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Evaluation of the Phytochemical and Antibacterial characteristics of leaf extracts of *Xanthium strumarium* L. against Bacteria

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ABSTRACT

Plants are one of the large sources of herbal medicines. Many plants have the capability to produce some bioactive constituents which give defense against microorganisms like bacteria and fungi and also from insects. *Xanthium strumarium* L. is a wild plant that grows like a weed. Leaves of *Xanthium strumarium* L. were subjected for extraction in 4 different solvents viz. methanol, chloroform, aqueous, and ethanol. The aim of this study was to investigate the phytochemical screening and antimicrobial activity of methanol, chloroform, aqueous, and ethanol extracts prepared from leaves of *Xanthium strumarium* L. The antibacterial activity was assessed by using the agar well diffusion assay against tested bacterial strain, *Escherichia coli* (CGSC 4312) and *Bacillus subtilis* (ATCC 23857). Phytochemical analysis has revealed that *Xanthium strumarium* L. has most of the significant phytoconstituents like Saponins, Terpenoids, Flavanoids, Phenol, steroids, Anthraquinones, Tannins, Alkaloids, Glycosides, and Carbohydrate. The zone of inhibition was measured and compared by standard antibiotic streptomycin. The outcome of the present work showed that chloroform extract 400 μ g showed maximum inhibition against *E. Coli* (23mm), and Ethanol extract 400 μ g showed maximum inhibition against *Bacillus subtilis* (24mm). Thus, this plant has many active compounds that can be used for the development of various potent drugs.



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INTRODUCTION

India has a rich history, which consists of plant and herbal based medicines. There are many medicinal plants in use throughout the world, with a large

range of action and degrees of potency. Most have a specific action on particular body systems and are known to be suitable for treating a certain type of disease (Sambamurty, 2006). *Xanthium strumarium* L. is an annual plant species that belongs to the Asteraceae family. The species is monoecious with the flower born in separate unisexual heads (Weaver and Lechowicz, 1983). *Xanthium strumarium* L. is a weed plant which is found in all states of India it occurs in waste sites agricultural lands and rural area (Weaver and Lechowicz, 1983). It is also found in roadsides and open grasslands (Tiwari et al., 2005). The plant has some medicinal properties. Due to this, it has been used in traditional medicine in India (Devkota and Das, 2018). All parts of the plant own some medicinal properties like diaphoretic, sedative, and diuretic. Plant extracts

from the part of leaves, root seeds, and fruits have medicinal value for the treatment of epilepsy, leucoderma, and insect bite (Kamboj and Saluja, 2010). According to Ayurveda *X. strumarium* has lots of medicinal properties like digestive, diuretic, appetizer, etc. The plant produces compounds as secondary metabolites and their derivatives like phenolic compounds, terpenes, alkaloids, tannins, glycosides, isoflavonoids, and flavonoids, which has antimicrobial characteristics (Simoes et al., 1999). Plant extracts or bioactive herbal compounds have been reported scientifically for their biological activities. Humans may be protected by phytochemicals from disease-causing pathogens (Farooq et al., 2014). In earlier chemical studies on *X. strumarium* carboxyatractyloside, isoxanthol, alkaloids, thiazinedione, were identified (Ma et al., 1998; Cumanda et al., 1991).

The aim of the present study is to determine the phytochemical analysis and antibacterial activity of various extracts of *X. strumarium* leaves against some pathogenic bacteria.

MATERIALS AND METHODS

Plant Material

X. strumarium leaves were collected from the Kota region, Bilaspur (C. G). Fresh and young leaves were collected for extraction.

Preparation of Extracts

The leaves of *Xanthium strumarium* were dried under shade at room temp. And then grinded in a homogenizer to form a powder 20 gm of dried plant powder was extracted with methanol, chloroform, petroleum ether, and aqueous successively each solvent taken in amounts 200ml each and separated using a soxhlet extractor. After then, the solvent present in the extract was evaporated at 50°C in the water bath. Then residual powder extracted from the left from solvent evaporation was dissolved in DMSO and stored at 4°C.

Phytochemical analysis

The qualitative phytochemical analysis of crude extracts was performed by standard methods described by (Harborne, 1998).

Antimicrobial activity

Antimicrobial activity of methanol, ethanol, chloroform, and aqueous extract of the *Xanthium strumarium* plant was determined by measuring the diameter (mm) of growth inhibition zone by agar well diffusion method. The microbial inoculums were inoculated aseptically and spread consistently on the surface of the pre-solidified nutrient agar plate. 6

wells of about 6.0 mm were aseptically punctured by using sterile cork borer. Plant extracts of different concentrations were poured in each well. Streptomycin was used as positive control while DMSO was used negative ad control. All the plates were inoculated at 37°C for 24 hrs. And the antimicrobial activity was observed and calculated.

Microbial Strain

The microbial strain *E. coli* (CGSC 4312), *Bacillus subtilis* (ATCC 23857) were used. These strains were constantly sub-cultured and maintained in nutrient agar. The diameter of the zone of inhibition (expressed in mm) was determined to test the sample of antibacterial activity. The procedure was repeated thrice, and the mean of the three experiments was recorded. For complete inhibition of bacterial growth, the MIC test was conducted to find out the lowest.

RESULTS AND DISCUSSION

The use of medicinal plants plays a vital role in covering the health needs in developing countries, and these plants act as a new source of antimicrobial agents, which gives pathogenic mechanisms against infection-causing bacteria. In this study, the four different crude extract of *Xanthium strumarium* were investigated for phytochemical and antibacterial activity.

Phytochemical analysis

Phytochemical screening of different *Xanthium strumarium* extracts, i.e., aqueous, ethanol, methanol, and chloroform extracts, showed the difference in their phytoconstituents due to use of different solvents. Secondary metabolites such as tannins, alkaloids, terpenoids, glycosides, saponins, flavonoids, phenol, carbohydrates, steroids, anthraquinones, were detected in above extracts. In the phytochemical screening of aqueous extracts, tannins, terpenoids, flavonoids, phenol, carbohydrates, steroids were found (Figure 4). While in ethanol extract, terpenoids, saponins, flavonoids were found (Figure 3). In methanol extract, evidence of Tannins, Terpenoids, Saponins, flavonoids, phenol, and steroids were found (Figure 2). The extracts of chloroform showed the presence of tannins, alkaloids, Saponins, flavonoids, and steroids were found (Figure 1), (Table 1).

Antimicrobial activity

Antimicrobial activity of the methanol, ethanol, chloroform, and aqueous extract of *Xanthium strumarium* plant was studied by the agar well diffusion method, and the result was characterized by recording diameter (mm) zone of inhibition around the

Table 1: Phytoconstituents present in different extracts of *Xanthium strumarium* leaf

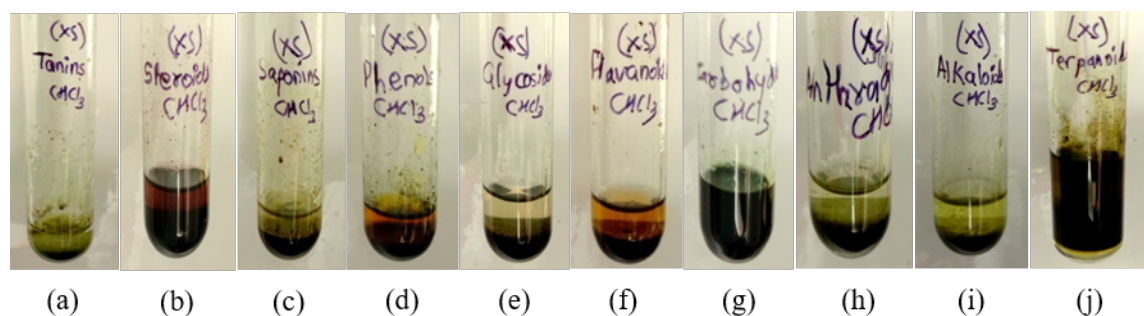
Phytoconstituents	Xanthium strumarium			
	Aqueous	Ethanol	Methanol	Chloroform
Tannins	+	-	+	+
Alkaloids	-	-	-	+
Terpenoids	+	+	+	-
Glycosides	-	-	-	-
Saponins	-	+	+	+
Flavanoids	+	+	+	+
Phenols	+	-	+	-
Carbohydrates	+	-	-	-
Steroids	+	-	+	+
Anthraquinones	-	-	-	-

Table 2: Antibacterial activity of different extracts of *Xanthium strumarium*

S.No.	Name of Bacteria	The diameter of zone of inhibition in mm (well size 6 mm)				
		<i>Xanthium strumarium</i> extracts				Positive control Streptomycin
		Methanol extract	Chloroform extract	Ethanol extract	Aqueous extract	
1.	Bacillus subtilis	20 mm	21 mm	24 mm	22mm	40 mm
2.	E.coli	20 mm	23 mm	20 mm	15 mm	30 mm

Table 3: Minimum Inhibitory Concentration of various extracts of *Xanthium strumarium*

S.No.	Name of bacteria	Minimum inhibitory concentration (MIC)			
		Methanol extract (μg)	Chloroform extract (μg)	Ethanol extract (μg)	Aqueous extract (μg)
1.	Bacillus subtilis	400 μg	500 μg	400 μg	500 μg
2.	E.coli	600 μg	350 μg	400 μg	600 μg

**Figure 1: Phytochemical test of chloroform extract of *Xanthium strumarium* (a) Tannin (b) Steroids (c) Saponins (d) Phenols (e) Glycosides (f) Flavanoids (g) Carbohydrate (h) Anthraquinones, (i) Alkaloids (j) Terpanoids**

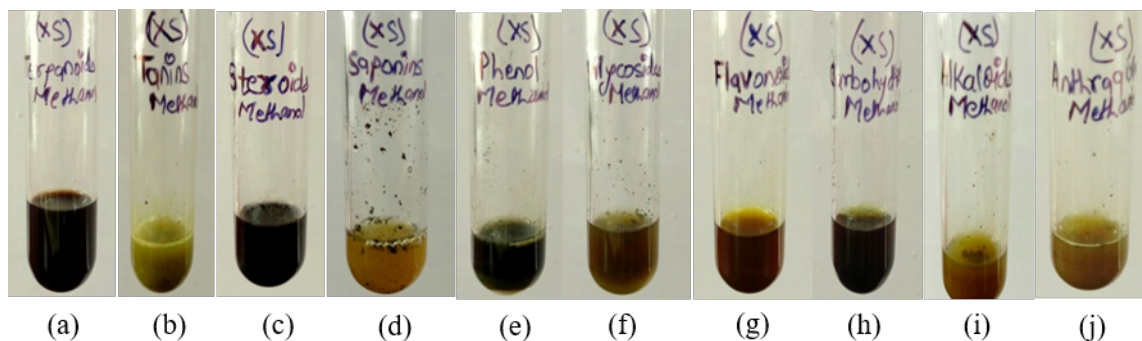


Figure 2: Phytochemical test of methanol extract of Xanthium strumarium (a) Terpanoids, (b) Tannins, (c) Steroids, (d) Saponins, (e) Phenols, (f) Glycosides, (g) Flavanoids, (h) Carbohydrate (i) Alkaloids (j) Anthraquinones

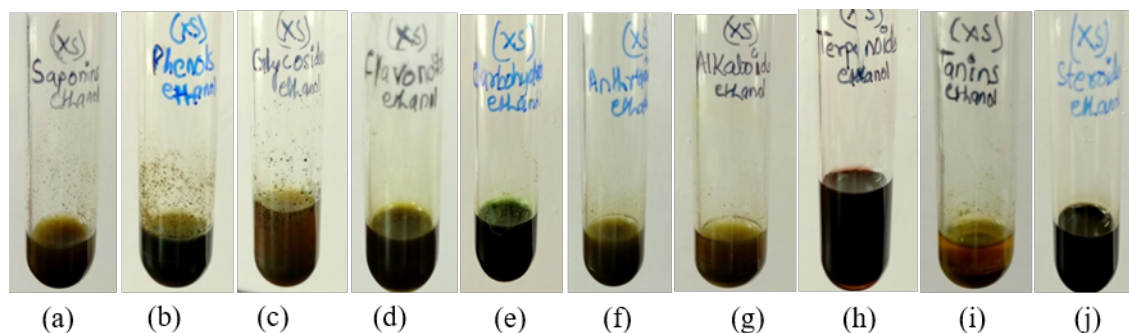


Figure 3: Phytochemical test of ethanol extract of Xanthium strumarium (a) Saponins (b) Phenols (c) Glycosides (d) Flavanoids (e) Carbohydrate (f) Anthraquinones (g) Alkaloids (h) Terpanoids (i) Tannins (j) Steroids

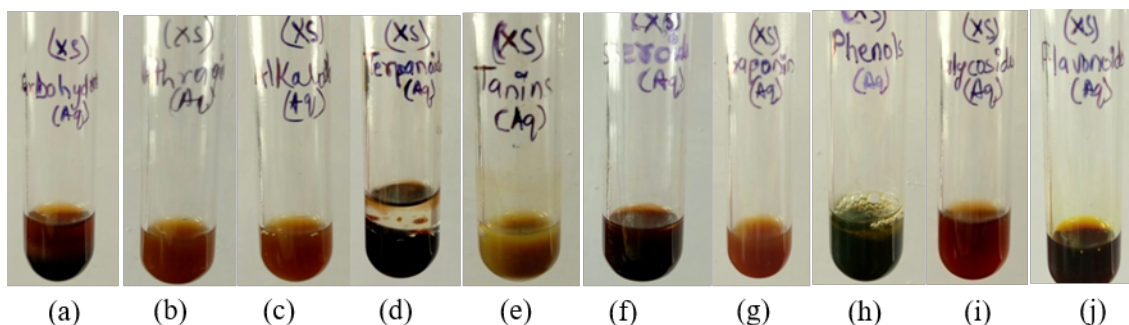


Figure 4: Phytochemical test of aqueous extract of Xanthium strumarium (a) Carbohydrate (b) Anthraquinones (c) Alkaloids, (d) Terpanoids, (e) Tannins, (f) Steroids, (g) Saponins (h) Phenols (i) Glycosides (j) Flavanoids

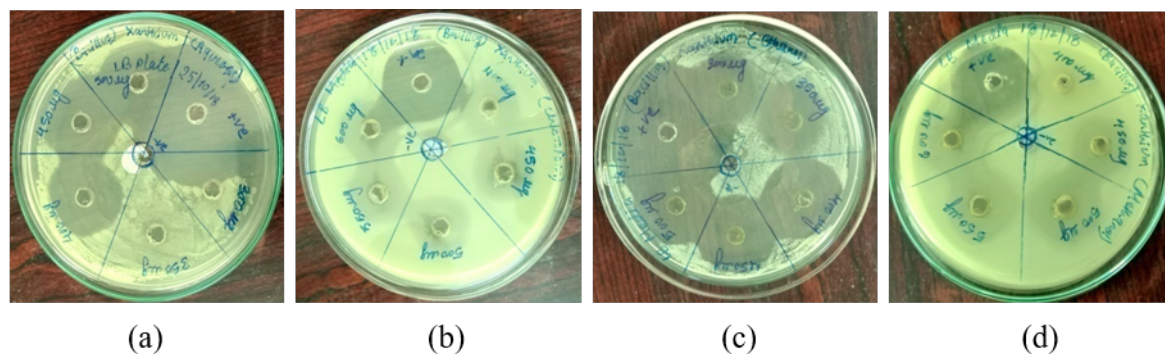


Figure 5: Antibacterial activity of different extracts of Xanthium strumarium against Bacillus subtilis (a) Aqueous extract, (b) Chloroform extract, (c) Ethanol extract, (d) Methanol extract

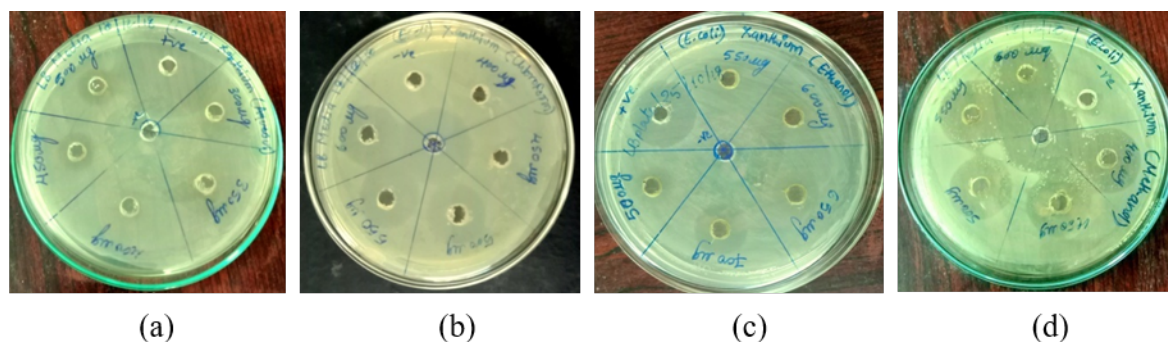


Figure 6: Antibacterial activity of different extracts of *Xanthium strumarium* against *Escherichia coli* (a) Aqueous extract, (b) Chloroform extract, (c) Ethanol extract, (d) Methanol extract

well (Table 2). The extracts were tested against both the bacteria. The antibacterial activity was determined by zone of inhibition of Aqueous, Chloroform, Ethanol and Methanol extracts of *Xanthium strumarium* plant against *Bacillus subtilis* and *Escherichia coli* has shown in (Figure 5) and (Figure 6). However, the highest inhibitory effect was shown by chloroform extract against *E. coli*.

The inhibition zone being 23 mm, and the methanol extract showed the least zone of inhibition of 20mm against the same. Similarly against *Bacillus subtilis* ethanol extract showed maximum zone of inhibition of 24 mm and aqueous extract showed the least zone of 15mm. (Figures 5 and 6). All the extracts of *Xanthium strumarium* showed significant antimicrobial activity at different concentrations against tested organisms (Table 3). The methanolic extract was effective against both *Bacillus subtilis* and *E. coli* at a concentration of 400 μ g and 600 μ g, respectively. Chloroform extract showed a zone of inhibition against *Bacillus subtilis* at 500 μ g concentration and inhibited *E. coli* at a concentration of 350 μ g. Ethanol extract inhibited the growth of both the organism at the same concentration of 400 μ g. The aqueous extract inhibited *Bacillus subtilis*, *E. coli* at a concentration of 500 μ g, and 600 μ g, respectively.

CONCLUSION

This current study demonstrates the *Xanthium strumarium* showed that the presence of bioactive constituents. This plant exhibited good antibacterial activity against pathogenic bacteria, which indicates its potential as a source of functional ingredients to produce the new potential antimicrobial drug. Therefore, synergistic use of medicinal plant extract should be encouraged to prevent drug-resistant bacteria and to treat emerging and re-emerging diseases caused by the pathogenic microorganism.

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