A Comparative Qualitative Phytochemical Analysis of Crude Extracts of Indigenous Plant *Xanthium strumarium* L. Aerial Parts

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ABSTRACT

Xanthium strumarium Linn. (Asteraceae) is an indigenous medicinal plant, found all over Asia. It is a common roadside hedge in Pakistan. The plant is abundant in bioactive metabolites attributing medicinal properties to it. Crude extracts of its aerial parts have been in practice in herbal medicine to treat various syndromes since inception such as fever, headache, sinusitis, herpes, arthritis, etc. Moreover, it also provides relief from cramping and limb numbness. *Xanthium stumarium* is a dynamic anthelmintic, laxative and digestive agent used to cure epilepsy, urogenital infections and hormonal disorders. Current study investigates qualitative analysis of primary and secondary metabolites present in aqueous, methanol and dichloromethane extracts of *X. strumarium* aerial parts. Preliminary screening showed polar crude aqueous and methanol extracts were a good source of flavonoids, tannins, terpenoids, alkaloids, carbohydrates, proteins and carboxylic acids whereas non-polar dichloromethane extract was rich in plant gums, a therapeutic potent constituent. This qualitative analysis of phytochemicals may be thought provoking to explore further bioactive phytochemicals from this indigenous *X. strumarium* mainly in benign medium (aqueous), so that low cost medicines can be developed to treat various health issues.

1. Introduction

Medicinal plants are one of the main components of traditional herbal medicine since inception. Not only pure compounds but their crude extracts are also effective against several diseases due to the synergistic actions of secondary metabolites (Jana & Shekhawat, 2010). Bioactive phytochemicals exert specific physiological effect on human body through nutrition and hence, prevent them from diseases with the help of strong defensive system (Cowan, 1999). These phytochemicals exist in almost all parts of a plant including root, stem, leaves, flower, fruit, seeds, etc. These are not only responsible for color, fragrance and flavor of a plant but also provide natural protection against several organisms (Chede, 2013). Phytochemicals are of two types, primary and secondary metabolites. Primary metabolites provide physical stability to plant cell, such as chlorophyll and protein while secondary metabolites are derived from primary metabolites, for instance, alkaloids, flavonoids, steroids, terpenoids, tannins and phenolic compounds (Wadood *et al*., 2013). Since secondary metabolites are biologically active against various diseases, therefore plants are one of the principal sources of raw material for drug synthesis and are utilized both in isolated crude or purified form in herbal medicine (Amin *et al*., 2016).

X. strumarium Linn. (commonly, cocklebur) is a medicinal plant of family Asteraceae (Jawerea *et al*., 2023). The plant has traditional medicinal value from ancient times (Amina *et al*., 2019). The plant is commonly found in moderate zones of Asia. In Pakistan, it is a common road side hedge (Syed, 1989). Its name expresses the color change of seedpods from green to deep yellow followed by brown upon ripening (Gajanan *et al*., 2022). The whole plant holds extreme medicinal values (Fahd *et al*., 2017). Moreover, the herb has also been conventionally experienced to treat sinusitis, headache, fever, scrofula, herpes, leukoderma, arthritis and cancer (Xiang-Wei *et al*., 2022). Ethnic Chinese used to get relief from nasal sinusitis, arthritis, headache, cramping and limb numbness (Mandeep *et al*., 2015). They also practiced its decoction to treat ulcers, malaria and sinus problems (Ranjan *et al*., 2020). In Ayurveda, the plant has been

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used as tonic, cooling, digestive, laxative, anthelmintic and antipyretic agent (Khuda *et al.*, 2012). In addition to this, it also improves appetite, voice and memory loss (Bhogaonkar & Ahmad, 2012). It is applied as antidotes in poisonous insects' bite, for epilepsy and salivation medication (Anjoo & Ajay, 2010). Many American innate tribes used to relieve diarrhea, vomiting and constipation through this plant (Masvingwe & Mavenyengwa, 1998). It owns bioactivities against wide range of disorders including antidiabetic, antioxidant, antibacterial, antiviral (Ahmet Beyatli, 2024), insecticidal, herbicidal and antitrypanosomal (Umer *et al.,* 2014), etc. The decoction of fruit is used to treat hormonal disorders, urinogenital infections and small pox (Krishan, 2000). The current report is a comprehensive study of our research published in 2017 (Aneela *et al.,* 2017). Herein the main objective of current investigation was the qualitative analysis of phytochemicals present in crude aqueous, methanol and dichloromethane extracts of *X. strumarium* so as to explore its medicinal potential against several disorders.

2. Materials and Methods*l*

2.1 Extraction

X. strumarium Linn. was collected and identified (G.H. No.86398, No. 01, KU) as reported in the earlier citations (Aneela *et al.*, 2012). The aerial parts of plant after thorough washing with water dried in shade, crushed, homogenized to fine powder using electrical grinder and then stored in sterile air tight bottles in dry place. The finely powdered plant material (100 g) was then extracted from analytical grade methanol, dichloromethane (DCM) and distilled water separately after soaking for one week each. Each extract was filtered and then concentrated in vacuum to obtain crude extract (50 mL approx.) . These were stored in sterile bottles for further phytochemical analysis in refrigerator.

2.2 Chemicals

Fehling solution A and B, ethanol, hydrochloric acid, sodium hydroxide, alcoholic alpha naphthol, sulphuric acid, acetic acid, acetic anhydride, picric acid, ammonium hydroxide, lead acetate, zinc sulphate, olive oil, methanol, chloroform, and *n*-hexane. All reagents used for analysis were of analytical grade and purchased from Merck.

2.3 Qualitative Analysis of Phytochemicals

The preliminary qualitative phytochemical analysis of the crude extracts of *X. strumarium* was performed using standard reported protocols.

2.3.1 Test for reducing sugars: *Fehling's test*

An equal amount of Fehling solutions A and B were added to 1 mL extract in a test tube and heated for 120 s. Red-brown precipitates indicated reducing sugar in the extract (Nekrasov, 1978).

2.3.2 Test for non-reducing sugar: Fehling's test

1 mL extract taken in a test tube was mixed with 0.5 mL concentrated HCl and refluxed for 20 min. After cooling, the solution was neutralized by 0.1 N NaOH. Fehling's reagent 1 mL was added and refluxed for another 10 min. The no appearance of red precipitates proved the absence of sucrose (non-reducing sugar) (Nekrasov, 1978).

2.3.3 Test for carbohydrates: *Molish's test*

1 mL of 1 % alcoholic alpha naphthol and 1 mL extract were shaken together followed by addition of concentrated sulphuric acid along the wall of test tube. Appearance of purple ring at the junction of both layers indicated the presence of carbohydrates in the extract (Nekrasov, 1978).

2.3.4 Test for phytosterols: *Liebermann-Burchard's test*

In a test tube, 1 mL extract was heated with 0.5 mL acetic acid and acetic anhydride. After heating, concentrated sulphuric acid was then added along the side wall of the test tube. Absence of green color confirmed the absence of steroids (Harborne, 1973).

2.3.5 Test for alkaloids: *Hager's test*

1 mL extract and 1 mL dilute HCl mixed in a test tube then filtered in another test tube and the filtrate was combined with saturated solution of picric acid. Creamy-yellow precipitation confirmed the presence of alkaloids (Tiwari *et al.,* 2011).

2.3.6 Detection of flavonoids

1 mL extract taken in a test tube was mixed with 0.5 mL of 10 % NH_4OH and concentrated H_2SO_4. Yellow

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color was observed which disappeared on standing. This confirmed the existence of flavonoids in the extract **(**Suradkar *et al*., 2017).

2.3.7 Detection of tannins: *Lead acetate test:*

1 mL extract and 1 mL lead acetate 10 % was mixed in a test tube. Appearance of white precipitates confirmed the presence of tannins in the extract (Umer *et al.,* 2014).

2.3.8 Detection of saponins: *Frothing test*

1 mL extract was warmed for 5 min and then shaken with distilled water until the formation of froth. The froth was then blended vigorously after addition of two drops of olive oil. A persistent emulsion was observed which indicated the presence of saponins in the extract **(**Sucheta *et al*., 2016).

2.3.9 Detection of terpenoids: *Salkowski's test*

1 mL extract and 1 mL chloroform were taken in a test tube and concentrated H_2SO_4 was added by the side wall of the test tube. Observation of reddish-brown ring at the interface showed the occurrence of terpenoids in the extract (Edeoga *et al.,* 2005)**.**

2.3.10 Detection of Quinones

Appearance of blue, green or red color upon addition of 0.1 N NaOH to 1 mL extract revealed the presence of quinones in the sample extract. (Um*er et al*., 2014).

2.3.11 Detection of amino acids and proteins: *Zinc sulphate test*

1 mL extract was made alkaline by 10 % NaOH and then shaked well with 1 mL of 10 % $ZnSO_4$ solution. White precipitates proved the existence of proteins in the extract (Tariq & Asma, 1992).

2.3.12 Detection of Carboxylic acid: Effervescence test

Observation of effervescence upon mixing 1 mL extract with 0.5 mL aqueous sodium bicarbonate solution in a test tube determined the presence of carboxylic acid in the test sample (Kumar *et al.,* 2013).

2.3.13 Detection of gum: *Adhesives test*

The extract and water 1 mL each was mixed vigorously. No swells and adhesives were observed. It indicated the absence of gums in the extract (Umer *et al*., 2014).

3. Results

In this research work, qualitative screening of phytochemical of various extracts of *X. strumarium* was carried out on comparison basis of solvent polarity, i.e. aqueous, methanol and dichloromethane extracts. The results obtained unveiled active medicinal phyto-constituents like alkaloids, terpenoids, flavonoids, phytosterols, quinones, tannins, saponins, carbohydrates, sugars (reducing and non-reducing), amino acids and gums with different standard chemical tests (Table 1). The phytochemical analysis showed that the methanol extract was rich in alkaloids, flavonoids, saponins and tannins. Analysis of aqueous extract showed occurrence of alkaloids, terpenoids, flavonoids, saponins, carbohydrates, reducing sugar and amino acid whereas tannins in less strength. The dichloromethane screening for phytochemicals exhibited presence of saponins, carbohydrates, reducing sugars, amino acids and gums. The study displayed that both polar (aqueous and methanol) and non-polar (dichloromethane) extracts of *X. strumarium* were rich in various bioactive metabolites. Hence, present investigation unveils medicinal potential of the plant under study.

Table 1. Phytochemical tests of different extracts of *X. strumarium*

Phytochemicals	Chemical tests	Water Ext.	Methanol Ext.	DCM Ext.
Reducing Sugars	Fehling test	+++	++	+
Non-reducing sugars	Fehling test	-	-	-
Carbohydrates	Molish's test	+	+	+
Phytosterol	Liebermann Burchard's test	-	-	-
Alkaloids	Hager's test	++	+++	-
Flavonoids	_	++	+++	-
Tannins	Lead acetate test	+	+++	-
Saponins	Frothing test	++	+++	+
Terpenoids	Salkowski' s test	++	+	-
Quinones	_	-	+	-
Amino acids and Proteins	Zinc sulphate test	+++	++	+
Carboxylic acid	Effervescence test	+++	+	-
Gum	Adhesives test	-	-	+

Key: (-) indicates absence, (+) shows presence, (++) for moderate concentration, (+++) is high concentration, DCM = Dichloromethane, Ext. = Extract

It is an established fact that bioactivity of medicinal plants is due to the presence of bioactive secondary

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metabolites in it. These metabolites exert significant role on human health and hence contributes to treat various diseases. **Alkaloids** are nitrogen containing heterocyclic compounds of important pharmacological activities. The alkaloids derived from *Cataranthus roseus* are hypoglycemic (Ijaz *et. al.,* 2021), from *Galanthus woronwii* are anti-inflammatory whereas steroidal alkaloids of *Sinomenium acutum* are neurodegenerative in nature (Babita *et al.,* 2022). **Tannins** are antimicrobial components. They inhibit pathogenic fungi by inhibiting cell protein synthesis. These are stringent and can heal wounds too. Tannin isolated from *Acacia* species and *Betula pendula* exhibited anti-microbial properties (Maria *et al.,* 2021). Similarly, **Flavonoids** are phenolic compounds possesses multiple therapeutic properties, occurs mainly in edible parts of plants. *Cannabis sativa*, *Brysonima crassa* and Citrus species are rich in flavonoids. These are used in herbal medicines to treat inflammation, allergies, cancer and microbial infections (Arpita *et al.,* 2022). Flavonoids are potent antioxidant and can control oxidative stress also (Giuseppe *et al*., 2007)**. Terpenoids,** a class of isoprenoids, exhibit antifungal, antimicrobial, anti-parasitic, anti-viral, anti-allergenic, anti-hyperglycemic, anti-inflammatory, immunomodulatory and insecticidal potential (Mahato & Sen, 1997). Moreover, **Steroids** regulate immune response and cholesterol, and promote nitrogen retention in osteoporosis as well (Pinar *et al.,* 2023). **Saponins** exhibit the distinctive property of red blood cells precipitation and coagulation (Okwu, 2004). **Carbohydrates** are valuable dietary supplements which exert a beneficial role on immune system (Madziga *et al*., 2010). **Amino acid** and **Proteins** are the building blocks of human body. These are essential for repairing and maintenance of body skeleton (Ojala *et al.,* 2000). **Quinones** are electron scavengers and helpful in preventing and treating various disorders such as osteoporosis and cardiovascular problems (Nahed *et al*., 2011). **Plant gums** are also of medicinal values which exhibit anti-inflammatory, anti-arthritic, anti-parasitic, gastro urinary, antitumor, anti-parasitic, antioxidant, and gastro protective activities (Ravi, 2017).

4. Conclusion

The present investigation for the existence of bioactive metabolites defines *X. strumarium* L. as a valuable medicinal plant. Different extracts especially aqueous (a benign and universally available green solvent), of this plant are a rich source of bioactive metabolites which can protect and prevent a number of diseases and has potential to fight against several syndromes. However, further research is still needed to explore therapeutic agents from this plant for maximum utilization of its bioactive constituents thus making it more beneficial for public health.

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