IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

In vitro evaluation of anti-asthmatic activity of *Stinging nettle* leafs

***[1]Chollangi Bharghavi,[2]Sabina Khatun,[3]Md Masud Alam,[4]Ragni kumari,[5]Ravi Kumar Vishwakarma**

1. Lovely Professional University, Jalandhar, Phagwara, Punjab-144001, India.
2. NIIMS University, Delhi highway, Jaipur, Rajasthan -303121, India.
3. R.K.D.F College of Pharmacy, SRK University, hoshangabad road, Bhopal, MP-462026, India.
4. School of pharmacy, LNCT University, Bhopal, Madhya Pradesh-462022, India.
5. Department of Pharmacology,Mangalayatan University, NCR 33rd milestone, aligarh-mathura highway,beswan,aligarh, UP-202145,India.

ABSTRACT

Stinging nettle (*Urtica dioica*) has a long history use as a medicinal herb found throughout the world that has used for medicinal purposes for centuries It has been used to treat asthma but synthetic drugs for the treatment of asthma and allergy in India found more side effects. Synthetic drugs used for the treatment of asthma and allergy in India but more side effects are reported. Over the centuries, they are using medicinal herbs in daily life and approximately 6000 plants species are known to have medicinal properties in India. As per the literature survey will be expressed medicinal plants and traditional systems of medicines, Ayurveda, Yunani, Siddha and Homeopathy for the treatment of asthma and allergy but no scientific validation. Several literatures are indicated that the herbal drugs have lesser adverse effects when compared to synthetic drugs. The Urtica dioica is not scientifically validated and which was traditionally using herb. The work provides scientific validation for use of leaves against asthma by revealing the chemical compounds may be present in the plant. The present study is attempts to develop a novel plant-based antihistamine work through anti asthmatic drug which will be evaluated by in vitro and in vivo.

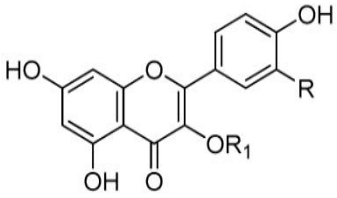
KEYWORDS- Mephramine ,anti-asthmatic activity; *Stinging nettle* leafs,

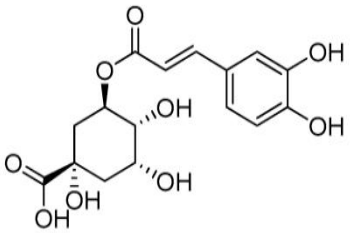
INTRODUCTION

Stinging Nettle (*Urtica dioica* L.) is one such species that is found widely in temperate and tropical Asia, Europe, northern America and northern Africa and consumed by traditional societies. *Urtica dioica* is a perennial herb that grows commonly in waste lands, gardens, farmers field (as weed), as hedges in terraced fields. It is distributed between 1200 to 3000 m in Himalaya from Jammu & Kashmir to Arunachal Pradesh (Wealth of India 1998). Nettle has been used for centuries in various folk medicine systems in China, Persia, Turkey, Russia, India and various other countries to cure humans and animals. For treatments it is used as extract (juice), in dried form, as tincture, ointment and/or as a supplement. It is used to treat allergies, kidney stones, burns, anemia, rashes, internal bleeding, diabetes, etc.Commonly called as Nettle, Common nettle or Stinging nettle, all over the world *Urtica dioica* is known with the different names.Allergy is one of the common diseases that affect mankind with diverse manifestations. Asthma is a respiratory disease characterised by recurrent

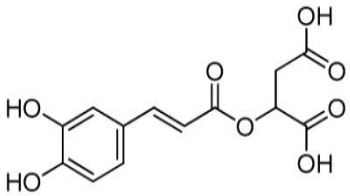
episode of chest tightness, cough, wheezing and difficulty breathing brought about by bronchial constriction, inflammation and excessive mucus secretion due to bronchial hyperesponsiveness. The sprevalence of allergy and asthma has risen in the recent years despite an improvement in the general health of the population. Allergic diseases are responsible for significant morbidity and have severe economic impact. Various epidemiological studies have identified the causes for an increase in the prevalence of upper and lower respiratory tract allergic diseases. Some of the postulated

reasons are increasing environmental pollution and increased predisposition of individuals producing excessive IgE through a major change in the gene pool, changing lifestyles, and an increasing awareness of the disorders.The application of nettle to cure diseases with good healing properties can be attributed to the presence of certain phytochemicals, such as flavonoids, lignans, fatty acids, sterols, polysaccharides, glycoproteins, carotenoids, plastocyanins, tannins and lectins (Sajfrtová et al., 2005, Ghaima et al.,2013). Efforts are being made to identify and isolate such phtochemicals from different parts of the plant that has direct effect (Krystofova et al., 2010). It is reported that nettle comprised polysaccharides, vitamin C and carotene, betasitosterol, and the flavonoids quercetin, rutin, kaempferol, and beta-sitosterol (Newall et al.,1996; Schottner et al., 1997; Konrad et al., 2000)The leaves comprised diterpene lactone and Phlogantholide A. Polar extracts of the nettle roots contain the lignans that have binding affinity to SHBG in the *in vitro* assay (Schottner et al., 1997).Other than the lignans nettle is reported to have lectins, sterols, phenylpropanes, ceramides,hydroxyl fatty acids, triterpenes, phenols,coumarins, fatty acids and carotinoids, flavonoids, amines, chlorophylls and carotinoids (Seliya and Kothiyal, 2014). The main components of essential oil in nettle are carvacrol (38.2%), carvone (9.0%), naphthalene (8.9%), (E)-anethol (4.7%),hexahydrofarnesyl acetone (3.0%), (E)-geranyl acetone (2.9%), (E)-ionone (2.8%) and phytol (2.7%) (Gul et al., 2012).





chlorogenic acid



caffeoyl malic acid

MATERIALS AND METHODS

The leaves of *urtica dioica* were collected.

Preparation of plant extract

Cold maceration technique was used for the extraction of plant material and a total of 200 g of *Urtica dioica* leaves the coarse powder was used. During the process 100 g of the coarse powder was soaked in an Erlenmeyer flask with 1 L of 50% of Ethyl Acetate and then placed on a shaker (Bibby Scientific Limited Stone Staffo Reshire, UK) tuned to 120 rpm with occasional shaking for 72 h at room temperature.The extract was filtered first using a muslin cloth and then Whatman grade No-1 filter paper and the marc was re-macerated for a second and third time by adding another fresh solvent. The filtrates were left overnight in a deep freezer and then lyophilized using freeze dryer. The dried plant extract was reconstituted with distilled water for oral administration.

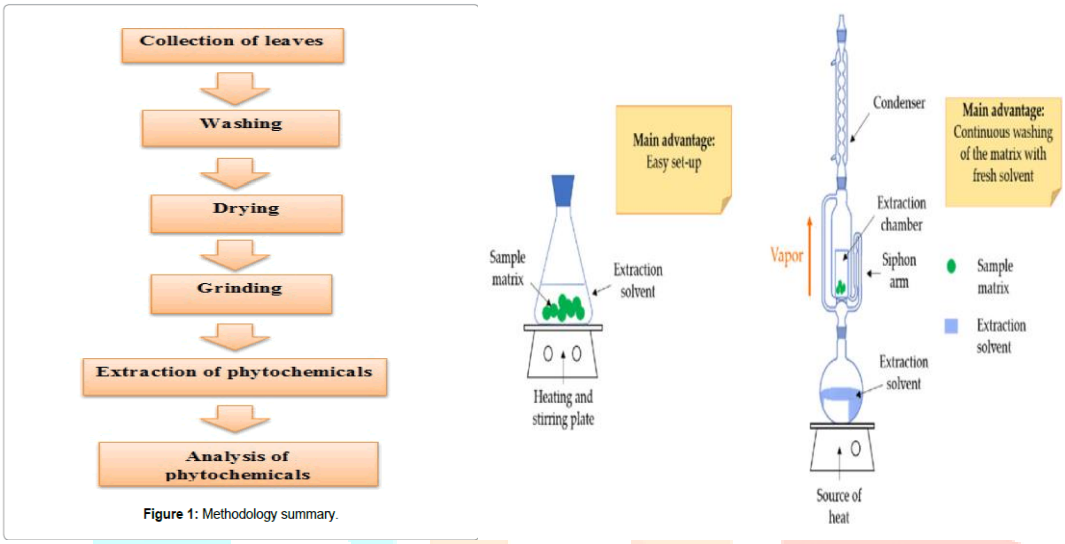


Figure 1: Methodology summary.

Phytochemical Test

Maeyer's reagent-0.355 g of mercuric chloride was dissolved in 60 ml of distilled water. 5.0g of potassium iodide was dissolved in 20 ml of distilled water. Both solutions were mixed and volume was raised to 100 ml with distilled water.

Test for alkaloids-About 0.5 to 0.6 g of the methanolic plant extract was mixed in 8 ml of 1% HCl, warmed and filtered. 2 ml of the filtrate were treated separately with both reagents (Maeyer's and Dragendorff's).

Test for steroids-About 0.5 g of the methanolic extract fraction of each plant was mixed with 2ml of acetic anhydride followed by 2 ml of sulphuric acid.

Dragendorff's reagent-Solution A: 1.7 g of basic bismuth nitrate and 20 g of tartaric acid were dissolved in 80 ml of distilled water. Solution B: 16 g of potassium iodide was dissolved in 40 ml of distilled water. Both solutions (A and B) were mixed in1:1 ratio.

Test for terpenoids-An aliquot 0.5 ml of methanolic extract was mixed with 2 ml of CHCl3 in a test tube. 3 ml of concentrated H2SO4 was carefully added to the mixture to form a layer.

Test for flavonoids-To the substance in alcohol, a few magnesium turnings and few drops of concentrated Hydrochloric acid were added and boiled for five minutes.

Test for tannins-The 0.5 g of powdered sample of each medicinal plant leaves was boiled in 20ml of distilled water in a test tube and then filtered. The filtration method used here was the normal.

Test for Phytosterol
1. Foam Test: 5 ml of the test solution taken in a test tube was shaken well for five minutes.
2. Olive oil test: - Added a few drops of olive oil to 2ml of the test solution and shaken well.

Test for glycosides
1.Keller -Killiani test: Added 0.4 ml of glacial acetic acid and a few drops of
5% ferric chloride solution to a little of dry extract. Further 0.5 ml of concentrated sulfuric acid was added along the side of the test tube carefully.
2. Hydroxyanthraquinone Test To 1 ml of the extract, added a few drops of
10% potassium hydroxide solution.

Test for Phytosterol

1. Foam Test: 5 ml of the test solution taken in a test tube was shaken well for five minutes.
2. Olive oil test: - Added a few drops of olive oil to 2ml of the test solution and shaken well.

Experimental animals
The Adult female *S*wiss mice weighing between (20-30 g) were used to calculate LD50 and female and male guinea pigs with an average weight of 220-250 g were used antihistamine study. They were housed in clean polypropylene cages and maintained under standard conditions of light (12 hours with alternative day/night cycles), relative humidity (60-70%) and temperature (26 ± 1 °C). The animals were fed daily with rodent pellet diet and tap water *ad-libitum* under strict hygienic conditions.

Histamine induced convulsion by using histamine chamber

Animals with nearly same pre convulsion time were selected and randomly divided into three groups of six animals each-
GROUP I – Asthmatic control-0.5% Histamine HCL aerosol.
GROUP II – Standard treatment 0.5% Histamine HCL aerosol with Mepyramine (8mg/ kg,p.o).
GROUP III - High dose (200mg/kg) of aqueous ethanolic extract of *urtica dioica.*

The experimental animals were kept in a closed chamber and exposed to an aerosol of 0.5% of histamine dihydrochloride and preconvulsion time was measured two hours after the above drug treatment, animals were exposed to histamine aerosol and pre convulsion time was noted. As soon as dyspnea occurs, it leads to the appearance of convulsion. animals were removed from the chamber and placed in fresh air to recover.
Percentage protection = {1-T1/T2} x 100 where, T1 = time in second for PCD before treatment; T2 – time in send for PCD after treatment.

RESULTS AND DISCUSSION

Urtica dioica were a light semisolid brownish color extract and the percentage yield was found to be 16.35%.

Phytochemical Analysis

S.No	Phytochemicals	Inference
1.	Steroids	+
2.	Alkaloids	+
3.	Terpenoids	-
4.	Tannins	+
5.	Flavonoids	+
6.	Glycosides	+
7.	Saponin	+
8.	Phytosterol	+

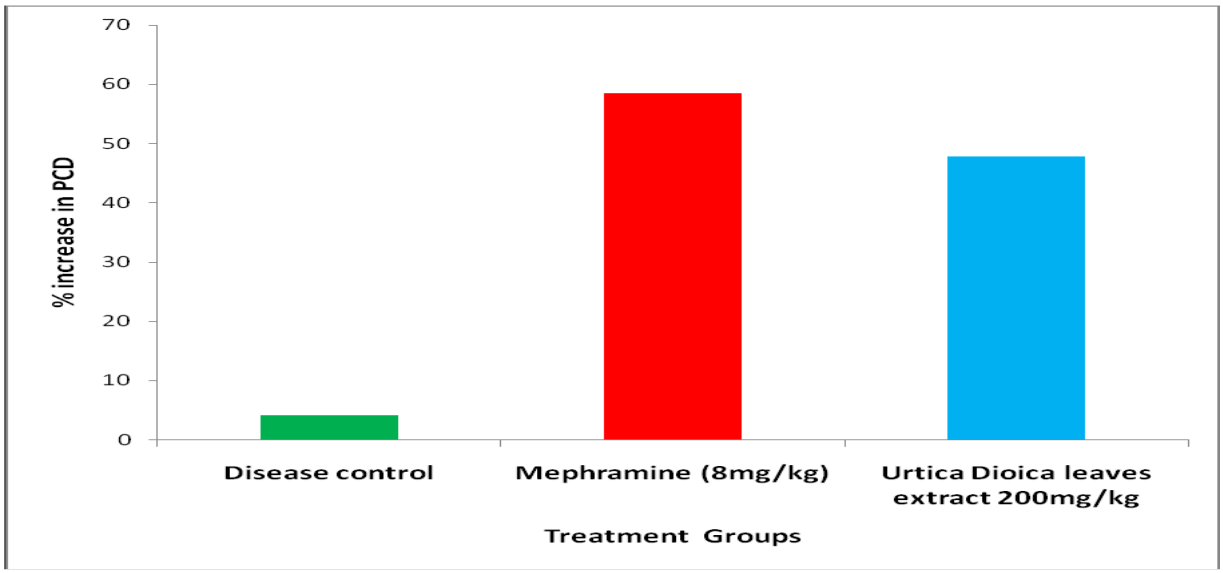
+, Presence of the compound

-, Absent

Histamine induced convulsion by using histamine chamber

S.No.	Treatment Groups	% increase in PCD
1.	Disease control	4.16 ±0.83
2.	Mephramine (8mg/kg)	58.5 ±0.22 *
3.	Urtica Dioica leaves extract 200mg/kg	47.83 ±0.47 *

CONCLUSION



This work will be useful to find new anti asthamatic drug with help of in vitro and in vivo models. Ethanolic extract will be possess highly substantial anti-asthmatic activity by significantly inhibited the histamine induced broncho constriction of guinea pig representing its H1 receptor antagonistic activity and support the plants by its anti-asthmatic properties.

REFERENCES

1. Evans WC, Trease GE (1996). In: Textbook of Pharmacognosy. 5th Edn., Saunders Publication, ELBS, p. 471.

2. Goyal RK (2003). In: Practical in Pharmacology, 3rd Edn., B.S., Shah Prakashan, p. 103.

3. Golan DE, Tashjian AH, Armstrong EJ, Golanter JM, Rose HS (2005). In: Principles of Pharmacology, Lippincott Wiliams and Wilkins, p. 649.

4. Hajhashemi V, Ghannadi A, Sharif B. Anti-inflammatory and analgesic properties of the leaf extracts and essential oil of Lavandula angustifolia Mill. J Ethnopharmacol. 2003;89: 67–71.

5. Ghannadi A, Hajhashemi V, Jafarabadi H. An investigation of the analgesic and anti-inflammatory effects of Nigella sativa seed polyphenols. J Med Food. 2005;8:488–493.

6. Bisser NG. Herbal Drugs and Polypharmaceuticals. Boca Raton, CRC Press; 1994. pp. 505–509.

7. Amresh G, Reddy GD, Rao C, Singh PN. Evaluation of anti-inflammatory activity of Cissampelos pareira root in rats. J Ethnopharmacol. 2007;110:526– 531.

8. Chandler F. Herbs, Everyday Reference for Health Professionals. Canada: Canadian Pharmacists Association and Canadian Medical Association; 2000. pp. 206–207.

9. Soares JR, Dinis TCP, Cunha AP, Almeida LM. Antioxidant activities of some extracts of Thymus zygis . Free Rad. Res. 2009; 26:469–478.

10. Dinis TCP, Madeira VMC, Almeida LM. Action of phenolic derivates (acetoaminophen, salycilate, and 5-aminosalycilate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 1994;315:161–169.

11. Slinkard K, Singleton VL. Total phenol analysis: automation and comparison with manual methods.Am. J. Enol. Vitic. 2015; 28:49–55.

12. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J. Agric. Food Chem. 1992;40:945–948.

13. Modarresi-Chahardehi, A.; Ibrahim, D.; Sulaiman, S.F.; Mousavi, L. Screening Antimicrobial Activity of Various Extracts of Urtica dioica. Rev. Biol. Trop. **2012**, 60, 1567–1576.

14. Bnouham, M.; Merhfour, F.Z.; Ziyyat, A.; Mekhfi, H.; Aziz, M.; Legssyer, A. Antihyperglycemic Activity of the Aqueous Extract of Urtica dioica. Fitoterapia **2003**, 74, 677–681.

15. Ranjbari, A.; Azarbayjani, M.A.; Yusof, A.; Mokhtar, A.H.; Akbarzadeh, S.; Ibrahim, M.Y.; Tarverdizadeh, B.; Farzadinia, P.; Hajiaghaee, R.; Dehghan, F. In Vivo and In Vitro Evaluation of the Effects of Urtica dioica and Swimming Activity on Diabetic Factors and Pancreatic Beta Cells. BMC Complement. Altern. Med. **2016**,

16. Gülçin, I.; Küfrevioˇ glu, Ö.I.; Oktay, M.; Büyükokuroˇ glu, M.E. Antioxidant, Antimicrobial, Antiulcer and Analgesic Activities of Nettle (Urtica dioica L.). J. Ethnopharmacol. **2004**, 90, 205–215.

17. Khalili, N.; Fereydoonzadeh, R.; Mohtashami, R.; Mehrzadi, S.; Heydari, M.; Huseini, H.F. Silymarin, Olibanum, and Nettle, A Mixed Herbal Formulation in the Treatment of Type II Diabetes: A Randomized, Double-Blind, Placebo-Controlled, Clinical Trial. J. Evid. Based Complement. Altern. Med. **2017**, 22, 603.

18. Moré, M.; Gruenwald, J.; Pohl, U.; Uebelhack, R. A Rosa Canina—Urtica dioica—Harpagophytum Procumbens/Zeyheri Combination Significantly Reduces Gonarthritis Symptoms in a Randomized, Placebo-Controlled Double-Blind Study. Planta Med. **2017**,83, 1384–1391.

19. Panth, N.; Paudel, K.R.; Gong, D.S.; Oak, M.H. Vascular Protection by Ethanol Extract of Morus Alba Root Bark: Endothelium-Dependent Relaxation of Rat Aorta and Decrease of Smooth Muscle Cell Migration and Proliferation. Evid. Based Complement. Altern. Med. eCAM **2018**.

20. Rehman, A.; Mehmood, M.H.; Haneef, M.; Gilani, A.H.; Ilyas, M.; Siddiqui, B.S.; Ahmed, M. Potential of Black Pepper as a Functional Food for Treatment of Airways Disorders. J. Funct. Foods **2015**.

21. Maiuolo, J.; Gliozzi, M.; Carresi, C.; Musolino, V.; Oppedisano, F.; Scarano, F.; Nucera, S.; Scicchitano, M.; Bosco, F.; Macri, R.; et al.Nutraceuticals and Cancer: Potential for Natural Polyphenols. Nutrients **2021**.

22. Devkota, H.P.; Adhikari-Devkota, A.; Paudel, K.R.; Panth, N.; Chellappan, D.K.; Hansbro, P.M.; Dua, K. Tea (Catechins Including -Epigallocatechin-3-Gallate) and Cancer. In Nutraceuticals and Cancer Signaling. Food Bioactive Ingredients; Jafari, S.M., Nabavi, S.M., Silva, A.S., Eds.; Springer: Cham, Switzerland, 2021; pp. 451–466.