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Phytochemical Screening and Cytotoxic Activity of *Martynia annua* L. Leaves Extracts

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Abstract

The present study was intended to reveal the metabolites presence and determine the cytotoxic potential of various extracts of *Martynia annua* (Martyniaceae) leaves. The shade dried and powdered leaves materials (50 g) were extracted successively with 300 ml of petroleum ether, ethanol, chloroform and acetone, by using Soxhlet extractor for 8 h at a temperature not exceeding the boiling point of the solvent. Phytochemical screening of the extracts was carried out according to the standard methods. In cytotoxic activity the hatched shrimps were taken for bioassay. The acetone and alcohol extracts showed many photochemical constituents and an alcoholic extract has higher cytotoxic activity. The qualitative phytochemical screening revealed the presence of carbohydrates, alkaloid, flavonoid, tannins, terpenoid, steroid, phenol, cardiac and saponin glycosides in alcoholic leaves extracts of *M. annua*. Maximum number of metabolites (9/13) presence was observed in the alcoholic extracts of *M. annua* followed by acetaone extracts (8/13). Cytotoxic activity was analysed in terms of brine shrimp lethality bioassay. The alcoholic and acetone extracts of *M. annua* was found to be most effective at which 50% mortality of brine shrimp nauplii occurred were found to be 239.48 and 328.21ppm respectively. The results of the present study revealed the cytotoxic Potential of *M.annua*.

Keywords: Phytochemical, Cytotoxic, *Martynia annua*.

Contents

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1. Introduction

Medicinal plants have been utilized nowadays in Ayurvedic, Homeo and Unani system of medicine. Many compounds used in today's medicine have a complex structure and synthesizing the bioactive compounds chemically at a low price is not easy. The increasing awareness about side effects of drugs had made the western pharmaceutical industries to turn towards the plant based Indian and Chinese medicine (1). These herbal medicines have been practiced worldwide and are now recognized by WHO as an essential building block for primary health care (2). Plant-derived substances have recently become great interest owing to their versatile applications. The cytotoxicity test has been used routinely in the primary screening of the crude extracts to assess the toxicity towards brine

shrimp. The bioassay has a good correlation with cytotoxic activity in some human solid tumours and with pesticidal activity. This *in vivo* lethality test has been successively employed for providing a frontline screen that can be backed up by more specific and more sophisticated bioassays, once the active compound has been isolated. A number of novel antitumors and pesticidal natural products have been isolated using this bioassay (3).

Martynia annua L. (Martyniaccae) is one of the medicinal herbs used by native people for various medicinal purposes, commonly known as scorpion (in Hindi, Bichchhu or Baghnukh). It has been used from ancient time in traditional medicine of India (4). The plant is native to Mexico but now well naturalized throughout India on waste lands. In folk medicine, the fruits are used for the treatment of asthma; the seeds are applied locally for itching and eczema. The leaves are given in epilepsy and its juice is gargled for sore throat (5). Decoction of whole plant is given in pneumonia and cold fever (6). The roots made into a poultice and applied in snake bite (7). Different parts of the plant studied scientifically reveals that the roots are anthelmintic, antifertile, leaves are analgesic, CNS depressant (7), antioxidant (9) and antibacterial (10) but no scientific data is available as far the antibacterial potential of crude fruits and flowers is concern. In addition, the literature survey reveals that plant contains alkaloids, tannins, glycosides, phenols and flavonoids (11, 12). Different anthocyanin glycosides have been identified from the flowers viz. pelargonidin-3-5-diglucoside, cyanidin-3-galactoside (13). With this knowledge, the present study was performed to evaluate the phytochemical constituents and cytotoxic activity in *M.annua* leaves using various extracts.

2. Materials and Methods

Preparation of leaves extracts of *Martynia annua* L

The matured and healthy leaves of *Martynia annua* L**.** were harvested from the wild and shade dried for 15 days. The shade dried leaves powedered using mechanical grinders. The 50 g of leaves of powder of *M.annua* successively extracted with 300 mL of petroleum ether, acetone, chloroform, ethanol by using the soxhlet extractor for 8 hours at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using Whattman filter paper (No.1) and then concentrated in vacuum at 40ºC using Rotary evaporator. The residues obtained were stored in a freezer -20º C until further tests.

Phytochemical Screening

The concentrated extracts were used for preliminary screening of various phytoconstituents *viz*. Carbohydrates, alkaloid, flavonoid, tannins, sterols, terpenoid, steroid, phenol, cardiac glycosides, anthraquinone glycosides, saponin glycosides, coumarin Glycosides and amino acid were detected by the standard method described by Brindha et.al. (1981)

Cytotoxic Activity

Hatching of Brine Shrimp

Artificial sea water (38g NaCl/1000mL tap water) was taken in a small tank and shrimp eggs were added to one side of the divided tank and the side was covered. The shrimps were allowed for 48 hours to hatch and mature as nauplii. During this period constant oxygen supply, temperature (around 37°C) and light supply was maintained. The hatched shrimps were taken for bioassay.

Application of test sample to the test tube containing brine shrimp nauplii

Thirty clean test tubes were taken and separated by 10mL in each test tube. Twenty five were for the samples in five different concentrations (5 test tubes for each concentration) and five test tubes for control. With the help of a Pasteur pipette ten living shrimps were dropped into each test tube (Mclughilin&Roger, 1991). Dried extract of *M.annua were* taken in different concentrations (2.5, 5, 7.5, 10 and 12.5 mg/10mL) to the sample tubes.

Preparation of Control group

Control group was added in cytotoxic activity to validate the test method and result obtained due to the cytotoxic activity of the test agent. Hence, 50µL of DMSO was added to control tubes containing 5 mL of mother solution and 10 shrimp nauplii to use as control groups. No extract were added to prepare control solution. If the brine shrimp in these test tubes show a mortality rate, then the test was considered as invalid as the nauplii died due to some reason other than the cytotoxicity of the compound.

Counting of nauplii: After 24 hours, the tubes were inspected using a magnifying glass and the number of survived nauplii in each vial were counted and observations were recorded for each vials. Using the recorded observations LC_{50}, 95% confidence limit, LC_{90} and chi square values were calculated.

3. Results and Discussion

The preliminary phytochemical studies confirmed that the alcoholic extracts of *M. annua* showed the higher percentage (69%) of metabolite constituents. The phytochemical investigation documented the high quantity of carbohydrates, alkaloid, flavonoid, tannins, terpenoid, steroid, phenol, cardiac glycosides and saponin glycosides in alcoholic leaves extracts of *M. Annua* (Table 1). Followed by, acetaone extracts of *M. annua* leaves showed eight (62%) metabolites existence. Out of 52 (4 x 13 x 1 = 52) tests for the presence or absence of the phytoconstituents,

29 tests gave positive results and the remaining 23 gave negative results. Out of thriteen metabolites tested, the coumarins, anthroquinnones glycosides and aminoacids were failed to show any positive result for their presence in any of the four extracts of *M. annua*.

The brine shrimp lethality bioassay was carried out with various extracts of *M. annua*. The alcoholic extracts of *M. annua* showed better toxicity towards the brine shrimp having an LC_{50} and LC_{90} values at 239.48 ppm and 628.23 ppm respectively (Table 2). The inhibitory effect of the extract might be due to the toxic compounds present in the active fraction that possess larvicidal properties. The result of brine shrimp lethality bioassay confirmed that alcoholic extracts of *M. annua* is pharmacologically active.

Table 1: Preliminary phytochemical screening of the various extracts of *Martynia annua L.* leaves

S.No	Test	Extract			
		Petroleum ether	Chloroform	Acetone	Alcohol
1.	Carbohydrate	+	+	+	+
2.	Alkaloid	-	-	+	+
3.	Flavonoid	+	-	+	+
4.	Tannins	-	+	+	+
5.	Sterols	+	-	-	-
6.	Terpenoid	-	+	+	+
7.	Steroid	+	-	+	+
8.	Phenol	+	+	+	+
9.	Cardia glycosides	+	+	-	+
10.	Anthraquinone Glycosides	-	-	-	-
11.	Saponin Glycosides	-	+	+	+
12.	Coumarin Glycosides	-	-	-	-
13.	Amino acid	-	-	-	-

Table 2: Cytotoxic activity of *Martynia annua L* leaves

Leaves Extract	LC_{50}	95% Confidence Limit		LC_{90}	¡ ^{2}
	(ppm)	Lower	Upper	(ppm)	
Petroleum ether	437.56	400.34	488.69	758.63	0.481
Chloroform	328.21	303.21	354.69	596.67	0.926
acetone	645.25	521.30	987.47	1397.81	2.102
Alcohol	239.48	197.12	274.63	628.23	2.327

Plant substances continue to serve as the viable source of drugs for the world population and several plant-based drugs are in extensive clinical use. For the past few decades, several plants have been widely used for the treatment of various diseases due to their antioxidant properties. Phytochemicals are chemical compounds formed during the metabolic processes of plants (Okwu, 2004). Phenolics are the most widespread secondary metabolites in the plant kingdom and have received much attention as potential natural antioxidants in terms of their abilities to act as both efficient radical scavengers and metal chelators (Lim *et al*., 2007; Sultana *et al*., 2007).

Flavonoids protect plants against various biotic and abiotic stresses and exhibit a diverse spectrum of biological functions and play an important role in the interaction between the plant and their environment (Pourcel *et al*., 2007). Alkaloids can work on the nervous system of the human body and used for analgesic, antispasmodic and bacterial effects (Okwu and Josiah, 2006). Plant sterols are membrane constituents as well as precursors for plant hormones and other secondary metabolites (Lindsey *et al*., 2003; Hartmann, 2004). Saponins have considerable commercial value and are processed as drugs and medicines, foaming agents, sweeteners, taste modifiers and cosmetics (Hostettmann and Marston, 1995). Tannin containing remedies are in use as antihelmintics (Ketzis *et al*., 2006), antioxidants (Koleckar *et al*., 2008), antimicrobials and antivirals (Buzzini *et al*., 2008). In the present study, the results of the phytochemical analysis of petroleum ether, chloroform, acetone and alcoholic extracts of *M. annua* revealed the presence of various primary and secondary metabolites with varied degree which may help in authenticating the plant along with nature of phytoconstituents present in it. The evaluation of the toxic action of plant extracts is indispensable in order to consider a treatment safe; it enables the definition of the intrinsic toxicity

of the plant and the effects of acute overdose. The *in vivo* brine shrimp lethality is a simple bioassay considered as a useful tool for primary screening of various kinds of bioactive compounds (Badisa *et al*., 2006; 2007; 2008). The result of the present study revealed the cytotoxicity properties of *M. annua* is the best source of cytotoxic secondary metabolites which is considered for compound isolation in order to detect future anti-tumor compounds.

4. Conclusion

The phytochemical constituents of *M. annua* leaves mainly showed the presence of alkaloids, terpenoids, flavonoids, tannins and steroids. These constituents are responsible for the medicinal characters. In addition, these compounds could serve as novel scaffolds in search for new drugs against cancer. Further investigations are required to expose the bioactive compounds of the *M. annua* leaves extracts responsible for cytotoxic properties.

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