chronic diseases and heart related problems. It is therefore advised to increase intake of flavonoid rich fruits and vegetables in daily diet to ensure sufficient supply of flavonoids (11). Plant flavonoids may also reduce the risk of thrombosis by inhibiting platelet aggregation and adhesion (12). In 1920 coumarin was first isolated from a definite kind of bean (13) but in 1822, Vogel isolated coumarin and purified it from the Tonka bean (*Dipteryx Odorata*). Nearly one thousand coumarins have been described in literature (14) Coumarin derivatives possess tumoristatic, anticoagulant, and immune stimulatory properties, and some derivatives are used in fluorescent labelling and as laser dyes (15). It is used in the perfumery, cosmetic and household products industry due to its pleasant bitter-sweet odour (14). In 1996 the term, biophenols was first introduced by Romeo and Uccella to describe bioactive phenols in olives (16). Phenol is used in different branches of industry including chemical production of phenolic resins, aniline, cresols, alkylphenols, xylenols, and other compounds, coal processing metallurgic and oil. It is also used in explosives, pesticides, textiles production and dyes. It penetrates the environment through vehicle exhaust and is used as antiseptic and reagent in chemical analysis (17). In 1951 Sitosterol was first described as a therapeutic agent for hypercholesterolaemia (18**).** Plant sterol or stanol esters lower total and LDL cholesterol concentrations by reducing the risk of coronary heart disease (19). Sterols are the precursors of steroid hormones and bile acids in humans, brassino steroids - phytohormones in plants and as the previous study showed that they are involved in important growth and developmental processes in living organisms. The steroids are among the most widely used class of drugs. Their role in the therapy of pulmonary, inflammatory, dermatological, and oncological diseases has been well described (20). Quinones play an important role in medicine. Imbalance occurs between the making and quench of free radicals from oxygen species so oxidative stress occurs. As we know that the mitochondria play a vital role in the formation of excess ROS. Quinones are electron carrier so that can target the mitochondria and restore electron transfer in deficiency states (21). Most of the saponins show different foaming properties. They are also added to liquid detergents, shampoos and beverages as emulsifier and long-lasting foaming agent. The saponins of plants have established in some pharmacological effects, such as antimicrobial, molluscicidal, anti-inflammatory, antidermatophytic, anthelmintic, cytotoxic activities and antitussives. The role of saponins in plants is to protect plant against attack by pathogens and pets (22,23).

Punica granatum is found in the Mediterranean areas of South East Asia. Due to its important biological actions, such as antioxidant, antimicrobial, anti-inflammation, and anti-cancer properties its extracts are used to avoid from cancer, irritation and from viral infections. In the leaves and flowers of *Pg*, high content of tannins and flavonoids are present (24). Traditionally the genus *Daphne* has been used for the treatment of cancer and skin disorders. Different species of the genus *Daphne* have good antimicrobial and cytotoxic effects (25). *Kochia prostrata* enhance availability of digest low-quality feed and indicated to be usable for livestock feed in winter period (26). *Isodon rugosus* is a deciduous shrub which is from the family Lamiaceae. The plant has medicinal uses like it is used in traditional medical practices in tooth ache and is very effective as an anti-diarrheal, hypoglycaemic, bronchodilator and antiseptic (27). The genus *Pistacia* belongs to the family Anacardiaceae, including in nut tree group. Some species of *Pistacia* have been used in folk medicine and these plants are used as anti-inflammatory,

antipyretic, antibacterial, antiviral in treatment of diarrhoea and throat infection (28).

Scientists around the globe are engaged in research on natural products and thousands of plant secondary metabolites of various functional groups have been discovered. Plants produce these compounds for communication with their biotic and abiotic environment along with protection from pathogens, UV radiation and herbivores. In the past two decades, almost two thirds of accepted new drugs were produced from medicinal plants (29).Qualitative phytochemical analysis of target plant species has been carried out in this research to confirm the presence or absence of phytochemicals in different organic extracts. Hence, in the present study phytochemical screening of some important medicinal plants was carried out to provide the basic information about the medicinal band nutritional importance of different herbs.

Materials and methods

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

Apparatus: Beakers, test tubes, pipette, filter papers, electronic balance, reagent bottles, spoon, glass rod, mortar and pestle, petri dishes, water bath, conical flasks, separatory funnels, stands, measuring cylinder, dropper, rotary evaporator, and masking tape.

Collection of plants material: The five different plants species were collected from the various regions of Hunza-Nagar during April 2014. The samples were dried in the laboratory at ambient temperature and was ground to powder. The plant was identified as *Daphne mucronata* Royle., *Punica granatum* L., *Kochia prostrata* L., *Isodon rugosus* (Wall. ex Benth.), *Pistacia khinjuk* Stocks. (Fig.), by Dr. Sher Wali Khan, a resident botanist at the department of biological sciences, Karakoram International University.

Extraction method: Dried and ground aerial parts of each plant *i.e. D. mucronata* (43 g), *P. granatum* (59 g), *K. prostrata* (56 g), *I. rugosus* (28 g), and *P. khinjuk* (63 g). was poured into reagent bottles and filled it with MeOH for soaking and let them for two weeks at room temperature. The mixture was filtered and added 250 ml hexane to filtrate. Transferred this mixture into the separating funnel, shacked well and placed on stand for 20 minutes to get clear layers. The Methanol layer was transferred into 500 ml conical flask. The process was repeated three time to get 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.

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 test 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% HCOH, 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 were carefully observed.

Hager's reagent: Appearance of a yellow precipitation 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 (5.0 g), potassium iodide (10.0 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 (10.0 g), disodiumhydrogenphosphate (35.0 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 presence of alkaloids.

Mandalian's reagent: A solution comprising ammonium vandate (0.5 g), 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 powder leaves were placed in a test tube and 10 ml of distilled water was added. Continuous shaking for 30 s and stain for 30 min lead to the formation of honey comb froth for saponins.

Foam test: 1ml 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 coloration.

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 plant extract 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: Ferric chloride test: Deep blue or black colour appeared my 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 to the health of individuals and communities. Gilgit-Baltistan is blessed with medicinal plants. Phytochemical analysis from different plant extracts shows the presence of constituents which are known to exhibit medicinal as well as physiological activities. Different phytochemicals have different function for example flavonoid are used as antimicrobial, antiallergic, anti-inflammatory, estrogenic, enzyme inhibition, antioxidant, and cytotoxic antitumor activity. Alkaloids may be used as stimulant of the central nervous system and strong narcotic pain killers. Steroids have hypertensive and cardio depressant potentials. Saponins have antimicrobial potentials. Tannins' have a wide range of anti-infective actions as well as diuretic, as a stimulant, diarrhoea and in tanning industry. Terpenoids are attributed for analgesic and anti-inflammatory activities. Saponins have been extensively used as detergents, as

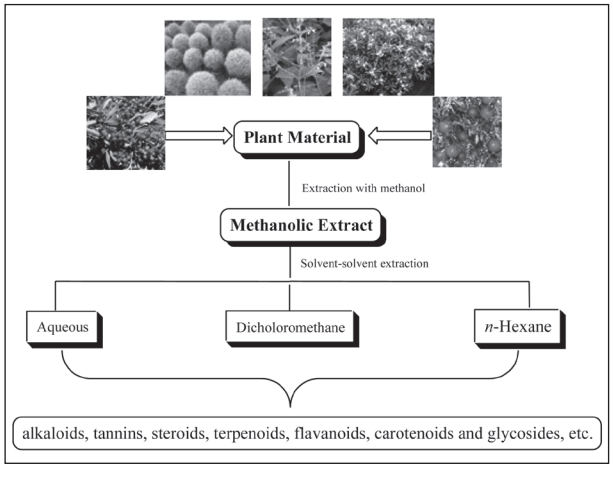

Fig 1. Scheme of phytochemical analysis from *D. mucronate, P. granatum, K. prostrate, I. rugosus, P. khinjuk*

pesticides and molluscicides, in addition to their industrial applications as foaming and surface-active agents and have beneficial health effects. Coumarin are used in laser dyes and for fluorescent labelling.

The present study was based on qualitative analysis of phytochemicals in different plant species collected from Gilgit-Baltistan region of Pakistan. The screening for secondary metabolites was carried out by using different solvent extractives from target plant species. The phytochemical profile of *Dm*, *Pg*, *Kp*, *Ir* and *Pk* was reported for the first time (Fig).

Alkaloids, flavonoids and tannins were found in all three extracts of *D. mucronata* whereas testes for steroids, sterols, saponin, and phenols were also positive for aqueous extract. Steroids, sterols, saponin, and phenols were not detected in hexane and dichloromethane extract of *Dm*. whereas quinones and coumarins were not detected in aqueous extract of *Dm*. The results of many tests for alkaloids in hexane extract of *Dm* were also negative. The dichloromethane and aqueous extracts of *Punica granatum* showed positive result for alkaloids, flavonoids, steroids, tannins, and saponins whereas phenols and quinones in both DCM and hexane and sterols and coumarins in aqueous extracts were absent (Table).

Kochia prostrata contain only alkaloids, and saponins whereas sterols, phenols and quinones were absent in all three extract fractions. Flavonoids and steroids were present in DCM and aqueous extract where these were not detected in hexane extract of the same plant. Hexane fraction contain tannin and coumarin while negative test result was observed for tannins in DCM and coumarins in hexane fractions (Table). The DCM, hexane and aqueous extracts of *P. khinjuk* were tested for nine different classes of phytochemicals including alkaloids, flavonoids, steroids, tannins, saponins, ster-

Table 1. Phytochemical profile of *D. mucronata*, *P. granatum*, *K. prostrata, I. rugosus*, *P. khinjuk*

Class of phytochemicals	Test/reagent	Extract type/plant species													
		n-hexane				dichloromethane					aqueous				
		Dm	*Pg*	*Kp*	*Pk*	*Dm*	*Pg*	*Kp*	*Ir*	*Pk*	*Dm*	*Pg*	*Kp*	*Ir*	*Pk*
Alkaloids	Kraut's	--	--	--	--	--	++	--	--	++	--	--	--	--	--
	Marqui's	++	--	++	++	++	++	++	++	++	++	++	++	++	++
	Hager's	--	--	--	--	++	--	--	++	--	--	--	--	--	--
	Marme's	--	--	--	--	++	++	--	--	--	++	++	++	++	++
	Scheiber's	--	--	++	--	++	++	++	++	++	++	++	++	++	++
	Erdmann's	--	--	--	--	++	--	++	--	--	++	++	++	--	--
	Mandalian	++	--	--	++	++	++	--	--	--	++	++	++	++	++
Flavonoids	$Pb(OAc)_4$	--	++	--	--	++	++	++	++	++	++	++	++	++	++
	NaOH	--	--	--	--	--	++	--	++	--	++	++	++	++	++
	H_2SO_4	++	++	--	--	++	--	++	--	--	--	++	--	++	++
Steroids	Salkowski	--	--	--	--	--	++	++	++	++	++	++	++	++	++
	Liebermann	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Tannins	KOH	--	++	--	--	--	++	--	--	--	++	++	--	--	++
	Braymer's	++	++	++	++	++	++	--	++	++	++	--	++	++	++
Saponins	Frothing	--	++	--	--	--	--	--	--	--	++	--	--	--	--
	Foam	++	++	++	--	++	++	++	--	--	++	++	++	++	--
Sterols	H_2SO_4	--	++	--	++	--	++	--	--	++	++	--	--	--	--
Phenols	$FeCl_3$	--	--	--	--	--	--	--	--	--	++	++	--	++	++
Quinones	HCl	++	--	--	--	++	--	--	--	--	--	++	--	--	++
Coumarins	NaOH	++	++	++	--	--	++	++	--	++	--	--	--	--	++

Key: ++ = present, -- = absent;
Dm: Daphne mucronata; Pg: Punica granatum; Kp: Kochia prostrata; Ir: Isodon rugosus; Pk: Pistacia khinjuk

ols, phenols, quinones, and coumarins. The aqueous extract showed positive results for most of the phytochemicals except saponins and sterols. In contrary, the hexane extract showed negative result for most of the phytochemicals except tannins and sterols. Flavonoids, saponin, phenols and quinones were present in DCM fraction where alkaloids, steroids, sterols, tannins, and tannins were not present in this fraction of *P. khinjuk*.

The DCM and aqueous fractions of *Isodon rugosus* showed positive results for alkaloids, flavonoids, steroids, and tannins, whereas sterols, quinones and coumarins were not present in both these fractions. The negative results were also observed for saponins and phenols in DCM extract of *I. rugosus*. Among the various solvents extracts the plant samples extracted with water showed more positive results followed by dichloromethane whereas *n*-hexane extracts contain very few classes of phytochemicals (Table).

Conclusions

The phyto-chemicals like flavonoids, saponins, tannins alkaloids, terpenoids etc. are known to show medicinal activity in addition to exhibiting physiological activities. *Daphne mucronata*, *Punica granatum, Kochia prostrata, Isodon rugosus,* and *Pistacia khinjuk* are the plants that contain phytochemicals like alkaloids, flavonoids, tannins, saponnins, steroids, sterol, quinones, phenol and coumarin. *Isodon rugosus* show good result for steroids, saponins and phenols. *Punica granatum* is rich for tannins and saponins. *Kochia prostrata* showed good results for coumarin, steroid and saponins. *Pistacia khinjuk* can be used as a good source of steroids and sterols. Alkaloid and flavonoids were present in all plants whereas polar fractions contain more phytochemicals as compared to least polar extracts. These results suggest that the phytochemicals from these plant species can be used for curing various ailments by isolation of new and novel lead compounds.

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