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Pharmacognostic, phytochemical and pharmacological study of *Martynia annua* Leaves" (Family: Martyniaceae)

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Abstract
Martynia annua (Devil's claw, Bichu), is a medicinal plant of the family of Martyniaceae, widely used for the treatment of epilepsy, tuberculosis, inflammation, etc The petroleum ether, chloroform, and methanol extracts of *Martynia annua* leaves were evaluated for analgesic effect in albino wistar rat using Eddy's Hot Plate Method and Hot water Tail Immersion test method. Analgesic activity of the extract was compared with the standard drug pentazocine 25 mg/kg. The extracts show significant analgesic activity at 2000 mg/kg. It was also observed that the methanol extracts exhibits greater analgesic activity as compared to petroleum ether and chloroform extract of the plant of *Martynia annua*.

Keywords: *Martynia annua*, Analgesic Activity, Hot Plate Method, Tail Immersion test method.

Introduction
Ayurveda, the ancient Indian therapeutic measure is renowned as one of the major systems of alternative and complementary medicine. As other herbal systems, greater parts of its medicaments are based on indigenous herbals. Plants are one of the most important sources of medicines. India is known as the "Emporium of Medicinal plants" due to availability of several thousands of medicinal plants in the different bioclimatic zones anti-inflammatory diseases including rheumatoid arthritis are still one of the main health problems of the world's population. Several modern drugs are used to treat these disorders but, their prolonged use may cause severe adverse side effects, the most common being gastrointestinal bleeding and peptic ulcers. Consequently, there is a need to develop new anti-inflammatory agents with minimum side effects. The use of natural remedies for the treatment of inflammatory and painful conditions has a long history starting with Ayurvedic treatment and extending to the European and other systems of traditional medicines. Plant drugs are known to play a vital role in management of inflammatory diseases [8, 9].

Martynia annua Linn. Is commonly known in Ayurveda kaakanassikaa belongs to family Martyniaceae. It small herb found in throughout India and it is native of Mexico. In Ayurveda, the plant is known as kakanasika, which is being used in Indian traditional medicines for epilepsy, inflammation and tuberculosis. The leaves and fruits are biologically active part of this plant. The leaves of the *Martynia annua* are edible and used as antiepileptic and antiseptic, applied locally to tuberculosis glands of the neck, the juice of the leaves as a gargle for sore throat and the leaf paste for wounds of domestic animals [12].

It is herbaceous, stout, erect, branched, clammy pubescent, annual plant growing to a height of 90 – 120 cm. Found throughout India, in waste places, rubbish heaps and along road sides. Flowers contain cyanidin-3-galactoside whilst p-hydroxy benzoic acid, snapic acid; and gentisic acids are present in Flowers. The leaves also contain chlorogenic acid; and fatty acids (such as palmitic acid, stearic acid and arachidic acid), P-hydroxy benzoic acid, snapic acid and fatty acids such as palmitic acid and stearic acid present in leaves. The seed also contain Arachidic acid, Linoleic acid, Malvalic acid, Oleic acid Palmitic acid, Stearic acid, Apigenin, Apigenin-7-O-beta- D- glucuronide. The fruit is considered alexiteric and useful in inflammations while ash of fruit mixed with coconut oil applied on burns. The fruits of *Martynia annua* used as local sedative and also used as antidote to scorpion stings to venomous bites and stings. Seed oil applied on abscesses and for treating itching and skin affections. The Ayurvedic Pharmacopoeia of India recommended the seed of *Martynia annua* for arresting of graying of hair [15].

Material and methods

Collection and Authentication of Plant

The Leaves of plant *Martynia annua* belonging to family Martyniaceae were collected from Kopergaon region, Tal: Kopergaon Dist: Ahmednagar. The plant was authenticated by P. A. Ingle, Joint director, Botanical survey of India, Pune. Voucher specimen number BSI/WRC/IDEN. CER. /2016/417 dated at 25/10/2016 species as *Martynia annua* L.

Preparation of extracts

The leaves of *Martynia annua* was collected, washed and dried at room temperature. Leaves were grinded into the fine powder, extracted with different solvents in decreasing order of solvent polarity i,e petroleum ether (40-60 ^{0}c), chloroform and methanol. The extract was dried in a vacuum oven to obtained constant weight.

Phytochemical evaluation

The Methanolic extract was used to analyze qualitatively various phytoconstituents such as alkaloids, glycosides, proteins, steroids, carbohydrate, phenolic compounds, tannins, and flavonoids using standard procedures

Table 1: Preliminary phytochemical screening of extracts.

Tests	Petroleum ether extracts	Chloroform extracts	Methanol extracts
Test for Steroids			
1. Salkowaski test	-	-	+
2. Liebermann- Burchant test	-	-	+
Test for Glycoside			
1. Brontragers test	-	-	-
2. Modified Brontragers test	-	-	-
3. Keller-killani test	-	-	-
Test for Carbohydrate			
1. Molisch's test	-	-	+
2. Barfoeds test	-	-	+
3. Benedicts test	-	-	+
Test for Proteins			
1. Millions test	-	-	+
2. Xanthoproteic test	-	-	+
3. Biuret test	-	-	+
4. Ninhydrin test	-	-	+
Test for Tannins			
1. Ferric chloride test	-	-	+
2. Dilute nitric acid test	-	-	+
Test for Flavonoids			
1.Shinoda test	+	-	+
2. Lead acetate test	+	-	+
Test for Saponin			
1.Foam test	-	-	+
2. Hemolysis test	-	-	+
Test for Alkaloid			
1. Dragandroffs test	-	+	+
2. Mayer's test	-	+	+
3. Hager's test	-	+	+
4. Wagner test	-	+	+

Pharmacological Activity

Acute toxicity study of Extract (LD_{50})

The present study was conducted according to the organization for economic cooperation and development (OECD) revised fixed dose procedure for acute toxicity testing (OECD guideline 420, 2001). Five healthy albino wistar rat of either sex (3-month old, 150–200 g b. wt.) were administered a limit dose of 2000 mg/kg of the extract. Animals were observed for mortality and clinical signs (behaviours: unusual aggressiveness, unusual vocalization, restlessness, sedation and somnolence; movements: twitch, tremor, ataxia, catatonia, paralysis, convulsion, fasciculation, prostration and unusual locomotion; convulsion: clonic, tonic, tonic–clonic, asphyxial and opisthotonus) for the first hour, then hourly for 3 h and finally periodically until 48 h. All of the experimental animals were maintained under close observation for 14 days, and the number of rats that died within the study period was noted. The LD_{50} was predicted to be above 2000 mg/kg if three or more rats survived.

Acute Oral Toxicity Study (LD50):- as per OECD revised fixed dose procedure for acute toxicity testing (OECD guideline 420, 2000) as follows

Groups	Treatment	Observations
Two groups 5 animals each (males or females) 14hrs fasted	One group 1st animal receive extract at a single dose of 2000 or 5000 mg/kg followed by 2nd and 3 more animals whereas equal volume of vehicle is given.	Made up to 14 days for presence of change in skin, eyes, mucous, respiratory, circulatory, autonomic, CNS activity and behavioural pattern.

Oral administration of extract at doses up to 2000 mg/kg produced no signs of toxicity. No mortality was observed up to 14 days. Thus, the median lethal dose (LD_{50}) of the extract was greater than 2000 mg/kg body weight.

Analgesic Activity

Animals

Animals were purchased from National Institute of Bioscience, Pune. Albino wistar rat 150–200 gm were housed

under standard laboratory conditions, in a group of six each. The animals maintained under standard husbandry conditions and had free access to diet and water. The animals were fasted overnight prior to the experiments. The distribution of animals in the groups, the sequence of trials and the treatment allotted to each group were randomized, throughout the experiment. All experiments are approved and conducted as per the guidelines of institutional animal ethical committee [7].

Analgesic Activity

1) Eddy's Hot Plate Method

The central analgesic activity of the test drug is studied against thermal stimuli using this method. The paws of mice & rats are very sensitive to heat even at temperature which does not cause damage to skin. They respond by jumping, withdrawal of paws & licking of paws. In these test an electrically heated hot plate (Orchid Scientific Eddy's Hot Plate) temperature was maintained at 55±0.^{0}c. The initial reaction time of all the animals of control and test groups were recorded by putting them on hot plate maintained at 55±0.^{0}c. Licking of paw or jumping was taken as the index of reaction to heat. Albino mice were divided into six groups of Pet. Ether extract, Chloroform extract and methanol extract at dose of 50mg/kg B.W; 100 mg/kg B.W: 200 mg/kg B.W: were administered by oral route & standard compound as Pentazocine lactate 10 mg/kg by intraperitoneal route. The animals were placed on hot plate and the time until either licking or jumping is recorded by stop watch. Cut off time was not more than 20 seconds. The delay Iresponse was recorded after administration of test or standard compound as Pentazocine lactate 10 mg/kg by intraperitoneal route [2].

% Inhibition = [A-B/A] x 100

Where A = paw volume of the right hind paw of mice in the control group at 3hr,

B = paw volume of the right hind paw mice in the test group at 3hr.

Table 2: Analgesic activity of Different extracts of leaves of *Martynia annua L.* by Hot Plate method.

Groups	Paw licking or Jumping response time in Sec at Min					
	Treatment	Basal	30	60	90	120
Control	D/W10ml/kg, p.o.	3.50±0.22	3.83± 0.30	4.00±0.25	4.16±0.30	4.16± 0.30
Standard	Pentazocine 25 mg/kg, i.p.	3.66±0.21ns	5.00±0.25**	6.83±0.47**	10.46±0.30**	13.33±0.33**
Extract Pet. ether	50 mg/kg	3.70±0.16ns	4.06±0.20*	6.10±0.29**	8.00±0.40**	10.23±043**
	100 mg/kg	3.81±0.16ns	4.60±0.20*	6.00±0.31**	9.33±0.40**	11.00±35**
	200 mg/kg	3.88±0.16ns	4.16±0.20*	6.55±0.31**	8.36±0.45**	10.40±044**
Extract Chloroform	50 mg/kg, p.o.	3.80±0.16ns	4.62±0.21*	6.16±0.31**	8.30±0.40**	10.23±042**
	100 mg/kg	3.80±0.16ns	4.12±0.18*	6.16±0.30**	8.00±0.41**	10.00±035**
	200 mg/kg	3.85±0.18ns	4.50±0.22*	6.16±0.30**	8.35±0.40**	10.39±046**
Extract Methanol	50 mg/kg, p.o.	3.83±0.16ns	4.66±0.21*	6.16±0.30**	8.33±0.42**	10.33±045**
	100 mg/kg	3.33±0.21ns	4.83±0.16*	6.33±0.33**	9.33±0.21**	11.00±36**
	200 mg/kg	4.00±0.01ns	5.00±0.01*	6.16±0.30**	9.33±0.16**	10.83±0.30**

(ns- nonsignificant,*<0.05, **p<0.01 values are mean ± SEM, n= 6, When compared with control by using one way ANOVA followed by Dunnette's multiple comparison test)

2) Hot water Tail Immersion test method

The hot water tail Immersion test unit serves to assess the tail flick reaction of mice. When their tail is immersed in a constant temperature bath. User can either conduct the study by holding, wrapping or placing the animal in a restrainer with the tail exposed. The animal's tail is then immersed into the water bath. The temperature range is from room temperature to 65 degrees Celsius. When the animal reacts by flicking their tail you can stop the timer by either the front panel push buttons or footswitch that is supplied, this unit will store and display the time of reaction [2].

Table 3: Analgesic activity of Different extracts of leaves of *Martynia annua* by Hot Water tail immersion method.

Groups	Tail Flick response time in sec at min.					
	Treatment	Basal	30	60	90	120
Control	D/W10ml/kg, p.o	3.16±0.30	3.83± 0.16	3.83±0.16	3.83±0.16	3.50± 0.22
Standard	Pentazocine 25 mg/kg, i.p.	3.66±0.21ns	5.16±0.25**	7.00±0.44**	10.50±0.34**	13.66±0.21**
Extract Pet.ether	50 mg/kg.	3.30±0.16ns	4.26±0.18*	6.10±0.29**	8.00±0.30**	10.23±035**
	100 mg/kg	3.71±0.16ns	4.40±0.21*	6.25±0.31**	9.23±0.30**	11.45±20**
	200 mg/kg	3.40±0.16ns	4.46±0.21*	6.55±0.31**	8.36±0.40**	10.40±044**
Extract Chloroform	50 mg/kg	3.21±0.16ns	4.62±0.14*	6.10±0.25**	8.30±0.40**	10.33±028**
	100 mg/kg	3.60±0.16ns	4.52±0.21*	6.18±0.30**	9.00±0.29**	11.00±019**
	200 mg/kg	3.40±0.25ns	4.50±0.22*	6.16±0.30**	8.35±0.40**	10.39±046**
Extract Methanol	50 mg/kg	3.33±0.33ns	4.83±0.16*	6.00±0.25**	8.50±0.50**	10.50±022**
	100 mg/kg	3.50±0.22ns	4.83±0.16*	6.50±0.34**	9.83±0.30**	11.50±0.22**
	200 mg/kg	3.33±0.21ns	5.00±0.01*	6.33±0.33**	9.33±0.21**	11.16±0.16**

(ns- nonsignificant,*<0.05, **p<0.01 values are mean ± SEM, n= 6, When compared with control by using one way ANOVA followed by Dunnette's multiple comparison test)

Result and Discussion

Methanolic extract of leaves of *Martynia annua L*. was screened for Analgesic Activity. The fraction isolated from methanolic extract of leaves is Bis (2-ethylhexyl) phthalate. The analgesic activity of extract of leaves of *Martynia annua L*. was performed. Analgesic activity of the extract was compared with the standard drug pentazocine. Results were analyzed for statistical significance with help of one-way ANOVA followed by Dunnet ˝test. A P value < 0.01 was significant. The methanol extracts exhibits greater analgesic activity as compared to petroleum ether and chloroform extract of the plant of Martynia annua.

Conclusion

From all the experiments done on leaves of *Martynia annua L*. It is concluded that the *Martynia annua L*. plant shows presence of alkaloids, glycosides, carbohydrate, tannins and phenolic compound, flavonoids, proteins, steroids and sterols. The phytochemical studies showed the presence of most of the biologically active compounds in the plant. **Bis (2-ethylhexyl) phthalate** was found as compound in methanolic extract. Methanolic extract of *Martynia annua L*. leaves was found to be significant in Analgesic activity.

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References

1. Vogel HG. Drug Discovery and Evaluation: Pharmacological Assays. 2nd ed. Springer-Verlag: Berlin Heidelberg. 2002; 369:796.
2. Dr. Pulok K. Mukherjee, Quality control herbal drugs, business horizon, New Delhi, 247, 555.
3. AK Nadkami, Indian Materia Medica, Popular Prakashan (P) Ltd. 2007; 430:277-278.
4. KR Kirtikar, BD Basu. Indian medicinal plants. 2006; II(52):601, 1546.
5. Khandelwal S De, IntJ Pharma Biomed Res. 2010; 1(5):150-157.
6. Stahl E. TLC: A laboratory handbook, 2nd edition, Springer International, 2005, 241-247, 688-689, 706-707, 863, 88.
7. Robert Turner: screening methods in pharmacology, academic press, Elsevier. 2009; l:162-163.
8. AN Kalia. Textbook of Industrial Pharmacognosy, CBS publisher, 2005, 143.
9. Kamboj VP. Herbal medicine, Curr Sci. 2000; 78:35-39. 16. Verma S, Singh SP. Current & Future Status of Herbal Medicine, veterinary world. 2006; 1:347-350.
10. Agrawal DP. Complementary and alternative medicine: An Overview. Curr Sci. 2002; 82:518-524.
11. Wagner H, Bladt S. Plant drug analysis: A TLC, 2nd edition, Atlas Germany, Springer publication, 1996, 335, 352-354, 363.
12. Katare V, Pathak AK, Kori ML, Chakraborthy B, Nandy S. Phytochemical and Pharmacognostic studies of *Martynia annua.* IRJP. 2012; 3(6):104-08.
13. The Ayurvedic Pharmacopeia of India, Govt. of India. 2001; 2(1):1.
14. RN Chopra, SL Nayar, LC Chopra. Glossary of Indian medicinal plants, Anonymous. Indian Medicinal Plants (a compendium of 500 species), Orient Longman Ltd, Hyderabad, 1995-2006; 4:236-238, 386-389.
15. Padmapryia M, Prabhitha KS, Devika R, Sugashini PK. Phytochemical Screening of *Martynia annua*. Ijppr. Human. 2016; 7(1):394-401.
16. http://www.painclinic.org/aboutpain-paintypes.htm.