MEDDOCS Open Access Publisher

ISSN: 2637-7721

Journal of Plant Biology and Crop Research

Open Access | Research Article

Phyto-Chemical Screening, Antibacterial and Anti-Inflammatory Activity of *Martynia Annua L.*

Ashwani K Dhingra[1]*; Bhawna Chopra; Rameshwar Dass

Guru Gobind Singh College of Pharmacy, Yamuna Nagar-135001, Haryana, India

***Corresponding Author(s): Ashwani K Dhingra**

Department of Pharmaceutical Sciences, Guru Gobind Singh College of Pharmacy, Near Guru Nanak Khalsa College, Yamuna Nagar-135001, Haryana, India

Fax: 01732-268381, Tel: +91- 9996230055

Email: ashwani1683@gmail.com

Received: Apr 04, 2020

Accepted: Jul 17, 2020

Published Online: Jul 22, 2020

Journal: Journal of Plant Biology and Crop Research

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Abstract

Objective: To carryout phyto-chemical screening in conjunction with *in vitro* antimicrobial and anti-inflammatory potential of *Martynia annua* Linn.

Methods: Organic solvents extracts of *Martynia annua* leaves were prepared sequentially by using petroleum ether, chloroform, ethyl acetate and ethanol by ultrasonication technique. The *in vitro* antimicrobial activity of various extracts was evaluated by using agar plate diffusion microbiological assay, while inhibition of protein denaturation assay was used for evaluation of anti-inflammatory potential.

Results: Preliminary phytochemical analysis confirmed the presence of alkaloids, glycosides, saponins, phenolic acid, tannins, carbohydrates, protein, terpenoids and flavonoids. The results clearly indicates the significant dose dependent antibacterial and anti-inflammatory activity of *Martynia annua* extracts.

Conclusion: The results of the present study provide the scientific basis for the traditional claims of *Martynia annua* Linn leaves as an antibacterial and anti-inflammatory drug.

Keywords: *Martynia annua*; Martyniaceae; Antibacterial; Anti-inflammatory

Introduction

The search of novel anti-inflammatory agent is not considered as an ending process, since most of the clinically used anti-inflammatory drugs such as NSAIDs, Coxibs, GCs *etc.* are allied with considerable toxicity. However, numerous approaches were used to overcome the toxicity level such as co-administration with suitable agent/substance which provides protection against toxicity as well to synthesise new potent and safe anti-inflammatory drug. Although the drug treatment has been improved steadily but yet, it is still a challenge for the medicinal chemists to identify more potent therapeutic agents to treat or reduce the symptoms of inflammatory diseases [1]. In addition, it is well documented that bacterial infections often produce pain and inflammation and therefore chemotherapeutic agent along with anti-inflammatory compounds are prescribed simultaneously to treat bacterial infections having inflammatory disorders. Unfortunately, none of the drugs possesses these two activities in a single component. Therefore, our aim is to find a compound having dual antimicrobial and anti-inflammatory activities.

Cite this article: Dhingra AK, Chopra B, Dass R. Phyto-Chemical Screening, Antibacterial and Anti-Inflammatory Activity of *Martynia Annua* L. J Plant Biol Crop Res. 2020; 3(1): 1025.

Moreover, medicinal plants are the richest bio-resources for their wide variety of chemical compounds which have important biological functions and enables as an herbal medicines having beneficiary role in the pharmacology [2]. The bioactive compounds (alkaloids, tannins, flavonoids etc.) present in traditionally used medicinal plants possess definite physiological action on the human body [3]. In addition, most of the all bioactive constituents are known to have therapeutic value as anti-constipative, spasmolytic, antibacterial, antifungal, anti-inflammatory, insecticidal and antioxidant [4].

The plant *Martynia annua* Linn. (family: Martyniaceae), is a herbaceous erect, branched, grandular hairy annual herb. Fruits are hard, woody with two sharp re-curved hooks and seeds are oblong [5]. The common names of plant include Devil's Claw (English), Bichhu (Hindi), Kakanasika (Sanskrit) and Vichchida (Gujarati). The leaves and fruits together contributes the most active biological part of the plant [6-7]. In Ayurveda the plant is known as kaakanassikaa and the ayurvedic pharmacopoeia of India recommended the seed of plant for arresting of graying of hair [8]. According to literature survey, the plant was found to possesses anticonvulsant [8], anthelmintic [9], analgesic and antipyretic [10], antibacterial [11], antifertility [12], antinociceptive and CNS depressant [13], antioxidant [14] and wound healing activity [15]. Therefore, the present study was carried out to investigate *in vitro* antibacterial and anti-inflammatory activity of different organic extracts of leaves of *Martynia annua* Linn.

Material and Methods

Plant material: The leaves of *Martynia annua* plant were collected between the months of August-September 2017, from the local area of Yamuna Nagar district of Haryana state, India. After collection, the leaves were washed thrice with water to remove dust or debris and air dried. After total dryness, the leaves were ground to coarse powder using a blender, passed through the 40-mesh sieve and stored in well-closed container.

Chemicals and drugs: The drug amoxicilline and diclofenac sodium were obtains as gift sample from Oscar Remedies Pvt. Ltd., Badi Majra,, Yamuna Nagar, Haryana 135001. All other chemicals were of analytical grade obtained commercially.

Extraction procedure: A weighed quantity (100gm) of the coarsely powdered plant material was sequentially extracted with petroleum ether (60-80°C), chloroform, ethylacetate and methanol using ultra sonicator technique. All the extracts obtained were concentrated and completely dried. The collected extracts were used for phyto-chemical screening and *in vitro* anti-bacterial and anti-inflammatory activity.

Analysis of phytochemicals: Quantitative analysis of all prepared extracts were carried out to investigate various phyto-constituents such as alkaloids, tannins, phenols, steroids, glycosides, saponins and flavonoids as per standard procedures and protocols [16-17].

***In vitro* antibacterial activity**

The *in vitro* antimicrobial potential of all the prepared extracts was carried out at Microbiology Laboratory, guru Gobind Singh College of Pharmacy, Yamuna Nagar-135001 (India) using the agar plate diffusion method using microorganisms cell suspension whose concentration was equilibrated to 0.5 Mc-Farland. The bacterial strains used in this present study are *Staphylococcus aureus, Bacillus subtilis*, *Klebsiella pneumonia* and *Escherichia coli*. All the bacterial strains were pre-cultured in nutrient broth overnight and then grown in Muller Hinton agar medium. The inoculums of microorganisms were prepared from bacterial culture. About 15-20 ml of Muller-Hinton agar medium was poured in the sterilized glass petridishes and allowed to solidify. One drop of each strain was spread over the medium by a sterile rod. Wells of 5 mm in diameter and about 2 cm apart were punctured in the cultured media using sterile cork borer. About 1 ml of plant extract was added to the wells. Inoculated plates were then incubated at 37°C for 24 hrs. Antibacterial activities were evaluated by measuring the diameters of inhibition zones. The Minimum Inhibitory Concentration (MIC) of petroleum ether, chloroform, ethylacetate and ethanol extract was determined as the lowest concentration of the plant extract inhibiting the visible growth of organism.

***In vitro* anti-inflammatory activity:**

The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of different organic extracts of *Martynia annua* so that final concentrations become 31.25, 62.5, 125, 250, 500, 1000 µg/mL [16]. Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37±2) °C in a BOD incubator (Labline Technologies) for 15 min and then heated at 70 °C for 5 min. After cooling, their absorbance was measured at 660 nm (SHIMADZU, UV 1800) by using vehicle as blank. Diclofenac sodium at the final concentration of (78.125, 156.25, 312.5, 625, 1250, 2500 µg/mL) was used as reference drug and treated similarly for determination of absorbance. The percentage protection from denaturation is calculated by using the formulae and it is tabulated.

% inhibition = (1- Vt / Vc) x 100

Where, Vt = absorbance of test sample, Vc = absorbance of control.

Result and discussion

Phytochemical screening

The phytochemical screening of different extracts of *Martynia annua* revealed the presence of medicinally active constituents as depicted in **Table 1**. The preliminary phytochemical analysis of *Martynia annua* leaves indicated the presence of alkaloids, glycosides, saponins, phenolic compounds, tannins, proteins and terpenoids in the solvents ethylacetate and ethanol. However, chloroform extract shows the presence of alkaloids, glycosides, saponins, phytosterol, quinones and terpenoids. In addition, Petrolium ether extract shows only presence of phytosterol, quinones and terpenoids.

***In vitro* antimicrobial activity**

The antibacterial activity of the tested extracts of *Martynia annua* showed significant dose dependent reduction in bacterial growth in terms of zone of inhibition (**Table 2**). In the present study, maximum growth of inhibition (28 mm) was observed in *Martynia annua* ethanolic leaf extracts at 100 µg/mL against *Staphylococcus aureus* which was followed by *Bacillus subtilis* (27 mm), *Klebsiella pneumonia* and *Escherichia coli* (25 mm).

Similarly, ethyl acetate extracts showed maximum growth inhibition (22 mm) at the concentration of 100 µg/mL against *Staphylococcus aureus*, followed by *Bacillus subtilis* (20 mm), *Klebsiella pneumonia* (16 mm) and *Escherichia coli* (14 mm).

In addition, chloroform extracts at the concentration of 50 and 100 µg/mL showed significant antibacterial activity, against all the tested organisms the petroleum ether extract of *Martynia annua* leaf were found to be inactive at 25 and 50 µg/mL and showed minimum zone of inhibition against all the tested microorganisms.

In vitro anti-inflammatory activity

The *in vitro* anti-inflammatory potential of different organic extracts of *Martynia annua* was evaluated against denaturation of egg albumin as depicted in **Table 3**. The present findings exhibited a concentration dependent inhibition of protein (albumin) denaturation by different extracts throughout the concentration range from 62.5µg/mL to 1000µg/mL. Diclofenac sodium at same concentration range was used as reference drug. However, out of all prepared extracts, the anti-inflammatory potential of ethanol, ethyl acetate and chloroform extracts was found to be more when compared with the standard drug diclofenac sodium. In addition, petroleum ether extracts also exhibited concentration dependent inhibition of protein denaturation; however, their effect was found to be less as compare to diclofenac sodium.

Figure 1: Phytochemical screening of the leaf extracts of Mar*tynia annua* Linn.

Sr. No.	Phyto-constituents	Tests	Pet. ether Extract	Chloroform Extract	Ethylacetate Extract	Ethanol Extract
1	Alkaloids	Mayer's test	-	+	+	+
2	Glycosides	Borntrager's test	-	+	+	+
3	Saponins	Froth forming test	-	+	+	+
4	Phenolic compounds	Lead acetate test	-	-	+	+
5	Tannins	$FeCl_3$ test	-	-	+	+
6	Phytosterols	Libermann Buchard test	+	+	+	-
7	Carbohydrates	Fehilings test	-	-	-	+
8	Proteins	Biuret test	-	-	+	+
9	Amino acids	Barfoed's Test	-	-	-	-
10	Flavanoids	Alkaline test	-	-	-	+
11	Quinones	Quinone test	+	+	-	-
12	Terpenoids	Terpenoid test	+	+	+	+

Figure 2: Antibacterial activity of leaf extracts of *Martynia annua L*. against human pathogenic microorganisms.

Sr. No.	Plant extracts	Concentration (µg/ml)	Zone of growth inhibition(mm)			
			Staphylococcus aureus	*Bacillus subtilis*	*Klebsiella pneumonia*	*Escherichia coli*
1	Ethanol Extract	25	22	24	20	19
		50	26	26	22	23
		100	28	27	25	25
2	Ethylacetate Extract	25	16	12	8	8
		50	20	14	10	10
		100	22	20	16	14
3	Chloroform Extract	25	12	9	9	10
		50	14	12	10	12
		100	18	21	18	16
4	Pet. ether Extract	25	Nil	Nil	Nil	Nil
		50	Nil	Nil	Nil	Nil
		100	10	8	6	8
5	Positive Control Amoxicilline	200	30	30	30	30
6	Negative Control (DMSO)	---	Nil	Nil	Nil	Nil

Figure 3: Anti-inflammatory activity of different organic extracts of leaves of *Martynia annua* Linn.

Sr. No.	Extract	Concentration (µg/mL)	Absorbance (660 nm)	% Inhibition
1	Control	---	0.099 ± 0.60	---
2	Petroleum ether	62.50	0.069 ± 0.60	30.30
		125.00	0.153 ± 0.80	54.55
		250.00	0.310 ± 0.30	213.13
		500.00	0.754 ± 0.50	661.62
		1000.00	1.666 ± 0.60	1582.83
3	Chloroform	62.50	0.362 ± 0.50	265.65
		125.00	0.475 ± 0.40	379.80
		250.00	0.853 ± 0.70	761.62
		500.00	1.531 ± 0.30	1446.46
		1000.00	4.0 ± 0.50	3940.40
4	Ethylacetate	62.50	0.497 ± 0.50	402.02
		125.00	0.510 ± 0.80	415.15
		250.00	0.650 ± 0.60	556.57
		500.00	1.110 ± 0.50	1021.21
		1000.00	1.963 ± 0.40	1882.83
5	Ethanol	62.50	0.452 ± 0.40	356.57
		125.00	1.412 ± 0.40	1326.26
		250.00	2.852 ± 0.30	2780.81
		500.00	3.754 ± 0.80	3691.92
		1000.00	4.540 ± 0.60	4485.86
6	Ibuprofen	62.50	0.087 ± 0.30	12.12
		125.00	0.075 ± 0.20	24.24
		250.00	0.049 ± 0.40	50.5
		500.00	0.309 ± 0.50	212.12
		1000.00	0.91 ± 0.40	819.2

Conclusion

The present study describes the phytochemical investigation along with *in vitro* antibacterial and anti-inflammatory activity of leaf extracts of *Martynia annua* Linn. Phytochemical investigation of plant extracts seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. Moreover, the antibacterial assay indicates significant dose dependent activity of ethanol, ethylacetate and chloroform extract against all bacterial strain. The ethanolic extract was found to be more active and represents maximum growth of inhibition (28 mm) against *Staphylococcus aureus*. However, the petroleum ether extract was found to be inactive at 25 and 50 µg/mL and showed minimum zone of inhibition against all the tested microorganisms.

In addition, denaturation of tissue proteins is one of the well-documented causes of inflammatory and arthritic diseases. Therefore protein denaturation bioassay was selected for *in vitro* assessment of anti-inflammatory potential of different organic extracts of leaves of *Martynia annua* Linn. The protein denaturation bioassay suggested that leaves of *Martynia annua* could be a potential source of natural anti-inflammatory agents having great importance as the prepared extracts showed remarkable activity as compare to the standard drug. Further definitive studies are necessary to ascertain the mechanisms and constituents behind its anti-inflammatory actions. Therefore, the leaves of plant can provide lead molecules which could be useful substrate for the synthesis of novel compound that can be use in the treatment of infections caused by the organisms. Further purification, identification and characterization of the active compounds would be our priority in future studies.

References

1. Dhingra AK, Chopra B, Dass R, Mittal SK. An Update on Anti-inflammatory Compounds: A Review. Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry. 2015; 14: 81-97.
2. Hutchings MR, Athanasiadou S, Kyriazakis I, Gordon IJ. Animals use foraging behavior to combat parasites. Proceeding Nutritional Society. 2003; 62: 361-365.
3. Okwu DE. Flavouring properties of spices on Cassava fulu: African Journal of Root-Tuber Crops. 1999; 3: 19-21.
4. Padmapriya M, Prabhitha K.S, Devika R, Sugashini PK. Phytochemical Screening Study of Martynia annua. International Journal of Pharmacy and Pharmaceutical Research. 2016; 7: 394-401.

5. Manandhar NP, Manandhar S. Plant and people of Nepal. Portland: Timber Pres. Inc, 2002: 311.

6. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants, National Institute of Science Communication, New Delhi. 1996: 162.

7. Satyavati GV, Gupta AK, Tandon N. Medicinal Plants of India, Volume II, Indian Council of Medical Research, New Delhi. 1987: 223-229.

8. Babu HB, Mohana LS, Saravana A K. Studies on phytochemical and anticonvulsant property of Martynia annua Linn. International Journal of Phytopharmacology. 2010; 1: 82-86.

9. Nirmal SA, Nikalye AG, Jadav RS, Tambe VD. Anthelmintic activity of Martynia annua roots. Indian Drugs. 2007; 44: 772-773.

10. Kar DM, Nanda BK, Pardhan D, Sahu SK, Dash GK. Analgesic and antipyretic activity of fruits of Martynia annua Linn. Hamdard Med. 2004; 47: 32.

11. Sermakkani M, Thangapandian V. Phytochemical and Antibacterial activity of Martynia annua L. against the different pathogenic bacteria. Journal of Herbal Medicine and Toxicology. 2010; 4: 221-224.

12. Mali PC, Ansari AS, Chaturedi M. Antifertility effect of chronically administered Martynia annua root extract on male rats. Journal of Ethnopharmacology. 2002; 82: 61-67.

13. Bhalke RD, Jadhav RS. Antinociceptive activity and CNS depressant activity of Martynia annua L. root. International Journal of Pharmaceutical Sciences. 2009; 1: 333-335.

14. Nagda D, Saluja A, Nagda C. Antioxidant activities of methanolic and aqueous extract from leaves of Martynia annua Linn. Journal of pharmacognosy. 2009; 1: 288-297.

15. Lodhi S, Singhai AK. Preliminary pharmacological evaluation of Martynia annua Linn leaves for wound healing. Asian Pacific Journal of Tropical Biomedicine. 2011; 1: 421-427.

16. Sangita C, Priyanka C, Protapaditya D, Sanjib B. Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein. Asian Pacific Journal of Tropical Biomedicine. 2012; S178-S180.