ejpmr, 2022,9(8), 328-342

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATION OF XANTHINE OXIDASE INHIBITORY ACTIVITY OF *PARTHENIUM HYSTEROPHOROUS*

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Article Received on 26/05/2022 **Article Revised on 15/06/2022** **Article Accepted on 06/07/2022**

ABSTRACT

Parthenium hysterophorus is one of toxic annual herbaceous weed. It is commonly known as 'altamisa', carrot grass, bitter weed, star weed, white top, wild feverfew, the "Scourge of India" and congress grass. *It* is a prolific weed belonging to *Asteraceae* family, producing thousands of small white capitul and each capitula yields five seeds on reaching maturity. It is world's most devastating and hazardous weeds. But many researches shows its medicinal benefits also. Gout is the arthritic syndrome due to the deposition of monosodium urate crystals. The attacks tend to come in discrete episodes, with normal joints in the intervening period, until late stages of the disease. Natural plants can be a good replacement for treatment of many disorders. The goal of the research work is to evaluate xanthine oxidase inhibitory activity of different extracts from whole plant of *Parthenium hysterophorous.* Research shows optimum activity of methanolic extract which opens gateways for its future uses in medicinal field.

KEYWORDS: Parthenium, Gout, Xanthine Oxidase Inhibitory Activity, Extracts.

1. INRODUCTION

In India, Parthenium weed was first described in 1810 but emerged as a serious problem after 1955, when it was introduced in contaminated cereal grains.[1] Since then, it has spread like wildfire throughout India and presently occupies over 5 million hectare land.[2] It is known to cause asthma, bronchitis, dermitis and hay fever in man and livestock. Several researchers have documented the allelopathic effects of the weed. It is also use as remedy for skin inflammation, rheumatic pain, diarrhoea etc. *Parthenium hysterophorus* is a prolific seed producer and widely distributed in Asia and Europe. *Parthenium hysterophorus* seed germination takes place over a broad range of fluctuating (12/2-35/25°C) temperatures.[3]

The weed prefers alkaline to neutral clayey soils for its growth.[4] Occurrence of *P. hysterophorus* negatively affects the diversity and composition of range land vegetation by depleting wealth of natural plant species in affected areas.[5]

Allelo chemicals production by the plants assists to regulate the soil microflora in their vicinity, physiochemical properties of their immediate surrounding environment and growth of competing plant species.[6]

1.1 Taxonomy of *Parthenium hysterophorus.*

Table 1: Taxonomy of *Parthenium hysterophorus.*

Kingdom	Plantae
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Order	Asterales
Family	Asteraceae
Genus	Asteraceae
Species	*Parthenium hysterophorus*

1.2 Chemical constituents present in plant

Isolation and structural elucidation of the active principles of *P. hysterophorus* is required to determine their chemical properties. Chemical analysis of *P. hysterophorus* has indicated that all its parts including "trichomes and pollen" contain toxins called sesquiterpene lactones (SQL).

P. hysterophorus contains a bitter glycoside parthenin, a major sesquiterpene lactone. Other phytotoxic compounds or allelochemicals are; hysterin, ambrosin, flavonoids (such as quercelagetin 3,7-dimethylether, 6-hydroxyl kaempferol 3-0 arabinoglucoside, fumaric acid.). (P-hydroxy benzoin and vanillic acid), caffeic acid, anisic acid, p-anisic acid, chlorogenic acid, ferulic acid, sitosterol and some unidentified alcohols.Parthenin,

hymenin and ambrosin are found to be the culprits behind the menacing role of this weed in provoking health hazards.[7]

Parthenium hysterophorus from different geographical regions exhibited parthenin, hymenin, coronopilin, dihydroisoparthenin, hysterin, hysterophorin and tetraneurin as the principal constituents of their sesquiterpene lactones.[8]

1.3 Health benefits and harmful effects

It is used to treat health issues like diarrhoea, neurologic disorders, urinary tract infections dysentery, malaria and as emmenagogue.[9] Ethno-botanically, some tribes use it as remedy for inflammation, eczema, skin rashes, herpes, rheumatic pain, cold, heart trouble and gynaecological ailments. As analgesic in muscular rheumatism, therapeutic for neuralgia and as vermifuge. This weed is also reported as promising remedy against hepatic amoebiasis.

Parthenium hysterophorus caused health problems like bronchitis, dermatitis, asthma and hay fever.[10] Parthenin and additional phenolic acids viz. caffeic acid, anisic acid, vanillic acid, chlorogenic acid, panisic acid and parahydroxy benzoic acids are the major components responsible for lethality to human beings and grazing animals.[11] Allergic eczematous, contact dermatitis and depression in humans coming in contact with this weed has also been witnessed.[12]

1.4 Gout[13-19]

Gout is the arthritic syndrome due to the deposition of monosodium urate crystals. The attacks tend to come in discrete episodes, with normal joints in the intervening period, until late stages of the disease. Gout attacks often are monoarticular, but polyarticular episodes can occur.

Primary gout is not associated with an identifiable cause, other than perhaps a family history. Secondary gout refers to the presence of a recognized cause or precipitating factor, such as lymphoma, the excessive use of alcohol, or the use of diuretics.

1.5 Cause of disease

Gout develops because of the build-up of purines in the body, either by decreased excretion (about 90% of cases of primary gout) or by increased production (about 10% of primary gout). When the concentration of urate exceeds its solubility, crystals precipitate, and the crystals are phlogistic. The crystals lead to activation of the classical and alternative pathways of complement, the influx of neutrophils into the joint, and the release of numerous inflammatory cytokines. In those patients who are over-producers of uric acid, their 24-hour urinary uric acid will likely be elevated, and they will be at risk of urate kidney stones as well as gouty attacks.

Any factors that raise nucleoprotein production will increase its breakdown -- ultimately, by the action of xanthine oxidase -- into uric acid.

Uricosuric drugs, which increase the urinary excretion of uric acid or XO inhibitors which block the terminal step in uric acid biosynthesis, can lower the plasma uric acid concentration, and are generally employed for the treatment of gout.[20,21]

Allopurinol is the only clinically used XO inhibitor in the treatment of gout.(22, 23) However, this drug suffers from many side effects such as hepatitis, nephropathy, and allergic reactions.(24) Thus, the search for novel XO inhibitors with a higher therapeutic activity and fewer side effects are desired not only to treat gout but also to combat various other diseases associated with the XO activity.

2. MATERIALS AND METHODOLOGY

2.1 Materials

The chemicals used for the project were purchased from Sigma Aldrich, Qualikem, Himedia Laboratories , Rankem and Central Drug House Pvt.Ltd. Various solvents used: Chloroform, Ethanol, Methanol, Ethyl Acetate, Butanol, Hexane. All chemicals used were of analytical grade. Allopurinol is used as standard antigout drug,

2.2 Plant used: The plant used for this research was collected from an open ground in Amritsar and was authenticated from Post Graduate Department Of Botany, Khalsa college, Amritsar.

2.3 Extraction procedure

The fresh whole plant of *Parthenium hysterophorus* was washed with distilled water to remove dust particles. The shade dried whole plants were powdered. The ground fine powder (100 gm) of the whole plant was extracted with methanol (300ml) at room temperature (30ºc) for three days after defatting. The extract was filtered through Whatman No: 1 filter paper and then concentrate using rotary vacuum evaporator to get final extract. Similarly hexane extract, chloroform extract and ethyl acetate extract were prepared.

2.4 Method for Biological evaluation

In vitro xanthine oxidase inhibitory activity has to be evaluated using xanthine oxidase assay.

The XOI activity will be assayed spectrophotometrically using xanthine as the substrate.[25] The assay mixture composing of 1 mL of the fraction (5-100 micro g/mL), 2.9 mL of phosphate buffer (pH 7.5) and 0.1 mL of xanthine oxidase enzyme solution (0.1 units/mL in phosphate buffer, pH 7.5), which will be prepared immediately before use.

After pre-incubation at 25 °C for 15 min, the reaction will be initiated by the addition of 2 mL of the substrate

solution (150 mM xanthine in the same buffer). The assay mixture will be incubated at 25°C for 30 min. The reaction then stopped by the addition of 1 mL of 1 N hydrochloric acid and the absorbance was measured at 290 nm using a UV spectrophotometer.[26]

Different concentrations of the fractions (5-100 µg/mL) will be dissolved in dimethyl sulfoxide (DMSO) and the final concentration of DMSO will be 5%, which did not affect the enzyme assay. Proper controls with DMSO will be carried out. Allopurinol (5-100 µg/mL), a known inhibitor of XO, will used as the positive control.

One unit of XO is defined as the amount of enzyme required to produce 1mmol of uric acid/min at 25 °C. XOI activity is expressed as the percentage inhibition of XO in the above assay system calculated as

$$\text{Inhibition (\%)} = (1\text{-}[B/A]) \times 100$$

where A represents the activity of the enzyme without plant extract and B is the activity of XO in the presence of plant extract.

3. RESULTS AND DISCUSSIONS

3.1 Ethyl acetate extract: Ethyl acetate extract shows significant xanthine oxidase activity. At 100µg/ml concentration it shows 50.67 % inhibition.

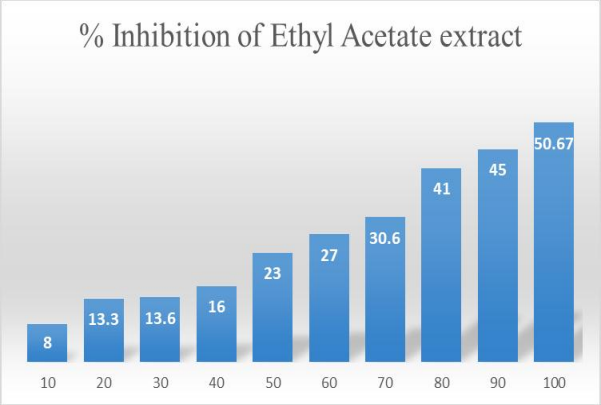

Fig. 1: XOI of Ethyl acetate extract.

3.2 Chloroform extract: Chloroform extract shows gradual increase in xanthine oxidase inhibition activity. It shows 46.67% inhibition at 100µg/ml concentration.

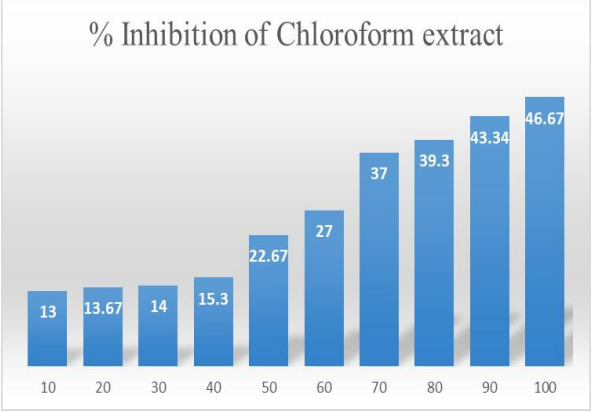

Fig. 2: XOI of Chloroform extract.

3.3 Hexane extract: Hexane extract shows lesser activity than other extracts. At 100µg\ml concentration it shows 19.34 % inhibition.

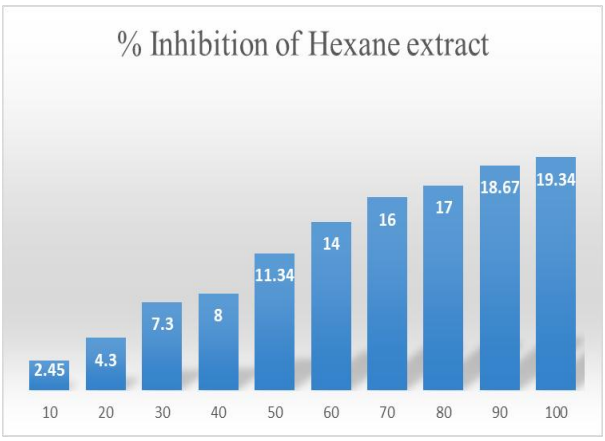

Fig. 3: XOI of Hexane extract.

3.4 Methanol extract: Methanol extract shows highest % inhibition than the other three extracts. It shows 61.1 % inhibition at 100µg/ml concentration.

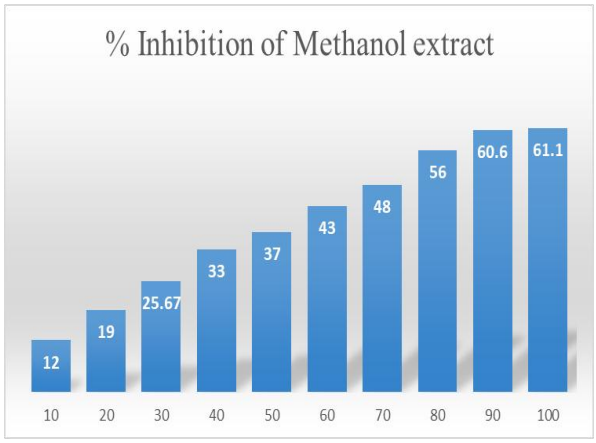

Fig. 4: XOI of Methanol extract.

3.5 Allopurinol: As a standard xanthine oxidase inhibitor allopurinol shows remarkable % inhibition. At 100µg/ml it shows 93.1 % inhibition.

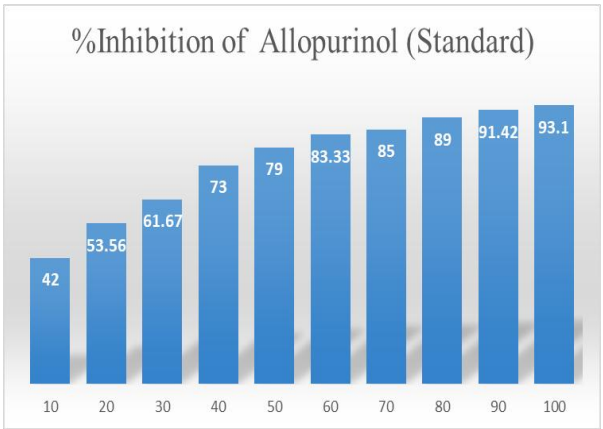

Fig. 5: XOI of Allopurinol standard.

3.6 Comparison of xanthine oxidase inhibitory activity assay results of extracts with standard

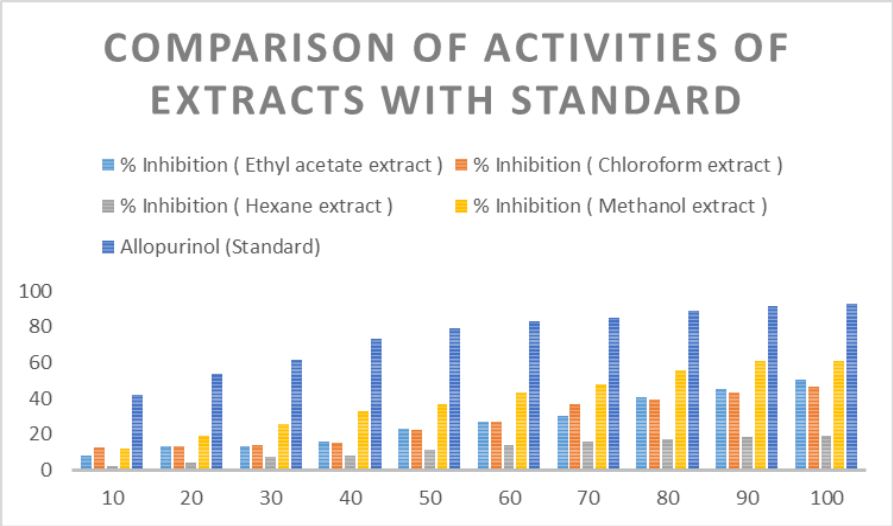

Fig. 6: Comparison of xanthine oxidase inhibitory activity assay results of extracts with standard.

On comparison of xanthine oxidase inhibitory activity of different plant extracts and the standard drug allopurinol, it was found that methanol and ethyl acetate have significant inhibition capacity than hexane and chloroform. Although no extract have inhibition power more than the standard drug allopurinol but still the results shows that methanol has significant xanthine oxidase inhibitory activity. At 100µg/ml methanol, ethyl acetate, chloroform, hexane and allopurinol shows 61.1%, 50.67%, 46.67%, 19.34% and 93.1% inhibition respectively.

3.7 IC_{50} values: The result shows following IC_{50} values of fractions, extracts, standard and compound1.

Table 2: IC_{50} values of extracts and standard.

S. NO	SUBSTANCE	IC_{50} VALUE
1	Methanol extract	72.89
2	Chloroform extract	117.7
3	Hexane extract	237.07
4	Ethyl acetate extract	103.34
5	Allopurinol	42.03

Lowest IC_{50} value is shown by the standard drug allopurinol then followed by methanol extract.

4. CONCLUSION

On comparison of xanthine oxidase inhibitory activity of different plant extracts and the standard drug allopurinol, it was found that methanol and ethyl acetate have significant inhibition capacity than hexane and chloroform. Although no extract have inhibition power more than the standard drug allopurinol but still the results shows that methanol has significant xanthine oxidase inhibitory activity. Further new constituents can be isolated from its extracts and can be evaluated against such disorders. Transforming a noxious weed into a boon for medicinal potentials would be a remarkable discovery.

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