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PHYTOCHEMICAL AND BIOLOGICAL EVALUATION OF *POLYGONUM AMPLEXICAULE* RHIZOME EXTRACT

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ABSTRACT

Medicinal plants have long been recognized for their applications in pharmaceutical, cosmetic, agriculture and food industry. The medicinal plants of genus *Polygonum* have far been used for a variety of purposes including the treatment of infectious diseases, inflammatory conditions, gastrointestinal disorders and cancer. *Polygonum amplexicaule* has folk medicinal use in Pakistan for such diseases. Since, the discovery of therapeutic agents depends on the knowledge of chemical constituents of the plant hence the present study was designed to evaluate the phytochemicals present in this specie's rhizome with respect to their importance in the field of phytomedicines. Different phytochemicals were analyzed qualitatively by using various assays. Antitumor and brine shrimp cytoxicity assay were performed along with tyrosinase inhibition assay to check the suitability of the extract for cosmetics. The qualitative analysis of phytochemicals reveals the presence of various secondary metabolites that includes alkaloids, steroids, cardiac glycosides, saponins, fixed oils and fats, phenolic compounds including flavonoids, saponins, tannins and terpenoids, gums and mucilages and anthraquinones, while phytosterols and phlabatannins have not been detected. The rhizome extract has shown significant cytotoxic and antitumor activities with 13.57µg/mL IC_{50} towards brine shrimp assay. The tyrosinase inhibition assay validates its suitability for cosmetics with 64.6% tyrosinase inhibition at 10µg/ml treatment. The phytochemicals evaluated in the rhizome extract of *P. amplexicaule* and its biological activities validates its folkloric use and prospects its future use towards isolation of therapeutic agents.

Keywords: Phytochemicals, biological screening, *P. amplexicaule,* Rhizome extract.

INTRODUCTION

Plants are very expedient and self-generating machines, producing a range of active poisonous and medicinal compounds. These active constituents are actually plant secondary metabolites and a particular combination of such metabolites may be taxonomically distinct attributing uniqueness of medicinal actions to particular plant species or even higher taxa (Ahmad *et al.,* 2011; Parekh *et al.,* 2007, 2006). These natural products can be obtained from any part of the plant i.e., roots, rhizomes, shoots, leaves, bark, flowers, fruits or seeds but it solely depends upon the nature of compounds as presence of some compounds may be restricted to some parts but not others or there may be concentration differences (Cragg and Newman, 2001).

According to World Health Organization consultative group, a plant (tree, herb or shrub, fresh or dried) with at least one of its parts constituting substances which can be employed for therapeutic purposes or which are precursors for the manufacturing of useful drugs is known as medicinal plant (Bernhoft, 2008). The use of medicinal plants as the treatment of several ailments dates back to prehistory and people of all continents have this old tradition (Mungole and Chaturvedi, 2011). About 80 per cent population around the world depends on traditional use of plant based pharmaceuticals for primary health-care needs, especially in the rural areas (Akinmoladun *et al.,* 2007). Their importance can be inferred from the fact that 12 out of the world's 25 popular pharmaceutical agents are derived from natural products (Ahmad *et al.,* 2011).

One of the most medicinally important plant family Polygonaceae, comprises approximately 48 genera and about 1200 species (Sanchez and Kron, 2008; Freeman and Reveal, 2005). The Polygonaceae family is consisted of numerous important medicinal plants with broad range of biological activities and intriguing phytochemical constituents (Sivakumar *et al.,* 2011). Regarding this family, several phytochemical surveys have been published disclosing the occurrence of bioactive phytoconstituents such as flavonoids, essential oils, tannins, triterpenoids, unsaturated sterols, steroidal sapogenins (saponins) and alkaloids (Ya-xiang *et al.,* 2008). Members of the genus *Polygonum* are distributed throughout the world and have found use as herbs and medicinal plants. Out of the 60 species distributed throughout the world, about 20 species of the genera

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Polygonum L. are found in Pakistan (Qaiser, 2001). Many species of the genus *Polygonum* are well-known for their therapeutic values throughout the world because of several beneficial substances.

Polygonum amplexicaule is a perennial herb with horizontal rhizomes found in shady grassy places in mountain slopes, forests on mountain slopes, grassy slopes in valleys and on forest margins (1000-3300m) of North Pakistan, Kashmir, India, Nepal and Bhutan (Lie *et al.,* 2003). *P. ampelxicaule*, commonly known as maslohar, is traditionally used as a whole plant (Robinson *et al*., 2011). In the form of tea, it is used to cure dysentery and heart problems while it is also used for the treatment of fever, menstruation, leucorrhoea and curing ulcer (Matin *et al.,* 2001). It has also been reported that *P. amplexicaule* D. Don's root sap is extracted and applied to cure fresh wound in the eyes (Uniyal *et al.,* 2006).

Since, in Pakistan, *P. amplexicaule* is not only used to cure dysentery and heart problems whereas it is also used for the treatment of fever, menstruation, leucorrhoea and curing ulcer (Matin *et al.,* 2001) and to the best of our knowledge, there are no reports about the phytochemical analysis of this species, so the current study was conducted to assess the qualitative and quantitative levels of nutrients that includes carbohydrates, proteins, specific vitamins and secondary metabolites which would be helpful in further drug development and to determine the precise concentration of phytochemicals and nutritional components of this plant species towards its folk medicinal use.

MATERIALS AND METHODS

Preparation of Plant material

Rhizomes of the *P. amplexicaule* were collected from Murree Hills Islamabad, washed, cut in to pieces, dried and grounded into a crude powder. For phytochemical analysis, the Crude Methanolic Extract (CME) was prepared by soaking 100g of the rhizome powder in methanol (300mL) for four days and then filtered by using Whatmann filter paper. The final product was concentrated at 45°C under low pressure in a rotary evaporator. Concentrated extract was stored in air tight bottles until analyzed. The aqueous extract (AE) of the sample was also prepared by the same procedure.

Phytochemical Analysis

Chemical constituents of the CME and AE were determined by qualitative and quantitative phytochemical analysis.

Qualitative Phytochemical Analysis

The pulverized rhizome sample, CME and/or the aqueous extracts were employed to carry out chemical tests for the identification of phytoconstituents by using standard procedures as described by Sofowora (1993), Harborne (1973) and Trease and Evans (1989).

Detection of carbohydrates: By dissolving the CME of the rhizome sample (100mg) in 5mL of water and filtering, the presence of carbohydrates was detected. Then, the filtrate was subjected to Molish's test, Fehling's test and Benedict's test.

Detection of proteins and free amino acids: The CME of the rhizome was subjected to Millon's test, Biuret test and Ninhydrin test for proteins and amino acids.

Detection of glycosides: To identify glycosides, after hydrolyzation with concentrated hydrochloric acid on water bath for 2hours, and then filtration, 50mg of CME of the rhizome was further used to Borntrager's test and Legal's test. Keller-Killani test and Baljet test were used to detect the presence of cardiac glycosides while Forth formation test was used to detect the presence of saponin glycosides (Venkateswarlu *et al.,* 2010). The presence of flavonol glycosides was detected by Magnesium and hydrochloric acid reduction test.

Detection of alkaloids: For the detection of alkaloids, aqueous rhizome extract (50mg) was stirred with few mL of dilute hydrochloric acid and filtered. The filtrate was then tested for Mayer's test, Wagner's test and Dragendroff's test (Harborne, 1973).

Detection of anthraquinones: For detection of anthraquinones, Borntrager's test was used (Harborne, 1973).

Detection of Fixed Oils and Fats: The presence of fixed oils and fats was detected by Spot test and Saponification test (Harborne, 1973).

Detection of Flavonoids: Sodium hydroxide test and concentrated sulphuric acid test detected the presence of flavonoids (Venkateswarlu *et al.,* 2010).

Detection of phlabatannins: An aqueous extract of the rhizome sample was boiled with 1 per cent aqueous hydrochloric acid. Deposition of a red precipitate was taken as confirmation for the presence of phlabatannins (Harborne, 1973).

Detection of phenolic compounds and tannins: The phenolic compounds were detected by Ferric chloride test, Gelatin test and Lead acetate test (Harborne, 1973).

Detection of phytosterols: The presence of phytosterols was detected by Libermann-Burchard's test and Salkowski reaction (Venkateswarlu *et al.,* 2010).

Detection of saponins: Foam test was applied for the detection of saponins in the rhizome sample (Harborne, 1973).

Detection of steroids: Two mL of acetic anhydride was added to 0.5g of aqueous rhizome extract with 2ml H_2SO_4. The color changing from violet to blue or green indicated the presence of steroids.

Detection of Terpenoids: Salkowski test was applied for determination of terpenoids in which formation of a reddish brown coloration of the interface was taken as positive sign for the presence of terpenoids.

Determination of Biological Activities:

Cytotoxic, antitumor and Tyrosinase Inhibition Assay of crude methanolic extract were determined.

Cytotoxicity by Brine Shrimp lethality assay: The method used for brine shrimp lethality bioassay was reported by (Ahmad *et al.,* 2007). Samples were prepared by dissolving 20mg of the CME in methanol to make 2ml (10,000ppm) stock solution. From the stock solution further concentrations (1000ppm, 100ppm and 10ppm) were made. As a positive control, MS-222 (Tricaine methane sulfonate) a common fish anesthesizer, was used at concentration of 1000, 100 and 10ppm. Artificial seawater was prepared by dissolving 28gm commercial sea salt in 1L distilled water with continuous stirring. Brine shrimps (*Artemia salina*) eggs were hatched in rectangular dish (22×32 cm) filled with prepared seawater. In the larger compartment, which was covered with aluminium foil to make the dark conditions the eggs (25mg) were sprinkled while the smaller compartment was illuminated. From the lightened side phototropic nauplii (brine shrimp larvae) were collected with the help of pipette that are separated by the divider from their shells after 24 hours of starting hatching. Against a light background the brine shrimp larvae can be counted macroscopically in the stem of pipette. The vials were maintained at room temperature 25°C to 28°C under illumination. The numbers of shrimps that were survived were counted with the help of magnifying glass after 24 hours. The resulting data was analyzed by probit analysis for the determination of LD_{50} value.

Antitumor Assay: The potato disc method was used for antitumor activity of plant extract as described and modified by Ahmad *et al.* (2007). Single colony from culture plate of *Agrobacterium tumefaciens* (At-10) was inoculated and culture was grown for 48hours at 28°C in shaking incubator. Sample was prepared of 10, 100 and 1000ppm. Positive control was vincristine. Ten petri plates were used for this assay. Two plates for each concentration (1000, 100 and 10ppm) and two plates for each control. The Lugol's solution was poured onto each disc for staining purpose and was allowed for 15minutes to diffuse. The destained portions of discs are actually tumors. Numbers of tumors per disc were counted. Following formula is used to determine the percentage inhibition of each concentration.

Inhibition (%) = 100 - [No of tumors with sample/No of tumors with control] x100

Tyrosinase Inhibtion Assay

After evaluation of phytochemicals in the CME of the *P. amplexicaule* rhizome, the sample was further evaluated for its suitability in the field of cosmetics by conducting Tyrosinase inhibition assay (Vanni *et al.,* 1990) for different concentrations (10, 100 and 1000ppm) of the CME of *P. amplexicaule*. Seventy units of tyrosinase were added to each 0.5mL of the plant extract. Then, 0.5mL of Sodium Phosphate buffer having pH 6.8 was mixed to the mixture and was incubated at 37°C for at least 10min. After incubation, the absorbance was measured spectrophotmetrically at 475nm. Kojic acid, the tyrosinase inhibitor was used as standard. The control reaction was accompanied with Sodium Phosphate buffer and tyrosinase. The IC_{50} value for each sample was calculated and % inhibition was found out by the following formula:

$$\text{Inhibition (\%)} = (A-B)/A \times 100$$

Where;

A = Spectrophotometric absorbance at 475nm (without test sample)

B = Spectrophotometric absorbance at 475nm (with test sample)

RESULTS AND DISCUSSION

The dried, powdered form of the rhizome of the *P. amplexicaule* was subjected to various chemical tests both qualitatively and quantitatively for the identification of pharmacologically active phytoconstituents.

Qualitative Analysis of Phytochemicals

The present study reveals the occurrence of various bioactive phytochemicals in the rhizome CME of *P. amplexicaule* as depicted by the table 1-2. The qualitative analysis of phytochemicals discloses the presence of carbohydrates, proteins and free amino acids, alkaloids, steroids, cardiac glycosides, saponins, fixed oils and fats, phenolic compounds including flavonoids, saponins, tannins and terpenoids, gums and mucilages and anthraquinones, while phytosterols and phlabatannins have not been detected.

Phytochemicals being the part of both traditional and modern system of medicament are participating actively in the treatment of various ailments. The qualitative analysis of the various bioactive substances allows us to detect the therapeutically active plant secondary metabolites, facilitating their quantitative estimation and qualitative separation as well. This analysis may lead the

Table 1. Results of qualitative analysis of carbohydrates, proteins and free amino acids in the rhizome of *P. amplexicaule.*

S. No.	Qualitative tests		Presence /Absence
1	Tests for Carbohydrates		
	Molish's Test		+
	Fehling's Test		+
	Benedict's Test		+
2	Tests for Proteins and free amino acids		
	Millon's Test		+
	Biuret Test		+
	Ninhydin Test		+
3	Tests for Glycosides		
	Glycosides	Borntrager's test	+
		Legal's test	+
	Cardiac glycosides	Keller-Killani test	+
		Baljet test	+
	Saponin glycosides	Forth formation test	+
	Flavonol glycosides	Hydrochloric acid reduction test	_

Table 2. Qualitative analysis of various phytochemicals present in rhizome sample of *P. amplexicaule.*

S. No.	Phytochemicals	Name of Test	Presence/Absence
1	Alkaloids	Mayer's test	+
		Dragendroff's test	+
		Wagner's test	+
2	Anthraquinones	Borntrager's test	+
3	Flavonoids	Sodium hydroxide test	+
		Sulphuric acid test	+
4	Gums and Mucilages		+
5	Phalabatannins		_
6	Phenolic compounds and Tannins	Ferric chloride test	+
		Gelatin test	+
		Lead acetate test	+
7	Phytosterols	Libermann-Burchard's test	_
		Salkowaski reaction	_
8	Saponins	Foam test	+
9	Steroids		+
10	Terpenoids	Salkowaski test	+

+) Positive result shows presence; -) Negative result shows absence

scientists to the drug discovery and development. The evaluation of chemical constituents is done qualitatively through various standard methods. In our study, most of the cases, the solvent of extraction was alcoholic in nature, either ethanol or methanol. Because of the higher polarity of these solvents, most of the phytoconstituents get easily dissolved in it, so they are mostly favored by many biochemists as a solvent for the extraction of numerous phytoconstituents (Sermakkani and Thangapandian, 2010; Ananthakrishnan, 2002).

The qualitative analysis is depicting the presence of almost all those vital phytoconstituents which have been responsible for numerous pharmacological activities of the medicinal plants. Various reports have confirmed the antimicrobial activity of alkaloids, flavonoids, phenolic compounds, saponins, tannins and terpenoids found in various species of genus Polygonum including *P. aviculare, P. barbatum, P. glabrum*, etc. (Sivakumar *et al.,* 2011; Salama and Marraiki, 2010; Mazid *et al.,* 2009; Cowan, 1999). As the presence of steroidal compounds in the medicinal plant is of great attention and worth for the pharmacy world owing to their relationship with compounds like sex hormones, the present sample may also be helpful because of the presence of steroids (Egwaikhide *et al.,* 2010). Another phytochemical, anthraquinone present in the rhizome is renowned for possessing anti-inflammatory, astringent, bactericidal,

Table 3. Brine shrimp bioassay results of crude methanolic extract of rhizome of *P. amplexicaule* [a-c]

S. No.	Treatments (mg/ml)	No of shrimps survived			Average	% Death	LD_{50} µg/mL
1	10	6	4	6	5.3	47	
2	100	4	2	1	2.3	77	
3	1000	0	0	0	0.0	100	13.57
4	Methanol	8	9	8	8.3	17	
5	MS-222	0	0	0	0	100	

a=Against brine shrimp in vitro. b= Data is based on mean values of three replicate. c= MS-222 reference drug.

Table 4. Antitumor activity of crude methanolic extract of rhizome of *P. amplexicaule*[a-e]

S. No.	Treatments (mg/ml)	Mean value of No of tumors, ± S.D	%age tumor inhibition
1	CME (10)	18±1.73	5.26
2	CME (100)	10.6±2.33	45.07
3	CME (1000)	2.3±0.33	88.08
4	Vincristine	0.0±0.0	100
5	DMSO	10±1.73	-

a= Potato disc assay. b= Vincristine as standard drug. c= DMSO as solvent and vehicle control.
d= % Inhibition = 100 - [No of tumors with sample/No of tumors with control x 100].
e= Mean value is replicate of three.

Table 5. Tyrosinase inhibition assay of CME of rhizome of *P. amplexicaule.*

S. No.	Concentrations of the rhizome extract	Mean value of Absorbance 475 nm	Percentage Inhibition = (A-B)/A ×100
1	10 µg/mL	0.313	64.6%
2	100 µg/mL	0.312	65.8%
3	1000 µg/mL	0.277	68.7%
4	Control	0.885	--

A = absorbance at 475 nm without test sample, B = absorbance at 475 nm with test sample, Kojic Acid was used as Standard.

adequate antitumor and purgative properties (Muzychkina, 1998). The qualitative analysis of various therapeutically active phytoconstituents validates the folkloric value of *P. amplexicaule* and this data suggests further evaluation of all those phytochemicals which have been found in the rhizome sample.

Biological activities:
Biological activities were determined as cytotoxic, antitumor, tyrosinase inhibition assay.

Cytotoxic Activity of *P. amplexicaule:* Cytotoxic activity of *P. amplexicaule* rhizome was determind by using brine shrimps assay. The results of the cytotoxicity of crude methanolic extract (CME) are given in table 3. Crude extracts of plants with LC_{50} values less than 250µg/mL were considered as significantly active and these extracts had the potential for further investigations (Rieser *et al.*, 1996). Different concentrations (10ppm, 100ppm and 1000ppm) of crude methanolic extract (CME) of *P. amplexicaule* rhizome showed the different percentage of brine shrimps death. As negative control methanol was used and as positive control MS-222 was used. Results have shown that the brine shrimp survival is inversely proportional to the concentration of extract. At 1000µg/mL concentration of CME the percentage death of shrimps was 100.

Hussain *et al.* (2010) checked the cytotoxicity of the six species of the family *Polygonaceae* by using the brine shrimp bioassay. The cytotoxic activity of the crude methanolic extract was determind at 1000, 100 and 10µg/mL against *Artemia salina*. Results of the assay demonstrated that *Rhuem australe* had zero value of LD_{50} and showed no cytotoxic activity. *Polygonum plebejum* showed the moderate activities with LD_{50} values of 35.0 and 11.06.

Hussain *et al.*, 2007 also used the brine shrimp assay for cytotoxic activity of *Fagonia cretica*. Results showed the cytotoxic activity of the extract with LD_{50} value of 118.89ppm. Wanyoike *et al.* (2004) determined the

cytotoxic activity of five plants used in Nairobi and Kenya for the treatment of malaria by using the brine shrimp lethality assay. Results showed that methanolic extract of *Cyathula polycephala, Pentas longiflora* and *Pittosporum lanatum* showed significant cytotoxic activities while the *Cyathula cylindrical* and *Albizia gummifera* showed no significant cytotoxicity. The presence of cytotoxic as well as antimicrobial activities enhanced the medicinal value of *P. amplexicaule.*

Antitumor activity of *P. amplexicaule* rhizome: Antitumor activity of *P. amplexicaule* rhizome was determind by potato disc method. Potato disc assay was carried out by using *Agrobacterium tumafaciens* strain At 10 for tumor induction and different concentrations of the crude extract were used for the tumor inhibition. Different concentrations (10ppm, 100ppm and 1000ppm) of crude methanolic extract (CME) of *P. amplexicaule* rhizome showed the different percentage of inhibition. Results are shown in table 4. DMSO was used as negative control and it did not inhibit the growth of tumors. Vincristine was used as a positive control and it had shown 100% tumor inhibition. Results have shown that percentage inhibition of tumors increases with increase in concentration of CME of *P. amplexicaule* rhizome.

Twenty percent and greater than twenty percent inhibition of tumors is considered as a significant value for plant extracts (Ferrigni *et al.*, 1982). Maximum inhibition 88 percent of tumors was observed by 1000ppm concentration of CME that was compareable to standard drug vincristine. Hussain *et al.* (2006) also used At 10 strain and checked the antitumor activity of methanolic extract of aerial parts of Fagonia cretica. Maximum percentage inhibition of Fagonia cretica was 77.04% at 1000ppm concentration. This showed that CME of *P. amplexicaule* rhizome has more antitumor activity as compared to *Fagonia cretica*. The antitumor potential of *P. amplexicaule* showed that it is useful for preparing the chemopreventive drugs in pharmaceutical industry.

Tyrosinase Inhibition Assay

The tyrosinase inhibition assay has been regarded as a key experiment for the investigation of suitability of a plant extract for cosmetic purposes as it involves the inhibition of tyrosinase enzyme which has been known as essential enzyme involved in melanin biosynthetic pathway. If a plant extract inhibits the tyrosinase activity, it is actually inhibiting melanogensis thus involved in skin-lightning process and thus may be a good choice in the cosmetics industry. The results of the tyrosinase inhibition assay have been presented in table 5 showing inhibitory action of the plant extract of greater than 50% at treatment of 10mg/mL which is considered as significant activity.

The accomplishment of healthy fair skin complexion and protection from darkening has always been the demand of cosmetic industry all over the world especially, in many parts of Asia. Many cosmetic companies have been involved in the preparations of elastase and melanogenesis inhibitors because of their prospective as active agent for skin-lightning and antiwrinkle properties. Nowadays, because of the many side-effects caused by artificial preparations, the increased demand for natural products including plant extracts has grown for antiwrinkle, depigmenting and other cosmeceutical purposes (Wang *et al*., 2006; Kiken and Cohen, 2002). Using plant extracts such as *Morus alba* (Lee *et al.*, 2002), *Areca catechu* (Lee and Choi, 1999), *Glycyrrhiza glabra* (Vanni *et al.*, 1990), cosmetic preparations have been used as skin-lightning agents.

Future Prospective

This study is a good start for disclosing the actual value of folkloric remedies as well as the isolation of valuable secondary metabolites from this plant species. This study suggests further investigation of pharmacologically active constituents and their extraction for drug discovery and development primarily in the field of antioxidant extraction as well as antimicrobial agents as present study has evaluated high phenolic and flavonoids contents in addition to alkaloids. Further investigations should also be required in vivo and in vitro for the cosmecutical preparations as this plant may be a good choice for cosmetic industry.

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REFERENCES

Ahmad, B., Khan, I., Bashir, S., Azam, S. and Hussain F. 2011. Screening of *Zizyphus jujuba* for antibacterial, phytotoxic and haemagglutination activities. Afr J. Biotechnol. 10:2514-9.

Ahmad, MS., Hussain, M., Hanif, M., Ali, S. and Mirza, B. 2007. Synthesis, Chemical Characterization and Biological Screening for Cytotoxicity and Antitumor Activity of Organotin (IV) Derivatives of 3,4-Methylenedioxy 6-nitrophenylpropenoic Acid. Molecules. 12:2348-2363.

Akinmoladun, Ibukun AC., Afor, E., Obuotor, OE. and Farombi, O. 2007. Phytochemical constituents and antioxidant activity of extract from the leaves of the *Ocimum graticcimum*. Sci Res Essay. 2:163-6.

Ananthakrishnan, TN. 2002. Chemodynamics of insect plant interactions. In: Insects plants and molecular interactions. 6:39-46.

Bernhoft, A. 2008. A brief review on bioactive compounds in plants. In: Bioactive compounds in plants – benefits and risks for man and animals. Aksel Bernhoft editor Oslo: The Norwegian Academy of Science and Letters.11-17.

Cowan, MM. 1999. Plant products as antimicrobial agents. Clin Microbiol Rev. 12:564-82.

Cragg, GM. and Newman, DJ. 2001. Natural product drug discovery in the next millennium. Pharm Biol. 39:8-17.

Egwaikhide, PA., Bulus, T. and Emua, SA. 2010. Antimicrobial activities and phytochemical screening of extracts of the fever tree, *Eucalyptus globulus*. E J E A F Che. 9:940-45.

Ferrigni, NR., Putnam, JE., Anderson, B., Jacobson, LB., Nichals, DE., Powell. RG. and Smith, CR. 1982. Modification and evaluation of the Potato disc assay and antitumor screening of Euphorbiaceae seeds. J. Nat. Prod. 45(6):679-86.

Freeman, CC. and Reveal, JL. 2005. Polygonaceae. Fl. N. Amer., Editorial Committee. Oxford University Press, Oxford, New York, USA. 5:216-21.

Harborne, JB. 1973. Phytochemical methods. Chapman and Hall, Ltd. London. 49-188.

Hussain, F., Badshah, L. and Dastigir, G. 2006. Folk medicinal uses of some plants of South Wazirastan Pakistan. Pak. J. PL. Sci. 12(1):27-40.

Hussain, F., Mukaram, S. and Sher, H. 2007. Traditional resource evaluation of some plants of Mastuj, district Chitral, Pakistan. Pak. J. Bot. 39(2):339-354.

Hussain, S., Jamil, M., Ullah, F., Khan, A., Ullah, F., Arfan, M., Ahmad, S. and Khatoon, L. 2010. Antimicrobial and antioxidant activities of the plant Heliotropium strigosum. Afri J Biotech. 9(45):7738-7743.

Kiken, DA. and Cohen, DE. 2002. Contact dermatitis to botanical extracts. American J Contact Dermatitis. 13:148-152.

Lee, KK. and Choi, JD. 1999. The effects of areca catechu L extract on anti-aging. International J Cosmetic Sci. 21:285-295.

Lee, SH., Choi, SY., Kim, H., Hwang, JS., Lee, BJ., Gao, JJ. and Kim, Y. 2002. Mulberroside F isolated from the leaves of *Morus alba* inhibits melanin biosynthesis. Biological and Pharmaceutical Bulletin. 25:1045-1048.

Lie, H., Wang, Z. and Liu, Y. 2003. Review in the studies on tannins activity of cancer prevention and anticancer. Zhong Yao Cai. 26(6):444-448.

Matin, A., Khan, MA., Ashraf, M. and Qureshi, RA. 2001. Traditional use of herbs, shrubs and trees of Shogran valley, Manshera, Pakistan. Pak J Biol Sci. 4:1101-7.

Mazid, MA., Datta, BK., Nahar, L., Bashar, SAMK., Bachar, SC. and Sarker, SD. 2009. Antinociceptive, anti-inflammatory and diuretic properties of *Polygonum barbaratum* (L.) Hara var. *barbata*. Rev Bras Farmacogn. 19:749-754.

Mungole, A. and Chaturvedi, A. 2011. Determination of antibacterial activity of two medicinally important Indian Taxa. Der Pharma Chemica. 3:83-89.

Muzychkina, RA. 1998. Natural anthraquinoues: Biological and physiological properties. Ed. Tolstikov, GA. PHASIS, Moscow, Russia.

Parekh, J. and Chanda, S. 2007. In vitro antibacterial activity of the crude methanol extract of Woodfordia fruticosa kurz. flower (lythraceae). Braz. J. Microbiol. 38:204-7.

Parekh, J., Karathia, N. and Chanda, S. 2006. Evaluation of antibacterial activity and phytochemical analysis of *Bauhinia variegata* L. bark. Afr. J Biomed. Res. 9:53-6.

Qaiser, M. 2001. Polygonaceae. In: Flora of Pakistan. Eds. Ali, SI. and Qaisar, M. Department of Botany, Karachi University and Missouri Botanical Garden, St. Louis, Missouri, USA. 205:76-111.

Rieser, MJ., Gu, ZM., Fang, XP., Zeng, L., Wood, KV. and McLaughlin, JL. 1996. Five novel monotetrahydrofuran ring acetogenins from the seeds of Annona muricata. J. Nat. Prod. 59:100-108.

Robinson, MM. and Zhang, X. 2011. Traditional medicines: global situation, issues and challenges. In: The World medicines situation report 2011 (3rd edi.). World Health Organization, Geneva. 1-14.

Salama, HMH. and Marraiki, N. 2010. Antimicrobial activity and phytochemical analysis of *Polygonum Aviculare* L. (Polygonaceae), naturally growing in Egypt. Saudi J Biol Sci. 17:57-63.

Sanchez, I. and Kron, KA. 2008. Phylogenetics of Polygonaceae with an emphasis on the evolution of Eriogonoideae. Syst Bot. 33:87-96.

Sermakkani, M. and Thangapandian, V. 2010. Phytochemical screening for active compounds in *pedalium murex* l. Rec Res Sci Tech. 2:110-114.

Sivakumar, P., Senthilkumar, KL. and Varma, JP. 2011. Phytochemical studies on *Polygonum glabrum* (willd.). IJPBS. 2:1.

Sofowora, A. 1993. Screening plants for bioactive agents. In: Medicinal plants and traditional medicine in Africa. Ed. Sofowora, A. Spectrum Books Ltd., Sunshine House, Ibadan. 81-93:134-156.

Trease, GE. and Evan, WC. 1989. Pharmacognsy (11th edi.). Brailliar Tiridel Can. Macmillian Publishers.

Uniyal, SK., Singh, KN., Jamwal, P. and Lal, B. 2006. Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalaya. J Ethnobiol Ethnomed. 2:1-14.

Vanni, A., Gastaldi, D. and Giunata, G. 1990. Kinetic investigations on the double enzymatic activity of the tyrosinase mushroom. Annali di Chimica. 80:35-60.

Venkateswarlu, BS., Chandira, RM., Ajay, T., Bhowmik, D., Jayakar, CB. and Kumar, KPS. 2010. Formulation Development and evaluation of Fast Dissolving Tablets of Carvedilol. J. Chem Pharm Res, 2:196-210.

Wang, KH., Lin, RD., Hsu, FL., Huang, YH., Chang, HC., Huang, CY. and Lee, MH. 2006. Cosmetic applications of selected traditional Chinese herbal medicines. J. Ethnopharmacol. 106:353-359.

Ya-xiang, F., Xiang-rong, H., Jun-chao, L. and Xiang-xin, L. 2008. Study on effective composition analysis and antibacterial effects of herb *Polygonum perfoliatum*. Prog Vet Med. 29:1.

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