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Full Length Research Paper

Phytochemical analysis and acute toxicity tests of two medicinal plant extracts

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Cassia fistula **L. (Caesalpiniaceae) is used in folk medicine for abdomeninal cramps, glands, liver, stomach and throat cancer, carcinomata, impostumes of the uterus, aperient, astringent, laxative purgative and vermifuge, burns, constipation, convulsions, delirium, diarrhea, dysuria, epilepsy, gravel, hematuria, pimples and glandular tumors.** ***Hippophae rhamniodes*** **(Elaeagnaceae) is used for nutritional purposes. This study was designed for the preliminary phytochemical screening and acute toxicity tests of** ***C. fistula*** **and** ***H. rhamniodes*** **as there is a lack of acute toxicity data available so far for both of these plants, although, they are extensively used in folk medicine. Phytochemical investigation of plant samples determines that alkaloids, saponins, anthraquinones, coumarins, flavonoids, tannins and glycosides were present in both plants except coumarins, which were absent in** ***H. rhamniodes.*** **The acute toxicity was studied in albino mice. Plant extracts at dose of 1, 3 and 10 g/kg to three test groups and normal saline at a dose of 10 ml/kg to a negative control group of albino mice were given. The extracts of both plants were found to be safe up to the dose of 10 g/kg. The results indicate that the studied plants presents potential for many pharmacological studies with high safety profile.**

Key words: Toxicity test*, Cassia fistula, Hippophae rhamniodes*.

INTRODUCTION

Many studies have shown that plants are used in the prevention of several diseases (Chu et al., 2002). The significance of plants in medicine remains even of greater importance with the current globalization to obtain drugs from plant sources due to high safety, efficacy and economy (Abubakar et al., 2011). There are two types of phytochemicals, that is, primary phytochemicals, such as sugars, amino acids, chlorophyll, proteins, etc., and secondary phytochemicals, such as alkaloids, essential oils, flavonoids, tannins, terpenoids, saponins and phenolic compounds. Most of the phytochemicals have valuable therapeutic activities, such as insecticidal, antibacterial, antifungal, anticonstipative, spasmolytic, antiplasmodial and antioxidant activities. The plants thus get their medicinal worth due to individual phytochemical constituents they contains (Iqbal et al., 2011). Toxicity is the science of poison. Organization for Economic and Development (OECD) defined toxicity as the advance effects taking place shortly (usually within 24 h) after oral administration of a single dose or multiple doses of a substance. Plant botanical interaction may lead to poising result in injury or even death. Toxicological data can be used to predict outcomes of plant botanicals used by human or animals. In ancient times, human being categorized plant botanicals as safe or toxic (Shirish, 2011).

Cassia fistula Linn. usually known as Indian Laburnum, is distributed in Asia, Mauritius, South Africa, Mexico, China, West Indies, East Africa and Brazil as an ornamental tree for its beautiful bunches of yellow flowers and it is recognized by the British pharmacopoeia (Mukhopadhyay et al., 1998). The main constituents present in seeds are tannins, fatty acids, isoflavonoids, flavonoids, glycosides, anthraquinones and phenolic compounds (Nayan et al., 2011). The seeds are reported to have demulcent and lubricating effect, bitter, acrid, cooling, emollient and useful in skin diseases, pruritus, burning sensation, dry cough and bronchitis (Sharma et

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Table 1. Phytochemical parameters of *C. fistula* and *H. rhamnoides*.

Phytochemicals	*C. fistula*	*H. rhamnoides*
Alkaloids	+	+
Glycosides	+	+
Saponins	+	++
Tannins	+	+
Anthraquinones	+	+
Flavonoids	++	+
Coumarins	+	−

Present: +, Strongly present: ++, Absent: −

al., 2005). The whole plant possesses medicinal properties useful in the treatment of skin diseases, inflammatory diseases, rheumatism, anorexia and jaundice (Kirtikar and Basu, 1991). The root is useful in skin diseases, leprosy, tuberculosis and glands' cures and burning sensations. The leaves are laxative, antipyretic, heal ulcers and used in rheumatism.

Hippophae rhamnoides is a deciduous species, widely distributed all over the Asia. The plant is a source of seed oil, which is much unsaturated and valuable, because of its light absorption and emollient properties, as an ingredient in cosmetics, phytopharmaceuticals or ultra-violet (UV) skin protecting characteristics. *H. rhamnoides* is also used in cancer therapy, cardio vascular therapy, liver diseases, skin diseases, gastrointestinal disorders (Alam, 2004). *C. fistula* and *H. rhamnoides* are used extensively in medicines; however, little is known about their toxicity.

In the present study we investigate the main phyto-chemicals and acute toxicity of *C. fistula* and *H. rhamnoides.*

MATERIALS AND METHODS

Chemicals

All chemical used (Bismuth nitrate, potassium iodide, acetic acid, mercuric chloride, hydrochloric acid, benzene, ammonia solution (35%), sodium hydroxide pellets, methanol, ethanol, aluminium chloride, potassium hydroxide, ferric chloride and glacial acetic acid) were of analytical grades and purchased from BDH, Sigma and Merck.

Plant

C. fistula leaves were collected from Abbasia Campus, the Islamia University of Bahawalpur, Pakistan, while *H. rhamnoides* berries were obtained from Pak Sea Buckthorn International, Skardo, Pakistan. The identification of these plants was performed at Cholistan Institute of Desert Studies, The Islamia University of Bahawalpur. The voucher specimens were preserved at the herbarium of Pharmacognosy Section, Faculty of Pharmacy and Alternative Medicine, The Islamia University of Bahawalpur for future reference.

Preparation of plant extracts

Shade-dried ground plant material of *C. fistula* leaves (150 g) was extracted with 70% methanol for 72 h at room temperature for each sample in a in 5 L beaker. The residues were extracted twice with the same fresh solvent, and extracts were combined.

H. rhamnoides berries were crushed and successively extracted. 320 g was macerated by a mixture of 2 L of analytical grade methanol and distilled water in 1:1 ratio in a glass beaker. The glass beaker was sealed with aluminum foil and kept at room temperature for three days. The macerated plant material was filtered through 8 layers of muslin cloth for coarse filtration. The coarse filtrate was then filtered through a Whatman No. 1 filter paper in order to get particle free extracts. The filtrate was evaporated under reduced pressure at 40°C in a rotary vacuum evaporator (EYELA, CA-1111, Rikakikai Co. Ltd. Tokyo, Japan).

Phytochemical screening

The preliminary phytochemical tests were performed according to the standard procedures of Evans (2008), Sofowora (1993) and Harborne (1973), and the results are summarized in Table 1.

Acute toxicity test

Acute toxicity test was performed on albino mice weighing about 18 to 26 g. Mice were kept in poly-carbonated cages. The crude extract was given in the doses of 300, 1000 and 10000 mg/kg of body weight orally to different groups of mice, each group consisting of six mice. The behaviors of animals were observed for 2, 4, 6, 8, 12 and 24 h. The number of animals that survived after 24 and 48 h were also noted. A normal control was also run parallel which was receiving normal saline (10 ml/kg). The mice received tap water and normal diet *ad libitum* (Sanmugapriya and Venkataraman 2006).

RESULTS AND DISCUSSION

Test for alkaloids (Mayer's reagent and Dragendoff's test)

In Mayer's reagent test for alkaloids, the test was carried out by subjecting 0.5 to 0.6 g of alcoholic plant extract in 08 ml of 1% HCl, boiled, filtered and Mayer's reagent was applied. Turbidity or occurrence of orange-red precipitate indicates the presence of alkaloids. In Dragendoff's test, 20 g of the plant extract was heated with MeOH:$CHCl_3$ (1:1) mixture and it was checked on a silica gel plates using the following solvent system: MeOH:$CHCl_3$ (1:9), EtOAc:$CHCl_3$ (2:8) and MeOH:NH_3 (100:3). Formation of dark yellow color with freshly prepared Dragendoff's reagent indicates the presence of alkaloids.

Test for flavonoids

Around 75 ml of the prepared plant extract was evaporated to dryness and was wash with ether to remove any fatty material. The defatted residue was dissolved in 30 ml of 80% ethanol and was filtered. This filtrate was used: 3 ml of the filtrate was mixed with 4 ml

of 1% $AlCl_3$ in MeOH in a test tube. Formation of yellow color precipitate indicated the presence of flavonoids (flavonols, flavones and chalcones). 3 ml of the filtrate was mixed with 4 ml of 1% KOH. A dark yellow color indicates the presence of flavonoids.

Test for tannins

0.5 mg of the plant extract was dissolved in 10 ml of distilled water and then filtered. Few drops of 1% $FeCl_3$ (ferric chloride) solution were added to the filtrate. Formation of a blue-black precipitate indicates the presence of hydrolysable tannins and green precipitate indicates the presence of condensed tannins.

$FeCl_3$ test for glycoside

5 ml of the concentration H_2SO_4 was added to 0.5 g of the plant extract and was boiled for 15 min. This was then cooled and neutralized with 20% KOH. The solution was divided into two portions. Few drops of $FeCl_3$ (ferric chloride) solution was added to one of the portions, and a green to black precipitate indicate the presence of phenolic aglycone as a consequence of hydrolysis of glycoside.

Frothing test for saponins

The plant extract was subjected to frothing test for the identification of saponins. 0.5 g of the plant extract was dissolved in boiling water in a test tube. After cooling shake the tube vigorously to forth and classify for saponins as: no forth, negative test; forth less than 1 cm, weakly positive; forth greater than 1 cm height, positive test; forth greater than 2 cm in height, strongly positive.

Test for anthraquinones

5 gm of the plant extract was shaken with 10 ml of benzene and was filtered. 5 ml of 10% NH_4OH solution was added to the filtrate, and was stirred. The formation of a pink, red or violet color indicates the presence of free anthraquinones.

Test for coumarins

For identification of coumarins, a piece of filter paper was moistened with NaOH, and then was kept over a test tube with 1 g of boiling plant extract. Yellow fluorescence under UV light indicates a positive test for coumarins.

Acute toxicity test

Mortality was the criteria for determining acute toxicity. No mortality was recorded even at high doses up to 10 g/kg body weight.

DISCUSSION

Results of the present study comprise of rational evidence and a scientific justification and support to the use of *C. fistula* and *H. rhamnoides* in traditional medicine. The preliminary phytochemical analysis of the leaves of *C. fistula* and berries of *H. rhamnoides* showed the presence of alkaloids, glycosides, saponins, anthraquinones, tannins, flavonoids and coumarins with the exception of coumarins which is absent in *H. rhamnoides* (Table 1). Many studies reported that plants containing flavonoids, tannins and sterols may be used for antiviral activity (Ramzi et al., 2006); so, both *C. fistula* and *H. rhamnoides* may be explored for anti viral activity. The presence of tannins shows that the plants can be used as purgative. They may also be used in the management of cough, asthma and hay fever (Gills, 1992).

The presence of tannins may also suggest the ability of these plants to play a major role as antidiarrhoeal and antihaemorrhagic agent (Asquith and Butler, 1986). Saponins are positive for both plants and have been shown to have enormous significance as antihyper-cholesterol, hypotensive and cardiac depressant properties.

Hence, these plants could be suitable for these purposes. Cardiac glycosides showed positive results for both plants indicating their possible use in cardiac failure (Trease and Evans, 2008). The existence of different secondary metabolites, especially saponins, tannins and flavonoids in plants has also been linked to the antimicrobial activities of the plants (Lewis and Ausubel, 2006; Cowan, 1999).

In the case of acute toxicity study, it was observed that all the animals survived after 24 h and even after 48 h. Hence, it was proved from the acute toxicity test that the crude extracts of both plants are quite safe and have no acute toxicity up to the dose of 10 g/kg of body weight orally which is considered a very high dose.

Conclusion

Concentration on natural products is increasing rapidly, and research into natural products has advanced tremendously in pharmaceuticals. Natural products have proved to be a rich source of therapeutic agents. In addition, these results indicate that *C. fistula* and *H. rhamnoides* could not only serve as a folk medicine, but may also be of importance in scientific ethno-botanical studies due to thier phytochemicals. In the case of toxicity study, it can be concluded the hydro-alcoholic extract of the leaves of *C. fistula* and berries of *H. rhamnoides* are safe upto very high doses. However it is recommended that: (1) further toxicity studies are required using different animal species and (2) order to establish the long-term effects of the extract, sub-acute and chronic toxicity tests is required.

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