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PRELIMINARY PHYTOCHEMICAL SCREENING AND ESTIMATION OF IN-VITRO ANTIOXIDANT PROPERTY OF XANTHIUM INDICUM J. KOENIG OBTAINED FROM NORTHEAST INDIA

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ABSTRACT
Northeast India has been a gathering around for various species of medicinal plants and most of the village and rural areas people still rely on herbal plant medicine. *Xanthium indicum* J. Koenig. has been widely used traditionally for the treatment of inflammation and other related diseases. The present study aims to analyse the phytochemicals present and determine the antioxidant property of the plant obtained for Northeast India. The preliminary phytochemical analysis showed the presence of alkaloids, carbohydrates, proteins and amino acids and flavonoids. Antioxidant activity was determined by DPPH free radical scavenging assay which showed an IC50 of 112.60 ± 0.62 µg/ml of *Xanthium indicum* extract. The outcome of the present study concludes that the plant *Xanthium indicum* showed significant antioxidant activity.

INTRODUCTION

Northeast India has a greater abundance of medicinal plants because of its wide range of flora and fauna. Various plants growing in this region of the country are widely used by the locals and traditional healers for treating various ailments and diseases [1]. North east region comprises approx. 8% of the total area of India but consists of more than 8000 species of flowering plants [2,3]. Herbal medicine has been the key to development of new drugs and has found a superior position in the social and cultural folk medicinal knowledge in the therapeutic system [4,5] *Xanthium indicum* J. Koenig commonly called 'Agora' belongs to the family Asteraceae and is commonly found in the river side areas.

It grows to about a meter high and is widely distributed in North America, Brazil, China, Malaysia and hotter parts of India.

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Traditionally *X. indicum* is used to treat poisonous bites of insects, rheumatism, long standing cases of malaria, allergic infection, bacterial and fungal infections and inflammations [6,7]. *X. indicum* are erect herbs, stem terete and scabrid. The leaves are 8-15 x 9-10 cm long, broadly ovate, shallowly lobed, base cordate, 3-ribbed, scabrous. The petioles are 8-10 cm long. Heads are axillary, bisexual heads in upper axils and sterile female heads in lower axils. Corolla is absent in female flowers but tubular in bisexual flowers. Achenes are 1.5 x 0.7 cm, paired on each head enclosed in a 2-beaked utricle and pappus absent. The plant flowers are in terminal capitulum, white and spiny and flowers throughout the year [8]. The present study aims to determine the phytochemical and antioxidant property of *X. indicum* obtained from North Eastern part of India.

MATERIALS AND METHODS

The major requirement used to perform the experimental work includes Ethanol purchased from Changshu Hongsheng Chemical Co. Ltd., 2,2-Diphenyl-1-Picrylhydrazyl from Sisco Research Laboratories. All the chemicals used in the study was of analytical grade.

Collection of Plant and Authentication

X. indicum J. Koenig is commonly available in North east India. The plant roots were collected from the Brahmaputra banks of Panikhaiti, Guwahati, Assam. A whole plant was collected for authentication in the month of August and the plant root was collected too. Following the standard protocols and procedures, herbarium was prepared and authentication was done from Guwahati University, Assam, India with the reference no. Herb/GUBH/2019/125.

Processing of Plant Material and Extraction

X. indicum J. Koenig roots was collected and washed properly. It was left for shade drying for 2 months (Fig. 1). After complete drying of the roots, they were further subjected for grinding (Fig. 2). Extraction was done by maceration process. Ethanol was used for extraction in the ratio of 1:10. A total of 1.6 kg of plant material was subjected for extraction process followed by filtration (Fig. 3). The extract was concentrated using Rotary Evaporator (Buchi Rota Vapor, Switzerland) (Fig. 4). After drying of the extract, the percentage yield was determined by the formula

$$\%Yield = \frac{Actual\ Yield}{Theoretical\ Yield} \times 100$$

Figure 1: Dried Roots

Figure 2: Grinded Roots

Figure 3: Filtration of extract

Figure 4: Drying of extract using Rotary Evaporator

Phytochemical Screening

Phytochemical analysis was carried out as per standard protocols [9]. Tests for detection of proteins and amino acid (Millions Test and Ninhydrin test), detection of carbohydrates (Molish's test, Fehling's test and Barfoed's test), tests for alkaloid (Mayer's test, Dragendroff's test and Hager's test), test for steroids and triterpenoids (Libermann-Burchard's test and Salkowski test), detection of Glycosides (Borntrager's test and Keller-Killiani test), test for flavonoids (Alkaline reagent test and ferric chloride test) were performed.

Antioxidant Activity

Free radical scavenging ability of the extract was tested by DPPH radical scavenging assay as per standard protocol. A solution of 0.1mM DPPH in methanol mixed with solution of extract in methanol at different concentrations (10,20,40,80,160 µg/ml). Absorbance was checked at 517 nm where ascorbic was used as standard [10]. Percentage inhibition was determined using the formula:

$$\%\ Inhibition = \frac{Control - Test}{Control} \times 100$$

RESULT AND DISCUSSION

After successful extraction and drying using rota evaporator, the amount of the extract was found to be 97 gram and the percentage yield was 6.06%. The extracts subjected to phytochemical analysis and the results are tabulated. (Table 1). The presence of proteins and amino acids, carbohydrates, alkaloids, steroids and triterpenoids, glycosides and flavonoids maybe the reason for the antioxidant property. The presence of flavonoids may indicate that the *Xanthium indicum* may also be useful as antimicrobial and wound healing when subjected for preclinical analysis.

Table 1: Phytochemical Analysis of *Xanthium indicum*

S No.	Phytochemical Presence	Test	Result
1	Proteins and Amino acids	Millon's Test	+
		Ninhydrin Test	+
2	Carbohydrates	Molish's Test	+
		Fehling's Test	+
3	Alkaloid	Mayer's test	+
		Dragendroff's test	+
		Hager's test	+
4	Steroids and Triterpenoids	Libermann-Burchard's test	-
		Salkowski test	-
5	Glycosides	Borntrager's test	-
		Keller-Killiani test	-
6	Flavonoids	Alkaline reagent test	+
		Ferric Chloride test	+

Table 2: Percentage inhibition of *Xanthium indicum* root extract

Sample No.	Concentration (µg/ml)	% Inhibition
1	10	13.14 ± 0.36
2	20	18.75 ± 0.91
3	40	23.92 ± 0.93
4	80	41.37 ± 0.26
5	160	65.30 ± 0.12

A substance which delays or inhibits the oxidative damage to cells is known as antioxidant. Free radical scavenging activity of *Xanthium indicum* extract was determined by using DPPH. The antioxidant activity was compared to standard ascorbic acid. The maximum inhibition was found to be 65.30 ± 0.12 at a concentration of 160 µg/ml. The IC 50 of the extract was found to be 112.60 ± 0.62 µg/ml when compared to standard ascorbic acid was found to be 3.48 ± 0.63 µg/ml. The extract showed significant antioxidant activity which explains that the extract of the roots may protect from oxidative damage to cells cause by free radicals during inflammation.

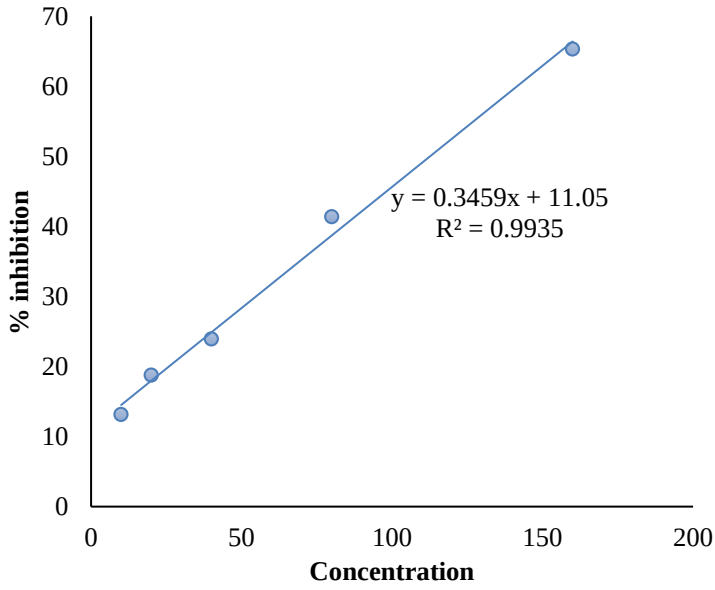

Figure 5: Graphical representation of % inhibition of *Xanthium indicum* root extract.

CONCLUSION

Xanthium indicum obtained from North East India has shown significant antioxidant potential which proves the plant might be useful against oxidative stress or scavenging free radicals. The presence of antioxidant property may also lead to anti-inflammatory property as claimed for traditional uses. The preliminary phytochemical activity study indicates the presence of alkaloids, proteins and amino acids, carbohydrates and flavonoids as major class of phytochemical. The presence of flavonoids may also indicate that this plant might also be useful in wound healing.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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