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PHYTOCHEMICAL ANALYSIS OF *Lepidium didymum*

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ABSTRACT

Phytochemical screening is a chief phase, which results in the isolation of novel and new compounds. In the current study, Lepidium didymum roots, stems and leaves extracts in N-hexane, ethanol and water were examined for phytochemicals in order to investigate various groups of phytochemicals. Secondary metabolites like cardiac glycosides, phlobatannins, tannins, steroids, flavonoids, saponins, terpenoids, proteins and carbohydrates in Lepidium didymum were investigated. N-hexane, ethanolic and Aqueous extracts of stems revealed the presence of flavonoids, carbohydrates, anthra-glycosides and saponins. Likewise, the N-hexane ethanolic, and aqueous extracts of roots revealed the existance of carbohydrates, terpenoids, anthra-glycosides, cardiac glycosides and saponins. Phlobatannins and Hager were not present from the given extracts in the plant. Hence, it was concluded that Lepidium didymum may be employed in medicinal purposes as it is rich in secondary metabolites.

Key words: Carbohydrates, cardiac glycosides, Lepidium, saponins, terpenoids.

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INTRODUCTION

Medicinal plants have a great role in improvement of health of community and individuals. Since ancient times, the world has relied upon the use of herbal drugs for the treatment of different type of diseases across (Prajapati and Prajapati, 2002; Shinwari *et al*., 2006). Over 80% of the population in the developing world depends upon the traditional medicinal plants extracts for the provision of health coverage (Fransworth and Soejarto, 1998; Latif *et al*., 2003). Study of

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medicinal plants and the invention of synthetic drugs is much needed, considering the indication of AIDS (acquired immune deficiency syndrome) like fatal diseases and the threats of newly arising diseases like bird flu and SARS (Severe acute respiratory syndrome) etc. Fractional plants and plant extracts have been a good source of natural phytochemicals and herbal medicines over the years (Robert and Lewis, 1998; Brodie, 2010). Data obtained from various studies showed that an estimated thirty five thousand to seventy thousand species of plants are used in folk medicines worldwide (Lewington, 1990). *Lepidium* L. is a large genus of about 250 species, cosmopolitan in distribution on all continents excepting Antarctica. *Lepidium* belongs to *Brassicaceae*, the Mustard family. One of the representative of the genus is *L. didymum* (common name lesser swine-cress), which is a herbaceous annual to biennial plant. The continent of South America is the site from where the origination and geographic distribution of *L. didymum* occurred. *Lepidium* plant is known very well for the production of a variety of natural products that are biologically active. These products include flavonoid, coumarins, phlobatannins and terpenoids etc (Kashani *et al*., 2012)

Species of genus *Lepidium* are well reputed medicinally and work as folk medicinal remedies for healing of various diseases (Abebe, 2013). In India, traditional medicine has valued the plant as a source for the treatment of wounds and allergies. The use of its seeds improves lung function in asthmatics thereby alleviating the asthma symptoms. In Mauritius, the application of *Lepidium sativum* leaf poultice externally on the head and the consumption of the decoction of the whole plant orally has been used for the treatment of headache. Oral consumption of the decoction also helps in relieving of fever (Archana and Anita, 2006). Research data have revealed the efficacy of *L. didymum* leaves for the treatment of other skin disease and diabetes. The present study was undertaken to screen different parts of *L. didymum* for qualitative chemical analysis so that these phytochemicals can be further quantified.

MATERIALS AND METHODS

Chemicals

N-hexane, Distilled water, chloroform, ethyl acetate, olive oil, ferric chloride, ammonia, hydrochloric acid, sulphuric acid, lead, benzene, ethanol, acetate, Hager reagent, glacial acetic acid, nephtol solution, ferric chloride, million reagent and iodine solution.

Glassware

Funnel, bottle, graduated cylinder, beakers, tripod stand, test tubes, beakers, pipette, spirit lamp, test tubes tray, stirrer and aluminum foil.

Plant collection

Leaves, stems and roots of *Lepidium didymum* were collected during October, 2015, from Pakha-ghullam area (ring road) in district Peshawar. Dr. Naveed Akhtar, Plant taxonomist, (Department of Botany, Islamia Collage, Peshawar, Pakistan) identified and characterized the plant to be *Lepidium didymum* L.

Extraction

The plant material was dried for one month in shade at room temperature. The dried plant material (roots, stems and leaves) of *Lepidium didymum* were crushed into a fine powder. The powdered material was soaked for 3 days in n-hexane, water and ethanol and subjected to extraction till plant material exhaustion.

Phytochemical investigation

Different tests were performed on the water, n- hexane and ethanol extracts of roots, stems and leaves of *Lepidium didymum* following the methods of Ali *et al.* (2014).

1. Reducing sugar (Benedict test)

Benedict reagents (2.5ml) and test solution (5ml) were heated for 5 minutes in water bath. Depending on amount of reducing sugar in the solution, the colour of the test solution changed to red, yellow or green (Ali *et al.*, 2014).

2. Flavonoid test

In this test, 3 ml of test solutions was taken and 5ml of NH_3 solution was added to it, followed by the addition of conc. HCl. The appearance of a yellowish color showed the flavonoids to be present.

3. Tannins test (lead acetate test)

3ml of lead acetate were mixed in a test tube with 3ml extract, formation of the precipitate marked the presence of tannins.

4. Terpenoids test

The appearance of reddish brown color, after the mixing of about 5 ml of the test solution, 2 ml of chloroform and 3 ml of concentrated H_2SO_4, showed the terpenoids to be present.

5. Phlobatannins test

Test solution (3ml) was mixed with 1% HCl, formation of red colored precipitate indicated the phlobatannins presence in the test solution.

6. Steroids test (Salkowski's reaction)

Test solution (2ml) was mixed with 2ml conc. H_2SO_4 and 2ml chloroform and shacked well. The acid layer showed yellow greenish florescence while chloroform layer appeared red indicating the presence of steroids.

7. Saponins test

Test solution (10 ml) was added to distilled water (5ml) and vigorously shaken. Thereafter, 3 drops of olive oil were added to it and shaken again. Formation of emulsion showed the existence of saponins.

8. Cardiac glycoside test

Here 2ml of glacial acetic acid containing 1 drop of ferric chloride solution was added to about 5 ml of test solution. Under layer of 1ml of concentrated H_2SO_4 formed violet or brown color which indicated a deoxy-sugar, characteristic of cardinolides sugars.

9. Anthraquinone glycoside (modified Borndragers test)

A few drops of ferric chloride and 5ml of dilute hydrochloric acid was added to 5 ml of test solution and heated for one minute in water bath then cooled. After cooling 2 ml of benzene were added to it and shaken for the separation of organic layer. An equal amount of NH_3 was then added to the solution. Presence of anthraquinone glycoside was indicated by red coloration.

10. Test for alkaloid (Hager's test)

Few drop of Hager mixture was added to 3ml of test solution and mixed. Areddish brown coloration showed the presence of alkaloid.

11. Carbohydrate test (Molish test)

About 3ml of alpha nephtol solution were added into 3 ml of test solution. After vigorous shaking, conc. sulphuric acid was added from the side of test tube. Presence of carbohydrates was shown by the appearance of violet coloration.

12. Starch test (Non-reducing sugar)

Three (3) ml of test solution were added to a few drops of iodine solution. A blue color appeared and vanished on steaming which showed the presence of starch.

13. Proteins test (Millions test)

About 5 ml of million reagents was added to 3ml of test solution and mixed, a white precipitate, which turned brick red on heating, was formed. This indicated the presence of proteins.

14. Anthocyanin test

Here test solution (2ml) was mixed to 2ml of ammonia and hydrochloric acid. The pink red color of the mixture turned into blue violet color which indicated the existence of anthocyanin.

15. Coumarins test (zohra *et al.* 2012)

About 3 ml of ten percent ammonium hydroxide was mixed with 2 ml of test solution which resulted in the formation of yellow color; this indicated the presence of coumarins.

16. Emodins test (Zohra *et al.* 2012)

3 ml of test solution was mixed with 2 ml of NH_4OH and 3 ml of benzene. Appearance of red color indicated that emodins are present.

Table-1: Qualitative phytochemical results of *Lepidium didymum* roots, stem and leaves in N-hexane, ethanol and water extracts

Components	Roots			Stem			Leaves		
	N-Hexane	Ethanol	Water	N-Hexane	Ethanol	Water	N-Hexane	Ethanol	Water
Anthocyanin	-	-	-	-	-	-	-	+++	++
Anthra-glycosides	+	++	+	+	++	++	+++	+	+
Benedict	-	++	++	-	+	-	-	++	++
Carbohydrates	+++	++	++	+++	++	++	+++	++	+++
Cardiac glycosides	+++	++	+	+	+++	-	+	+	-
Coumarins	-	+++	+	-	+++	++	+	+	++
Emodins	-	-	-	-	-	-	-	+	+
Flavonoids	-	++	+	++	+++	+	+	++	+++
Hager	-	-	-	-	-	-	-	-	-
Phlobatannins	-	-	-	-	-	-	-	-	-
Proteins	-	-	+++	-	-	++	-	-	+++
Saponins	+++	++	++	++	++	++	+++	-	++
Starch	-	-	-	-	-	-	-	-	-
Steroids	-	-	-	-	+	-	-	++	-
Tannins	-	+++	-	-	+	+	+	+	++
Terpenoids	+	++	+	++	++	-	-	-	-

- = absence of phytochemicals; += presence of phytochemicals;
++ = moderate concentration; +++ = high concentration

RESULTS AND DISCUSSION

Table-1 represents the qualitative phytochemical results of *Lepidium didymum* roots, stem and leaves extracts in the N-hexane, ethanol and water. These results showed different compounds in *L. didymum* that are medicinally active. Anthraquinone glycosides and Carbohydrates were present in all parts of the plant (Table-1). Hager, phlobatannins and starch were, however, absent at the given extracts in the plant. In N-hexane extract Benedict test was negative for the whole plant while positive in ethanolic and aqueous extracts of roots and leaves.

Tests for proteins were positive in the roots, stems and leaves only in the aqueous extracts. Tests for steroids were positive for stem and leaves in the ethanolic extract only. Emodins and anthocyanin were absent at all extracts in roots and stems. Except the ethanolic

extract from leaves, saponins were present at all extracts in all the plant parts. Tannins tests were positive in all leaves extracts however, it was negative in the N-hexane extract in roots and stems as well as water extract in roots. The table showed that except Hager, phlobatannins and starch, various plant organs showed positive results for many phytochemicals that are medicinally active.

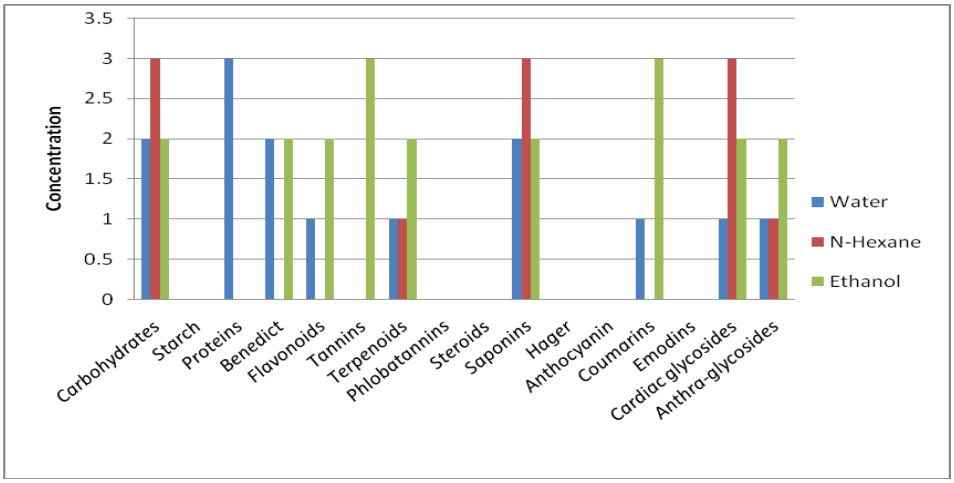

Figure 1. Phytochemicals of roots extracts in ethanol, N-hexane and water. Each bar represents the conc. of coloration for the phytochemicals in these extracts.

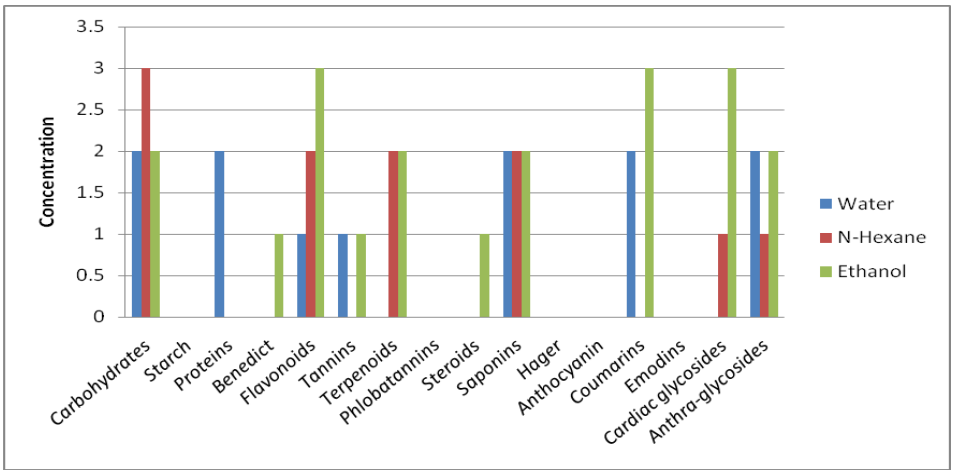

Figure 2. Phytochemicals of stems extracts in ethanol, N-hexane and water. Each bar represents the conc. of coloration for the phytochemicals in these extracts.

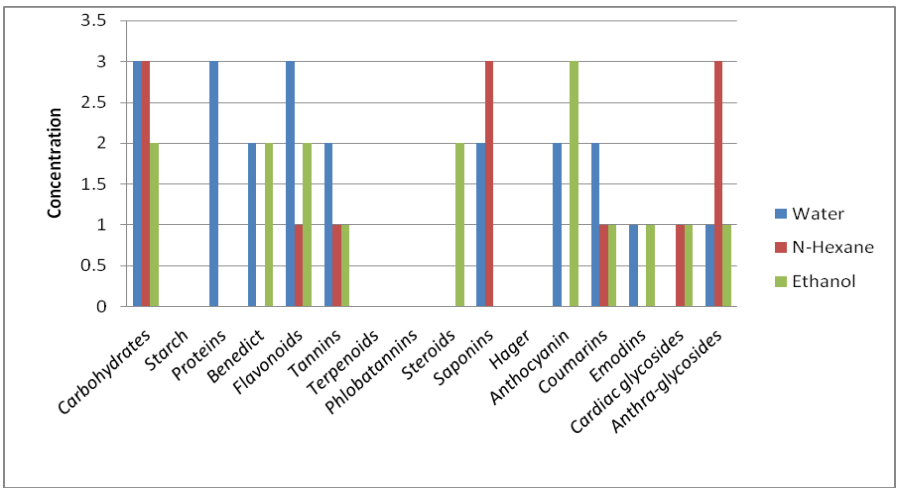

Figure 3. Phytochemicals of leaves extracts in ethanol, N-hexane and water. Each bar represents the conc. of coloration for the phytochemicals in these extracts.

Phytochemicals exploration of the plant extracts has shown the presence of medicinally as well as physiologically active compounds (Sofowora, 1993). Qualitative phytochemical screening test of crude extracts in methanol/chloroform has shown the presence of phytochemicals like carbohydrates, cholesterol, terpenoids, flavonoid, glycosides, steroids, phytosterols, tannins, phenols, proteins, saponins and alkaloids in different plant species (Berehe and Boru, 2014). *Brassica* genus is known to contain different phytochemicals like carbohydrates, alkaloids, glycosides, flavonoids, proteins and tannins (Talreja and Moon, 2014).

Examination of *L. didymum* extracts showed the presence of various phytochemicals like flavonoids, tannins, phenols, glycosides, saponins, terpenoids and steroids. One of the major groups of plant metabolites are phenolic compounds (Singh *et al.,* 2007). According to Han *et al*. (2007), phenolic compounds have biological assets such as antiaging, anti-apoptosis, anti atherosclerosis, anti-inflammation, anti-carcinogenic, cardiovascular protection and enhancement in endothelial functions, as well as angiogenesis events and inhibition of cell proliferation. Medicinal plants contain phenolic compounds which have antioxidant properties (Krings and Berger, 2001; Brown & Rice-Evans, 1998). Natural antioxidant major source is from medicinal plants, which are present as phenolic compounds like flavonoids, tocopherols and phenolic acids, etc. (Ali *et al*., 2008). Tannins are known to bind to proteins that are rich in proline thereby affecting protein synthesis. Plants in reaction to microbial infection produce

hydroxylated phenolic substance called flavonoids, which when used in vitro, show antimicrobial properties against wide array of microorganisms. Flavonoids shows their antimicrobial properties by forming complexes with bacterial cell wall as well as extracellular and soluble proteins (Marjorie, 1996). They are antioxidant and display strong anticancer activities (Okwu, 2004; Benavente-García *et al*., 1997; Salah *et al*., 1995).

Saponins, which are renowned for their inhibitory effect against inflammation, were also reported from the plant extracts (Just *et al*., 1998). Saponins are also shown to be coagulating and precipitating red blood cells. Other characteristics shown by some saponins iclude creation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo *et al*., 2000). Steroids compounds are also of significance because of the ability to complex with sex hormones (Okwu, 2001), and antibacterial activity (Raquel, 2007). Cardiac glycosides, reported in plant extracts have been employed for centuries as stimulants in the cases of cardiac failures (Trease and Evans, 2001). Terpenoids were also found in *L. didymum* especially in stems and roots. These are known for having antimicrobial potential (Habtemariam, 1993). Hence presence of terpenoids in the roots provides protection against bacterial infections.

CONCLUSION

Data obtained from the present study reveals that *L. didymum* is a rich source of phytochemical compounds. The presence of such a large array of bioactive constituents enables plant for its significant medicinal value. Our present facts and figures are in accordance with the literature, which confirms that those phytochemicals have strong effects on physiology and hence used in cure of different diseases. The practice of customary medication is strongly suggested for *L. didymum*. More work is recommended for the isolation, purification and characterization of those medicinally active constituents which are medicinally active.

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