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Qualitative phytochemicals screening of the endemic plant *Buxus papillosa* C.K. Schneid.

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Abstract: *Buxus papillosa* C.K. Schneid. is a member of the Buxaceae family and it is endemic to Pakistan. *B. papillosa* C.K. Schneid. have some medicinal properties is known as the effective remedy against some diseases like malaria, rheumatism and skin diseases. To the phytochemical screening of *B. papillosa* C.K. Schneid. we used dried powder of leaves and made the extracts of acetone, methanol, chloroforms and water, all had the presence of alkaloids, phenolic compounds, flavonoid, protein, saponins, carbohydrates, fats, and oils. In this study we investigated the phytochemical compounds of *B. papillosa* C.K. Schneid.

Keyword: Buxus papillosa

INTRODUCTION

Buxus papillosa C.K. Schneid. belongs to family Buxaceae and order Buxales, which is endemic to Pakistan. In Pakistan, only two species are found while about 70 species are distributed in Eurasia, Middle East, South and Tropical Africa. *B. papillosa* C.K. Schneid.is a 3-5 m tall (shrub or small tree) and it presents crooked stem with erect branches. The leaves are narrowly lanceolate or oblong elliptic (Abdul Ghafoor, 1974). All plants of the kingdom plantae have a great source of medicinal importance. Medicinal plants have a great value of drugs potential with both have safe and side effects. *Oldenlandia corymbosa, Bryophyllum pinnatum, Xanthium strumarium, Ipomea aquatica, Terminalia bellerica, Tinospora cordifolia,* and *Ricinus communis* these seven plants have been used as an effective drug theoretically for anticancer, antimicrobial, antihepatotoxic compounds. The bioactive components of medicinal plants like alkaloids, tannins, terpenoids, carbohydrates, steroids, and flavonoids have great effect on human health. The primary and secondary compounds are extremely diverse both taxonomically and chemically (Yadav and Agarwala, 2011).

All plants have secondary metabolites and they have specific tastes, colors, and odors (Bennett *et al.*, 1994). Some medicinal plants have been studied phytochemically. *Momordica charantia* is a medicinal plant. which is used against diabetes to lower sugar level in the blood. *M. charantia* (bitter gourd) has a wide range of alkaloids and terpenoids (momordicin-28, momordenol, momordicinin, momordol, and momordicilin). *Morus nigra* (mulberry) belongs to family Moraceae had shown great medicinal properties such as anti-inflammatory activities (Kim *et al.*, 1999). Phytochemical

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investigations yielded 20 phytochemical compounds in *Psidium guajava* (Guava) extract (Osman *et al*., 1974, Begum *et al*., 2002). Member of Rosaceae family *Prunus persica* (peach) also has great medicinal importance and it is widely used in countries of Africa as an anti-fungal effect (Coccioni *et al*., 2002). Pomegranate (*Punica granatum*) has shown great effect against dysentery, diabetes, cough, diarrhea, bronchitis, fever, AIDS, inflammation, ulcers, malaria, prostate cancer, asthma, bleeding disorders, atherosclerosis, hypertension, male infertility, hyper lipidemia, and obesity (Panhwer *et al*., 2007). In Pakistan *Fagonia cretica* is used for snakebites (Cakilcioglu *et al.*, 2011).

Phytochemicals in the plant are playing a vital role in defense and inhibition mechanisms against many diseases and microbes (Bansode, 2016). *B. paplliosa* C.K. Schneid. also has some medicinal properties against some diseases like malaria, rheumatism, and skin diseases (Atta-ur-Rahman *et al*., 1989). *B. papilliosa* C.K. Schneid. contains more than 50 steroidal alkaloids and triterpenoids (Attaur-Rahman, 1990; Naz, 1995). Buxahejramine, buxakashmiramine and buxakarachiamine three phytochemicals isolations and characterization have also been reported along with the triterpenoidal bases cyclovirobuxeine-A, cycloprotobuxine-C, cyclomicrophylline-A and semperviraminol (Orhan *et a.,* 2004). In this study, we performed a phytochemicals qualitative analysis of *B. paplliosa* C.K. Schneid.

PROTOCOL AND METHODS

Plant material: B. papilliosa C.K. Schneid, was collected from the northern area of Pakistan, fresh and healthy plant leaves were collected and wash into tap running water then air dried in the shade after air-dried collected all leaves in to the polythene bags. Voucher specimens of the *B. paplliosa* C.K. Schneid. has been deposited into the Karachi University Herbarium (KUH), Center for plant conservation.

Extract preparation: After air-drying leaves were ground in the blander. Weight 10gm of powder into a conical flask and added 20mL of distilled water then mixed well and left on a shaker until overnight. This process repeated the same for the preparation of different extracts of acetone, chloroform, methanol, and water. After that, all solutions filtered with the help of filter paper and collected extract power into the other separated bottles for the use of phytochemical analysis.

Physico-chemical analysis/ Evaluation: The parameters of physico-chemical test such as loss and drying weight of ash, solubility in water and nitric acid were checked (Esha *et al.*, 2016).

Alkaloids detection (Evans 1997): Taken solvent-free extract 50 mg of acetone, Methanol, Chloroform and Water with few drops of hydrochloric acid and filtered after the following tests were done carefully.

Mayer's test:In the few mL of filtered added few drops of Mayer's reagent (Potassium Iodide and Mercuric chloride) along the side of the test tube. The formation of white or yellow precipitate indicates the positivity of the test, then observed the result.

Wagner's reagent (Wagner, 1993): In to the filtrate was added few drops of Wagner's reagent (iodine and potassium iodide) along the side of the test tube. Reddish-brown color indicates the presence of alkaloids.

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Hager's Test (Wagner *et al*., 1996): Added a few drops of Hager's reagents (saturated aqueous solution) along the side of the test tube yellow color precipitate indicates the presence of alkaloids.

Phenolic compounds and flavonoids detection:

Ferric chloride test (Mace, 1963): Extract (45-50 mg) treated 5-6 mL of distilled water then added the few drops of neutral 5% ferric chloride solution along the side of the test tube. The presence of a dark green color indicates the phenolic compounds.

Lead acetate: Extract treated with 3 mL of 10% lead acetate solution. The presence of bulky white precipitate indicates the presence of phenolic compounds.

Alkaline reagent: Added a few drops of 10% ammonium hydrochloride solution into the extract along the side of the test tube. The yellow fluorescence color indicates the presence of flavonoids.

Protein and amino acid test (Fisher, 1968; Ruthmann, 1970):

Millon's test (Rasch and Swift, 1960): 2 mL of the filtrate and added Millon's reagent. The white precipitate indicates the presence of proteins.

Biuret test (Gahan, 1984): 2 mL of filtrate and added 2% copper sulphate solution. After that added 95% of ethanol, followed by the additional of potassium hydroxide pellets. Pink color ethanolic layer indicates the presence of proteins.

Ninhydrin test (Yasuma and lchikawa, 1953): Into the filtrate added 2 drops of ninhydrin solution. The purple color indicates the presence of amino acids.

Saponins (Kokate, 1999): 0.6 mL of extract shake well with 3ml of water. The presence of foam indicates the presence of saponins.

Carbohydrates and Glycosides (Ramakrishnan *et al*., 1994): For preparation of subjected filtrate taken 100 mg of extract and dissolved in 5 mL of water and filtered.

Molisch's test: 2 mL of filtrate and added few drops of alcoholic solution of α-naphthol are added after well shaken of mixture added few drops of concentrated sulphuric acid along the side of the test tube. violet color indicates the presence of carbohydrates.

Fehling's test: After boiling, 1mL of filtrate on the water bath with the addition of 1 mL each Fehling's solution A and B. The indication of red color precipitate shows the presence of sugar.

Benedict's test: 0.5 mL of Benedict's reagent into the 0.5 mL of extract and the mixture was boiled 2 minutes colored precipitate indicates the presence of sugar

Borntrager's test: 2 mL of hydrolysate are taken and 3 mL of chloroform are added and then shaken well after few minutes separated the chloroform layer and added 10% of ammonia. The presence glycosides indicate by the pink color of the solution.

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Fats and oils test:

Spot test: Take a small quantity of extract and press it between two filter papers. The stain of oil indicates the presence of oil.

Saponification test: Add a few drops of 0.5 N alcoholic potassium hydroxide solution and phenolphthalein along the side of the test tube then heated the mixture for few minutes on to the water bath. The presence of soap indicates oil and fixed oil.

RESULTS AND DISCUSSION

Our results are in agreement with previous findings. The genus Buxus represents more than 200 new steroidal alkaloids (Cordell, 1981). Many alkaloids of *Buxus papillosa* C.K. Schneid. especially triterpenoids are known to have inhibitory activity against acetyl cholinesterase and butyryl cholinesterase and are known for pharmacological activities (Atta-Ur-Rahman, 2001). In this study, we analyzed the presences of phytochemicals in *B. paplliosa* C.K. Schneid. like alkaloids, phenolic compounds, flavonoids, carbohydrates, glycosides, fats, protein, and amino acid. We detected phytochemicals from the extracts of different solvents (acetone, methanol, chloroform and water). The phytochemical screening of alkaloidal was achieved best with Wagner's reagent test rather than Hager's, and Mayer's reagent test. Identification of phenolic compounds and flavonoids were best achieved with the help of lead acetate and alkaline reagent test while ferric chloride gave the moderate result. The proteins and amino acids tests yielded moderates results with Millon's reagent, Biuret reagent while achieved better results from Ninhydrin reagent test. Carbohydrates and glycosides tests were obtained with Molisch's, Fehling's, and Borntrager's test while the best result achieved by Benedict's test. Saponins were detected with the help of foam test. The presence of fats and oil in *B. paplliosa* C.K. Schneid. were achieved by spot and saponification tests (Table 1). The Physico-chemical analysis was successfully carried out by loss and drying weight of ash, solubility in water and nitric acid (table 2). The physical state of ash was achieved into the fine powder and grey in color while the highest solubility was found in water which is 8.8% rather than acid.

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Table 1. Qualitative phytochemical screening of *B. paplliosa*

S.NO.	Phytochemical test	Acetone	Methanol	Chloroform	Water
1.	**A. Alkaloidal Tests**				
	Mayer's Reagent	+	++	++	++
	Wagner's Reagent	+++	+++	+++	+++
	Hager's Reagent	++	++	+++	+++
2.	**B. Phenolic compounds and flavonoid Tests**				
	Ferric chloride	+++	+	+++	++
	Lead acetate	+++	+++	+++	+++
	Alkaline reagent	+++	+++	+++	+++
3.	**C. Protein and amino acid test**				
	Millon's reagent	-	-	+	+
	Biuret reagent	+	++	-	-
	Ninhydrin reagent	-	+++	++	+
4.	**D. Saponins**				
	Foam Test	+++	+++	+++	++
5.	**E. Carbohydrates and Glycosides**				
	Molisch's test	++	++	+++	+++
	Fehling's test	--	++	-	+++
	Benedict's	++	+++	++	+++
	Borntrager's	++	++	++	+++
6.	**F. Fats and Oils test**				
	Spot test	+++	++	++	++
	Saponification test	+++	+++	++	++

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Table 2: Physio-chemical analysis of *B. paplliosa* leaves

S.NO	Physio-chemical Tests	Results
1	Physical state of ash	fine powder
2	Color of ash	Grey
3	% of loss on drying	8 %
4	% of ash content	6.7 %
5	% of moisture content	5.5 %
6	Nitric Acid insoluble	0.92%
7	Water soluble	8.8%

CONCLUSION

The leaves of *B. paplliosa* C.K. Schneid. showed the presence of certain chemical compounds such as alkaloids, phenolic compounds, flavonoid, protein, saponins, carbohydrates, fats, and oils. There is no doubt that these phytocontents are medicinally important.

DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare

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