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PHYTOCHEMICAL SCREENING AND IN VITRO ANTIDIABETIC AND ANTI-INFLAMMATORY ACTIVITY OF HERBAL EXTRACT OF *XANTHIUM STRUMARIUM*

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Abstract

This study deals with the Phytochemical screening and in vitro antidiabetic and anti-inflammatory activity of herbal extract of *Xanthium strumarium.* The leaves of the plant were collected & subjected to extraction by pet ether & hydroalcoholic solvent. Further the hydroalcoholic extract was screened for qualitative & quantitative estimation of phytochemicals along with TLC. Further the anti- diabetic & anti -inflammatory effect was also evaluated. The results showed that the percentage yield of pet ether extract of *Xanthium strumarium* was found to be 2.53% while for hydroalcoholic extract it was found to be 8.67%. Phytochemical screening revealed the presence of carbohydrate, proteins, saponins, flavonoids & tannins & phenolics. The TLC profile has revealed that presence of flavonoids in the tested plant extract. The phenolic & flavonoid content in hydroalcoholic extract of *Xanthium strumarium* was found to be 0.352mg/100mg & 0.674mg/100mg of dried extract. The anti -diabetic activity of plant was estimated by alpha amylase assay. The IC 50 value for standard acarbose was found to be 35.33% while for Extract of *Xanthium strumarium* it was found to be 335.60 %. The anti -inflammatory activity was performed by using Diclofenac sodium as standard. The IC 50 for Diclofenac was found to be 230.13 % while for hydroalcoholic extract of *Xanthium strumarium* the IC 50 value was found to be 322.09 %. So it can be concluded that *Xanthium strumarium* have potent anti -inflammatory & Anti diabetic effect.

Key words: *Xanthium strumarium,* Antidiabetic, Anti-inflammatory, Evaluation

Introduction

The introduction of plant derived drugs in modern medicine has been linked to the uses of plant derived materials as an indigenous cure in traditional system of medicine. Some of the plants have been found to possess significant antibacterial, antifungal, anticancer, antidiuretic, anti-inflammatory and anti-diabetic properties. Plant derived drugs are used to cure mental illness, skin diseases, tuberculosis, diabetes, jaundice, hypertension and cancer. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed [1,2].

Chemically prepared drugs may act quickly, but they have side effects which affect human body negatively in the long run, whereas, medicinal plants work in an integrated or pro-biotic with little or no adverse effects on the body. The development of human culture, the use of medicinal plants has had magical-religious significance and different points of view regarding the concepts of health and disease which existed within each culture. For the past 3000 years, a large number of plants are used in health care practices, such as in Traditional Medicine in China, India and Africa, most of which contains therapeutic values which has been ascertained as such by Western standards Plants have provided humans with many of their essential needs, including life-saving pharmaceutical agents. However, medicinal plants are threatened as a result of human impact and uncontrolled wild collection, it is therefore recommended that deliberate efforts towards domestication and cultivation are essential for continuous supply of these plant species. These plants may be used as a huge amount of raw material for pharmaceutical industries for manufacturing the medicines. In addition to the requirement for conservation of medicinal plants it has also become essential to protect and patent the traditional knowledge [3,4] .

Xanthium strumarium L. (Family: Compositae) a medicinal plant commonly found as a weed, is widely distributed in North America, Brazil, China, Malaysia and hotter parts of India. The herb is traditionally used mostly in treating several ailments. Extracts of the whole plant, especially leaves, roots, fruits and seeds have been applied in traditional medicine for the treatment of leucoderma, poisonous bites of insects, epilepsy, salivation, long-standing cases of malaria, rheumatism, tuberculosis, allergic rhinitis, sinitis, urticaria,

rheumatoid arthritis, constipation, diarrhoea, leprosy, lumbago, pruritis, bacterial and fungal infections. The plant is consid-ered to be useful in treating long-standing cases of malaria, rheumatism, diseased kidneys, and tuberculosis. It is also used as an adulterant for Datura stramonium. The fruits of X. stru-marium has the properties like anodyne, antibacterial, antifun-gal, antimalarial, Antirheumatic, antispasmodic, antitussive, cytotoxic, hypoglycemic and stomachic. They are used in-ternally in the treatment of allergic rhinitis, sinusitis, catarrh, rheumatism, rheumatoid arthritis, constipation, diarrhea, lumbago, leprosy and pruritus . A decoction of the root has been used in the treatment of high fevers and to help a woman expel the afterbirth and a decoction of the seeds h been used in the treatment of bladder complaints by local peoples. The dried leaves of X. strumarium are a source of tan-nin, that's the reason is poisonous to grazing animals [5-7] .This study deals with Phytochemical screening and in vitro antidiabetic and anti-inflammatory activity of herbal extract of *Xanthium strumarium*.

Material and Methods

Collection of plant materials

The leaves of *Xanthium strumarium* were collected from Bhopal in the period of June, 2022 considering the seasonal conditions for obtaining maximum phytoconstituents.

Methods

Extraction

Collected plant drugs namely *Xanthium strumarium* leaves were cleaned properly and washed with distilled water to remove any kind of dust particles. Cleaned and dried plant drugs were converted into moderately coarse powder in hand grinder. Powdered plant materials were weighed (75.86 gm) and packed in (250 milliliter) air tight glass Bottle. The plant drug was defatted with petroleum ether for about 12 hrs. The defatted plant drugs were subjected to extraction by ethanol and water (ethanol: water; 80:20) as solvent for about 24 hrs. The liquid extracts were collected in a tarred conical flask. The solvent removed from the extract by evaporation method using hot plate. The extracts obtained

with each solvent were weighed to a constant weight and percentage w/w basis was calculated [8,9].

Preliminary phytochemical screening

Preliminary phytochemical screening means to investigate the plant material in terms of its active constituents. In order to detect the various constituents present in the hydroalcoholic extract of leaves of *Xanthium strumarium*, were subjected to the phytochemical tests as per standard methods [10,11].

Thin Layer Chromatography

TLC was produced with the aim of identifying the individual substances in a mixture and also testing for purity or for separation of mixtures. The height of the solvent front and center of spots were measured in the form of R_f value. The R_f value indicates the position the position at which a substance was located in the chromatogram [12].

Total phenol content estimation

10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this extract was for the estimation of phenol. 2 ml of extract and each standard was mixed with 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexes for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a spectrophotometer [13].

Total flavonoids content estimation

10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm

In vitro* anti diabetic activity of hydroalcoholic extract of *Xanthium strumarium

Inhibition of alpha amylase enzyme

10 mg acarbose was dissolved in 10 ml methanol, and various aliquots of 100- 1000µg/ml were prepared in methanol. 10 mg of dried extract was extracted with 10 ml methanol. 500 µl of this extract solution was used for the estimation of enzyme inhibition. A total of 500 µl of test samples and standard drug (100-500µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle [14].

Evaluation of *in vitro* anti-inflammatory activity

Anti-inflammatory activity of the *Xanthium strumarium* extract was evaluated by protein denaturation method as described by Padmanabhan and Jangle . Diclofenac sodium, a powerful non steroidal anti-inflammatory drug was used as a standard drug. The reaction mixture consisting of 2 mL of different concentrations of *Xanthium strumarium* extract (100-500 µg/mL) or standard diclofenac sodium (100-500 µg mL-1) and 2.8 mL of phosphate buffered saline (pH 6.4) was mixed with 0.2 mL of egg albumin (from fresh hen's egg) and incubated at (37±1)°C for 15 min. Denaturation was induced by keeping the reaction mixture at 70°C in a water bath for 10 min. After cooling, the absorbance was measured at 660 nm by using double distilled water as blank [15].

Results and Discussion

The percentage yield of pet ether extract of *Xanthium strumarium* was found to be 2.53% while for hydroalcoholic extract it was found to be 8.67%. Phytochemical screening revealed the presence of carbohydrate, proteins, saponins, flavonoids & tannins & phenolics. The TLC profile has revealed that presence of flavonoids in the tested plant

extract. The phenolic & flavonoid content in hydroalcoholic extract of *Xanthium strumarium* was found to be 0.352mg/100mg & 0.674mg/100mg of dried extract. The anti diabetic activity of plant was estimated by alpha amylase assay. The IC 50 value for standard acarbose was found to be 35.33% while for Extract of *Xanthium strumarium* it was found to be 335.60 %. The anti -inflammatory activity was performed by using Diclofenac sodium as standard. The IC 50 for Diclofenac was found to be 230.13 % while for hydroalcoholic extract of *Xanthium strumarium* the IC 50 value was found to be 322.09 %. So it can be concluded that *Xanthium strumarium* have potent anti -inflammatory & Anti diabetic effect.

Table 1: % Yield of crude extracts

Extracts	Colour	Consistency	% Yield (w/w)
Xanthium strumarium			
Pet ether	Dark brown	Semisolid	2.53%
Ethanol: water; 80:20	Brown	Solid	8.67%

Table 2: Preliminary qualitative phytochemical tests for *Xanthium strumarium* extract

Phytoconstituents	Phytochemical tests of hydroalcoholic extract
i) Primary Metabolites	
Carbohydrates	**Present**
Amino acids	Absent
Proteins	**Present**
Fats and oils	Absent
ii) Secondary metabolites	
Steroids	Absent

Triterpenoids	Absent
Volatile oils	Absent
Gums and mucilage	Absent
Glycosides	Absent
Saponins	**Present**
Flavonoids	**Present**
Tannins & Phenolics	**Present**
Alkaloids	Absent

Table 3: TLC of leaves of *Xanthium strumarium*

S. No.	Extract	Rf Value
	Mobile phase (Toluene: Ethyl acetate: Formic acid; 5:4:1)	
1.	Quercetin Leaves of *Xanthium strumarium* Long UV=3 spot Short UV=4 spot Normal light =1 spot	0.44 0.46, 0.54, 0.78 0.48, 0.52, 0.72, 0.88 0.48

Table 4: Estimation of total phenolic and flavonoids content of *Xanthium strumarium*

S. No.	Extract	Total phenol content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1	Hydroalcoholic	0.352	0.674

Table 5: % Inhibition of acarbose and hydroalcoholic extract of *Xanthium strumarium*

S. No.	Concentration (µg/ml)	% Inhibition	
		Acarbose	Extract of *Xanthium strumarium*
1	100	51.19	30.74
2	200	70.10	37.41
3	300	74.20	49.12
4	400	85.18	56.22
5	500	88.75	62.53
IC_{50} (µg/ml)		**35.33**	**335.60**

Table 6: % Inhibition of Diclofenac sodium and *Xanthium strumarium* extract

Concentration (µg/ml)	% Inhibition	
	Diclofenac sodium	*Xanthium strumarium* extract
100	30.85	20.85
200	45.4	32.51
300	60.04	40.45
400	75.74	64.87
500	90.25	75.69
IC_{50}	**230.13**	**322.09**

Conclusion

This study demonstrated that the aerial parts extract of X. strumarium contains a wide variety of secondary metabolites that expressed antioxidant activity and anticancer ability on HepG2 cancer cell line based on the experiments performed. However, more scientific

evidence is needed to comprehensively evaluate the bio- logical effects of the aerial parts extract of X. strumarium This study demonstrated that the hydroalcoholic extracts of aerial parts extract of *X. strumarium* contains a wide variety of secondary metabolites that expressed anti diabetic activity and anti -inflammatory ability based on the experiments performed. However, more scientific evidence is needed to comprehensively evaluate the bio-logical effects of the aerial parts extract of *X.strumarium*

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