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Research Article

Phytochemical Investigation and Anti-Arthritic Activity of Hydroalcoholic Extracts of *Trichosanthes dioica*

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ABSTRACT

Trichosanthes diocia Roxb. is a Cucurbitaceous perennial herb widely distributed in tropical areas of Asia and Australia. This herb is cultivated across the globe firmly as a vegetable source. *Trichosanthes diocia* are used in traditional medicinal system as anti-inflammatory agent, liver tonic, cardiotonic, skin infection, antiulcer, anti-diabetic etc. Number of phytochemical and pharmacological work has been carried out on deferent parts of *Trichosanthes diocia*, prime objective of the study to find out Anti-Arthritic activity of hydroalcoholic extract of *Trichosanthes diocia*. Hydroalcoholic extracts are rich of flavonoids, Vitamins, Alkaloids, proteins and saponins. Although flavonoids are also responsible for inflammatory induced Antiarthritic activity, presence of secondary metabolite phytoconstituents encourages the current work. Results of the current study shows that *Trichosanthes diocia* Roxb. having significant anti-arthritic activity.

Keywords: Anti-arthritic; Hydroalcoholic extract; *Trichosanthes diocia*; Phytoconstituents

INTRODUCTION

Globally around 80,000 plant species are used for medicinal and aromatic purposes. Although there is wide use of herbal medicine, traditional knowledge of the use of medicinal plants is influenced by rapid urbanization, migration, climate change, and the increasing number of modern healthcare systems throughout the world [1-4]. About 90% herbal raw drugs used in the manufacture of vegetable drugs are obtained from the wild source which is limited. With the increasing esteem of herbal medicine and ayurveda, use of medicinal plants is expected to rise globally. Since the side effects and cost of synthetic drugs are higher it demands increased utilization of herbs [5]. Rheumatoid Arthritis (RA), classified as autoimmune affecting approximately 5% of the human population [6]. RA is a chronic inflammatory polyarthritis, affecting multiple diarthroidial joints in a Characteristic distribution, and leading to pain, joint deformities and a reduced quality of life. RA characterised by extensive synovitis resulting in erosions of articular cartilage and marginal bone that lead to joint destruction [7]. More than 100 of rheumatic diseases are characterized by inflammation and pain, are called as autoimmune diseases because they occur when the immune system shows significant activity with response to serious infection. Clinically RA manifests with a symmetric polyarthritis characterized by pain, swelling, loss of function and a morning stiffness lasting more than one hour [8]. A common onset synovitis involves the metacarpophalangeal, the proximal interphalangeal, the wrist and the metatarsophalangeal joints, although all the joints may be affected. Several constitutional symptoms can precede the onset of RA, such as fatigue, malaise, weight loss, fever and depression [9].

Trichosanthes dioica (*T. dioica*) Roxb. A herb of Cucurbitaceae family and commonly known as "Sespadula" in English "Parwal" or Patal in Hindi. In India fruits of the parval are used as vegetable in almost all seasons [10]. Their Anti-Inflammatory, antipyretic, diuretic, cardiotonic, and laxative and many more pharmacological activity are already established, Study of extensive ethnobotanical survey and its Anti-Inflammatory activity excites us to go for Anti-Arthritic activity of its hydro alcoholic extract because it contains more relevant phytochemicals as compared to other extracts [11].

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MATERIALS AND METHODS

Identification of *Trichosanthes dioica*

Trichosanthes dioica Roxb. fruits are collected freshly from nearby villages of Varanasi. Vouchered herbarium specimen of *Trichosanthes dioica* were prepared and preserved (Cucurbitaceae 2014/9) in faculty of science, department of botany, Banaras Hindu University, Varanasi (U.P.), India. Plants are Shade dried, reduced to powder and preserved for further use.

Animals

- Male wistar rats were chosen for the study.
- The animals were used after an acclimatization period of 7 days to the laboratory environment.

Preparation of extracts

Accurately weight 1.232 kg of powdered drug was taken in soxhlet apparatus, maceration was carried out till the extraction completed with petroleum ether up to 80°C. It was filtered hot and solvents were removed by distillation under reduced pressure. Marc left after petroleum ether extraction was dried in hot again loaded in soxhlet with ethanol (70%) until extraction completed. It was filtered hot and solvents were removed by distillation under reduced pressure and the hydroalcoholic extracts were stored for further use (Table 1).

Table 1: Extractive values and appearance of various extracts.

	Percentage Yield			Visual Results		
Types of Extract	Petroleum ether extract	Hydroalcoholic Extract	Aqueous Extract	Petroleum ether extract	Hydroalcoholic Extract	Aqueous Extract
Trichosanthes diocia Roxb.	3.2	25.1	27.5	Semisolid	Solid	Solid

Table 2: Presence of various phytoconstituents.

Name of phytoconstituents		Pet. Ether Extract	Hydro alcoholic Extract	Aqueous Extract
Presence of Alkaloid	Hager's test	-	+	-
	Mayers	-	+	-
Presence of Glycosides	Borntrager's test	-	+	-
	Keller killiani test	-	+	-
	Legal's test	-	+	+
Presence of Carbohydrates	Benedict's test	-	+	+
	Fehling's test	-	+	+
	Molish test	-	+	-
Presence of Phenols and Tannin	Bromine water Test	-	+	+
	Lead acetate Test	-	+	+
	$FeCl_3$ Test	-	+	-
Presence of Flavonoid	Shinoda Test	-	+	+
Presence of Protein	Biuret Test	-	-	-

Presence of phytoconstituents like flavonoids, tannins, phenols, alkaloids and glycosides, fats and carbohydrates were investigated by using preliminary phytochemical screening of *Trichosanthes dioica* and the maximum contents were observed in hydroalcoholic extracts of *Trichosanthes dioica* [12] (Table 2).

Estimation of phenolic content of *Trichosanthes diocia*

The hydroalcoholic extract of *Trichosanthes diocia* Roxb. was evaluated total phenolic content concentrations. Folin-Ciocalteu method was used for estimation of total phenolic content in extracts of *Trichosanthes diocia* data expressed as gallic acid was equivalents. For calculating absorption data gallic acid was dissolved in water and absorbance were recorded. Total phenolic content of hydroalcoholic extract of *Trichosanthes diocia* were found to be 94.65 ± 1.05 mg GAE/gm [13-15] (Table 3).

Table 3: Total Phenol content of hydroalcoholic extract of *Trichosanthes diocia.*

Type of Extract	Total Phenol content (mg GAE/gm)
Hydroalcoholic Extract	94.65 ± 1.05

Determination of flavonol content of *Trichosanthes diocia*

Spectrophotometric methods by using aluminum chloride were used for estimation of flavonoid content and the contents were expressed in terms of quercetin equivalents. Standard curve of quercetin was plotted by dissolving it in distilled water. Content of flavonoids identified in the hydroalcoholic extract of *Trichosanthes diocia* are shown in Table 4. The concentrations of flavonoids in hydroalcoholic extract of *Trichosanthes diocia* Roxb. 74.11 mg QE/gm [16-19].

Table 4: Flavonol content of hydroalcoholic extract of *Trichosanthes diocia.*

Type of extract	Amount of flavonol content (mg QE/gm)
Hydroalcoholic Extract	74.11 ± 0.64

Estimation of anti-arthritis activity of *Trichosanthes diocia*

Adjuvant induced chronic arthritis model: There are many experimental models available but autoimmune arthritis are mainly accelerated by mycobacterium infection via T-Cell intermediate pathway. For our study arthritis was induced by using injection of dead mycobacterium in combination with liquid paraffin. After treatment with FCA there is significant increase in paw volume of rat was observed when it is compared with hydroalcoholic and standard drug treated rat. Dose of 25 mg/kg of hydroalcoholic extract shows significant decrease in paw edema of rat. After a time interval of 28 days it was found that hydroalcoholic extract of *Trichosanthes diocia* reduces paw volume significantly and which is totally dose dependent. Reductions in paw volume after treatment with Hydroalcoholic extract of *Trichosanthes diocia* were found to be 0.39 ± 0.51 mL. Indomethacin which is used as standard drug reduces paw volume was found to be 0.23 ± 0.91 mL [20-29].

Before the start of experiment rats were divided into 5 groups each group contains 6 rats. On starting day 0.1 mL of FCA (Freund's complete adjuvant) were injected sub plantrly in left paw of rats. Standard drug Indomethacin and hydroalcoholic extract were injected after the next day of FCA injection and it will continue till 28th day. Left paw was marked with marker and paw volumes were recorded with the help of plethysmometer after injection routinely on 7th and 14th, 21st and 28th day of experiment (Table 5).

Control group marked as Group I: Arthritic infected rats treated with distilled water.

Group II: Arthritic infected rats treated with Indomethacin i.e., Standard drug with the dose of 10 mg/kg.

Group III: Arthritic infected rats treated with Hydroalcoholic extract of *Trichosanthes diocia* with the dose of 25 mg/kg.

Table 5: Effect of hydroalcoholic extract of *Trichosanthes diocia* in paw volume.

	Observation of Paw volume				
Name of the Group	On 0th Day	On 7th Day	On 14th Day	On 21st Day	On 28th Day
Control Group	0.21 ± 0.32	0.73 ± 0.47	0.98 ± 0.96	1.12 ± 0.25	0.98 ± 0.42
Indomethacin treated Group	0.27 ± 0.54	0.53 ± 0.83*	0.45 ± 0.63*	0.32 ± 0.59*	0.23 ± 0.91*
Hydroalcoholic extract treated Group	0.22 ± 0.14	0.63 ± 0.35	0.55 ± 0.47*	0.45 ± 0.47*	0.39 ± 0.51*

Study of hematological parameters

Table 6: Hematological study of hydroalcoholic extract of *Trichosanthes diocia*.

Name of the Group	Readings of RBC level	Readings of WBC level	Hb level	ESR level
Control Group	3.25 ± 1.24	23.17 ± 0.84	8.31 ± 1.53	35.26 ± 1.32
Indomethacin Treated Group	6.12 ± 0.46*	7.63 ± 1.31*	11.74 ± 0.77*	2.31 ± 0.27*
Hydroalcoholic Extract Treated Group	4.32 ± 1.52*	07.22 ± 1.06*	09.05 ± 0.45*	3.24 ± 1.25 *

After the administration of hydroalcoholic extract of *Trichosanthes diocia* on Freund′ s adjuvant treated rats it was observed that the hemoglobin (Hb) and Red Blood Cell Count (RBC) levels are increased significantly when it is compared to distilled water treated rats. Whereas the White Blood Cell (WBC) and Erythrocyte Sedimentation Rate (ESR) count were

significantly decreased after the administration of *Trichosanthes diocia* Hydroalcoholic extract [30-32] (Table 6).

DISCUSSION

After preliminary phytochemical screening it was observed that hydroalcoholic extract contains maximum amount of phytoconstituents which are responsible for inflammation and arthritic activity therefore we have chosen hydroalcoholic extract, Anti-inflammatory activities are already established therefore we have not gone through Anti-inflammatory activity. Based on earlier established data we have carried out our work for rheumatoid Arthritis. Rheumatoid arthritis is significantly characterized as inflammatory process therefore on folk medicinal information and previous study we have conducted Rheumatoid Arthritis activity.

RA is an autoimmune disease, therefore immunologically mediated FCA induced arthritic model is considered for ongoing study. Treatments of animals with FCA results and induction of systemic inflammation.

FCA insertion developed a chronic swelling of joints, erosion of joint cartilage, remodeling and bone destruction which results complete destruction of joint stability and mobility in the arthritic rats. Swelling of Ankle joints was observed which is termed as edema [33].

In our study, experimental arthritis were developed with repeated sub plantar injection of 0.1 mL of FCA up to 28 days which was characterized by development of tissue edema and reported highest on 7th day.

The progression of arthritis was confirmed in our study by scoring total arthritis lesions. Polyphenol content and presence of tannins also reveals anti-inflammatory action of *Trichosanthes diocia*. Therefore inhibition of lipid peroxidation and enzyme activity like cyclooxygenase, lipoxygenase may be due to tannins and polyphenols components [34].

In our study, arthritic control rats showed a decreased level of RBC and Hb where as ESR ratio are increased. Above symptoms are indications of anemia. Groups which are treated with Hydroalcoholic extract showed a significant recovery from anemic condition. Increase in leukocyte count in rats may be due to the stimulation of immune system against FCA therefore hydroalcoholic extract treated groups shows immunomodulatory effect. Resulting immunomodulation effect indicates the anti-arthritic activity of Hydroalcoholic extract of Trichosanthes diocia. Hydroalcoholic extract of Trichosanthes diocia are responsible for its anti-arthritis activity which are also supported by presence of various phytoconstituents.

CONCLUSION

Trichosanthes diocia Roxb. are rich in secondary metabolite such as alkaloid, glycoside, flavonoids, polyphenol etc. Polyphenol and flavones are responsible for various pharmacological activities. Therefore in our study an attempt was made to determine phytoconstituents and relate their anti-arthritis activity. Although anti-inflammatory activity are previously established therefore only for confirmation we have conducted phenol content and flavonol content study, presence of flavones on our hydroalcoholic extract supports our study. Hydroalcoholic extract of *Trichosanthes diocia* shows moderate anti-arthritis activity. This is also supported by presence of various plant metabolites like alkaloids, tannins, phenols and flavones. For estimation of possible mechanism responsible for anti-arthritis activity further study in cellular level and isolation of hydroalcoholic fractions are required.

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REFERENCES

1. Craig WJ. Health-promoting properties of common herbs'. American Journal of Clinical Nutrition. 1999; 1: 491-499.
2. Abdullahi AA. Trends and challenges of traditional medicine in Africa. Afr J Tradit Complement Altern Med. 2011; 8: 115-123.
3. Bernal J, Mendiola JA, Ibáñez E, Cifuentes A. Advanced analysis of nutraceuticals. J Pharm Biomed Anal. 2011; 55: 758-774.
4. Jordan SA, Cunningham DG, Marles RJ. Assessment of herbal medicinal products: Challenges, and opportunities to increase the knowledge base for safety assessment. Toxicol Appl Pharmacol. 2010; 243: 198-216.
5. Rokaya MB, Münzbergová Z, Timsina B. Ethnobotanical study of medicinal plants from the Humla district of western Nepal. J Ethnopharmacol. 2010; 130: 485-504.
6. Firestein GS (2003) Evolving concepts of rheumatoid arthritis. Nature 423:356-361. Halberg P. History. In: Klippel J, Dieppe P. (eds), Rheumatology. (2nd edn), London: Mosby, 1998: 1-4.
7. Mimori T. Clinical Significance of Anti-CCP Antibodies in Rheumatoid Arthritis. Intern Med. 2005; 44: 1122-1126.
8. Costerbader KH, Chang SC, Laden F, Puett R, Karlson EW. Geographic variation in rheumatoid arthritis incidence amongst women in the United States. Ann Intern Med. 2008; 168: 1664-1670.
9. Scott DL, Steer S. The course of established rheumatoid arthritis. Best Pract Res Clin Rheumatol. 2007; 21: 943-67.
10. Rahman MAA, Moon SS. Isoetin 5-methyl ether, a cytotoxic flavone from Trichosanthes kirilowii. Bull. Korean Chem Soc. 2007; 28: 1261-1264.
11. Khare CP. Encyclopedia of Indian medicinal plants. Berlin, Heidelberg; New York: Springer-Verlag; 2004; 457-458.
12. Kumar M, Mondal P, Borah S, Mahato K. Physico-chemical evaluation, preliminary phytochemical investigation, fluorescence and TLC analysis of leaves of the plant Lasia spinosa (Lour) Thwaites. Int J Pharm Pharm Sci. 2013; 5: 306-310.
13. Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nature Protocols. 2007; 2: 875-877.
14. Pasko P, Barton H, Zagrodzki P, Gorinstein S, Folta M, Zachwieja Z. Anthocyanins, total polyphenols and antioxidant activity in amaranth and quinoa seeds and sprouts during their growth. Food Chem. 2009; 115: 994-998.
15. Chlopicka J, Pasko P, Gorinstein S, Jedryas A, Zagrodzki P. Total phenolic and total flavonoid content, antioxidant activity and sensory evaluation of pseudocereal breads. LWT - Food Science and Technology. 2012; 46: 548-555.

16. Barros L, Ferreira MJ, Queiros B, Ferreira ICFR, Baptista P. Total phenols, ascorbic acid, β-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. Food Chem. 2007; 103: 413-419.
17. Norizan N, Ahmat N, Syed SA, Nazri NAA, Ramli SSA, Kasim SN, et al. Total phenolic content, total flavonoid content, antioxidant and antimicrobial activities of Malaysian Shorea. Res J Med Plant. 2012; 6: 489-499.
18. Gorinstein S, Medina Vargas OJ, Jaramillo NO, Salas IA, Ayala ALM, Arancibia-Avila P. The total polyphenols and the antioxidant potentials of some selected cereals and pseudocereals. European Food Research and Technology. 2007; 225: 321-328.
19. Cheung LM, Cheung PCK, Ooi VEC. Antioxidant activity and total phenolics of edible mushroom extracts. Food Chem. 2003; 81(2): 249-255.
20. Meera S, Kumar NS, Guptatyam VS. Screening of anti-arthritic, anti-inflammatory and analgesic activity of a polyherbal formulation. Int J Pharmacol. 2008; 4: 398-402.
21. Kaneria MS, Naik SR, Kohli RK. Anti-inflammatory, antiarthritic and analgesic activity of a herbal formulation. Indian J Exp Biol. 2007; 45: 278-284.
22. Yende SR, Sannapuri VD, Vyawahare NS, Harle UN. Antirheumatoid activity of aqueous extract ofPiper nigrum on Freund's Adjuvant-induced arthritis in rats. Int J Pharma Sci Res. 2010; 1: 129-133.
23. Kataoka H, Horiyama S, Yamaki M, Oku H, Ishiguro K, Katagi T, et al. Anti-inflammatory and anti-allergic activities of hydroxylamine and related compounds. Biol Pharm Bull. 2002; 25: 1436-1441.
24. Patil RB, Nanjwade BK, Manvi FV. Effect of *Sesbania grandiflora* and *Sesbania sesban* Bark on carrageenan induced acute inflammation and adjuvant-induced arthritis in rats. Pharma Sci Monitor: An Int J Pharmaceut Sci. 2010; 1: 75-89.
25. Ahmad SF, Khan B, Suri KA, Satti NK, Qazi GN. Amelioration of adjuvant-induced arthritis by ursolic acid through altered Th1/Th2 cytokine production. Pharmacology Resources. 2006; 53: 233-240.
26. Brand DD. Rodent models of rheumatoid arthritis. Comparative Medicine. 2005; 55: 114-122.
27. Brand DD, Latham KA, Rosloniec EF. Collagen-induced arthritis. Nature Protocols. 2007; 2: 1269-1275.
28. Ekambaram S, Perumal SS, Subramanian V. Evaluation of anti-arthritic activity of Strychnos potatorum Linn seeds in Freund′ s adjuvant induced arthritic rat model. BMC Complement Altern Med. 2010; 10: 1-9.
29. Mubashir K, Bashir AG, Ghazanfar K, Akbar S. Evaluation of Antiarthritic Potential of Methanolic Extract of Gentiana kurroo Royle. Arthritis. 2014; 2014: 1-6.
30. Chesbrough M, McArthur J. Laboratory Manual of Rural Tropical Hospitals. The English Language Book Society and Churchill Livingstone: London. 1972.
31. Austin JH, Drabkin DL. Estimation of Haemoglobin. J Biol Chem. 1935; 112: 67-69.
32. David G, Sykes AJ. Westergren and Wintrobe methods of estimating ESR compared. Br Med J. 1951; 2: 1496-1497.
33. Singh S, Majumdar DK. Effect of fixed oil of Ocimum sanctum against experimentally induced arthritis and joint edema in laboratory animals. Int J Pharmacog. 1996; 34: 218-222.
34. Yend SR, Sannapuri VD, Vyawahare NS, Harle UN. Antirheumatoid activity of aqueous extract of Piper nigrum on Freund's Adjuvantinduced arthritis in rats. Int J Pharma Sci Res. 2010; 1: 129-33.