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# Evaluation of antibacterial activity of *Xanthium strumarium* L. against pathogenic bacteria

**Karzan Omer Qader**

University of Sulaimanya-College of Science, Department of Biology,

**Sahar A.A Al-Saadi**

University of Basra -College of Science, Department of Biology,

**Risala H. Allami**

Al-Nahrian University-College of biotechnology-Iraq

Corresponding author email: [ririallami@yahoo.com](mailto:ririallami@yahoo.com)

**Abstract**---Bacterial infection is one of the most serious intrinsic bioactivity, by their ability to dissolve or diffuse global health issues in 21 century. Thus the present study was undertaken to investigate the antibacterial activity of *Xanthium strumarium*. Plant was extracted by using soxhlet extraction by using ethanol as a solvent extractor. Antibacterial activity of *Xanthium strumarium* was estimated against various bacterial pathogens. The results showed that ethanol extract of *Xanthium strumarium* exhibited antibacterial activity against gram positive and gram negative bacteria. Ethanol extract gave highest effect against *Klebsiella spp* and *P. aregenosia*. Then lower effect on *S. aureus* and *Bacillus subtilis*. The recent study showed that *X. strumarium* ethanolic extract can be used as microbial growth inhibitor against both gram positive and negative bacteria. Gram positive bacteria were found to be more resistant than gram negative bacteria, Also the studies determines that *P. aregenosia* was the most bacterial sensitive that produced the highest rate of inhibition zone, while the minimum inhibition zone with the same concentration was recorded by *Staphylococcus aureus*.

**Keywords**---fixed oil, *Xanthium strumarium*, antibacterial activity, Ethanol, soxhlet extraction method.

## Introduction

Herbal medicine is a growing area of health care that demands attention. Plants have played a significant role in maintaining human health and improving quality

of human life for thousands of years and have served human as valuable components of medicines.

*Xanthium strumarium* is one of the important medicinal plant commonly known as Cocklebur, is widely distributed in North America, Brazil, China, Malaysia, and hotter parts of India (Kamboj *et al.*, 2010). *Xanthium* belonging to Asteraceae family which include more than 20 species in the world and three species with one varietas in China. *X. strumarium* is annual herb approximately 20-90 cm on height, its stems are erect ,branched often speckled and have a short white hairs scattered across the surface .leaves are green , cauline mostly alternate with petiole and margins has toothed (Fan *et al.*, 2019). *Xanthium strumarium* has many medicinal properties like cooling, laxative, fattening, anthelmintic, tonic, digestive, antipyretic, improves appetite, voice, complexion anodyne, antirheumatic, appetizer, diaphoretic, diuretic, emollient and sedative. The plant is useful in treating long-standing cases of malaria, rheumatism, diseased kidneys, tuberculosis (NASIR *et al.*, 2012). The fruits of *Xanthium strumarium* has the properties like anodyne, antibacterial, anti-fungal, antimalarial, antirheumatic, antispasmodic, antitussive, cytotoxic, hypoglycemic and stomachic. They are used internally in the treatment of allergic rhinitis, sinusitis, catarrh, rheumatism, rheumatoid arthritis, constipation, diarrhea, lumbago, leprosy and pruritus (Fan *et al.*, 2019).

A decoction of the root has been used in the treatment of high fevers and to help a woman expel the after birth and a decoction of the seeds has been used in the treatment of bladder complaints by local peoples. The dried leaves of *Xanthium strumarium* are a source of tannin. However, *Xanthium strumarium* is poisonous to grazing animals (NASIR *et al.*, 2012). To extract the fixed oil from *Xanthium*, soxhlet extraction is mostly used to extract various types of essential oils the process is cheaper than other extraction methods, it does not require any solvent and is safer than other methods. Analytes with medium to low volatility which may play a role for the aroma and quality of oil extracted from the plant material are extracted with this technique (Scherer *et al.*, 2014).

The correct choice of solvent is important to obtain a good yield from the extraction as well as to prevent the loss of volatiles, the solvent that used in this method is ethanol. Advantage of this method is that can usually extract and carried out for a long period and the property of oils produced by this method are not altered. But disadvantage of this technique is that, due to the long heating period, the analytes are exposed to high temperatures which may lead to thermal degradation of some compounds. The recovered sample is diluted and must be concentrated further by evaporation (Govender *et al.*, 2010).

Previous studies have reported that *X. strumarium* induces intoxication and can be lethal to cattle, sheep, pigs and humans (Scherer *et al.*, 2014). Also the consumption of fruits and cotyledonary stage leaves leads to hepatic necrosis and myocardial injury in humans. The toxic principle in *X. strumarium* poison was isolated and identified as carboxyatractyloside (CAT) (Cutler *et al.*,1983). The aim of this study we used *X. strumarium* leaves as a bioactive agent against some clinical bacterial isolates.

## Materials and Methods

*Xanthium strumarium* that used in this study was purchased from university of Sulaimany and Sharbazher (Figure 1, A).

### Sample preparation:

Pretreatment the raw *X. strumarium* leaves contained some dirt particles and other adhering substances like small sand particles. Cleaning was conducted on the material in order to remove these substances as much as possible, and grinds to reduce the sample particle size. (Kumar *et al.*, 2017).

### Extraction of *X. strumarium* by using soxhlet extraction:

100 gram of dried *Xanthium strumarium* were introduced into the distillation flask which is gradually filled with 300 ml ethanol and water as a solvent for about 24 hours. When the liquid reaches an overflow level, a siphon aspirates the whole contents of the thimble\_holder and unloads it back into the distillation flask, carrying the extracted analytes in the bulk liquid (Figure 1, B). This operation is repeated until complete extraction is achieved, and assembly can be considered as a batch system then samples were left under fume hood (De Castro *et al.*, 2000).

### Preparation of bacterial culture

For antimicrobial test, four different types of bacterias were used to determine the activities of the ethanolic extract of *X. strumarium*. *Staphylococcus aureus*, *Bacillus subtilis* as gram positive while *Pseudomonas aerogenos*, and *klebsiella pneumoniae* as gram negative bacteria from shar hospital, maternally hospital, and microbiology laboratory. Bacterial types were cultured on Mueller Hinton Agar incubated 24 hours at 37°C. Antimicrobial activity of the ethanolic extract of *X. strumarium* was tested by well diffusion method. Antimicrobial activity was evaluated by measuring the inhibition zones expressed in millimeters against tested organisms. The inhibition zone was measured by using a ruler and recorded for statistical analysis.

### Test to determine the antimicrobial activity

The antimicrobial activity of fixed oil was tested by using well diffusion method and Mueller Hinton Agar plates were used (Figure 1, C and D). Each well added with (8,13,15) µl of fixed oil for each of bacterial species and incubated for 24 hours at 37°C . All the process was carried out under laminar air flow. Evaluated by measuring the inhibition zone expressed in millimeters against tested organisms.

### Statistical analysis

The data of the study were analysis by one-way analysis of variance (ANOVA). A significance level < 0.05 was considered statistically significant.



Figure (1): Steps of work A- whole plants; B- extract by Soxhlet apparatus; C- incubator D- well diffusion method by using petri dishes .

## Results & Discussion

Ever increasing demands from consumers for use of natural agents as additives and food preservatives, and the increased incidence of new and re-emerging infections, has led to a search for new and more effective antimicrobial compounds that have diverse chemical structure and novel mechanism of action. Plants are an invaluable source of pharmaceutical products, because they have an almost infinite ability to synthesize compounds with different antimicrobial activity against various pathogenic and opportunistic microorganisms (Scherer *et al.*, 2014). Each extracts were tested against the four isolates and identified bacteria (*S. aureus*, *P. aeruginosa*, *Klebsilla pneumoniae* and *Bacillus subtilis*). The antibacterial activity of the extracts was recorded as the mean diameter of the resulting inhibition zones of growth measured in (millimeters).

Diameter of inhibition zone of ethanolic extract of *X. strumarium* is reputation against strain of bacteria is summarized in Table (1).

Table (1): Diameter of inhibition zone of ethanolic extract of *X. strumarium* is reputation against strain of bacteria

Bacteria	Size of inhibition zone			
	Concentration of ethanolic extract			
	25	50	75	100
<i>Klebsiella pneumoniae</i> Gve-	8.6	10.	13.3	15.5
<i>Bacillus subtilis</i> Gve+	7.8	9.8	10.3	12.5
<i>Pseudomonas aerogenos</i> Gve-	7.5	10.8	13.5	16.2
<i>Staphylococcus aureus</i> Gve+	7.2	10.2	10.8	9.5

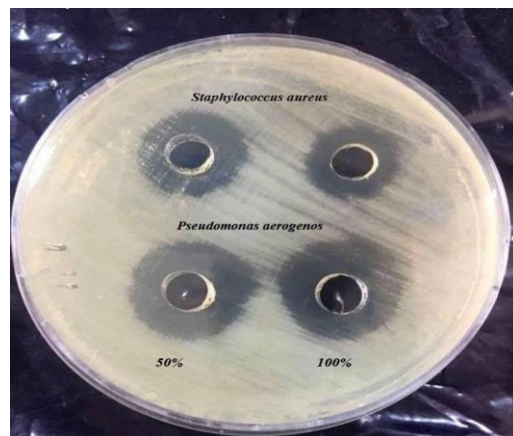
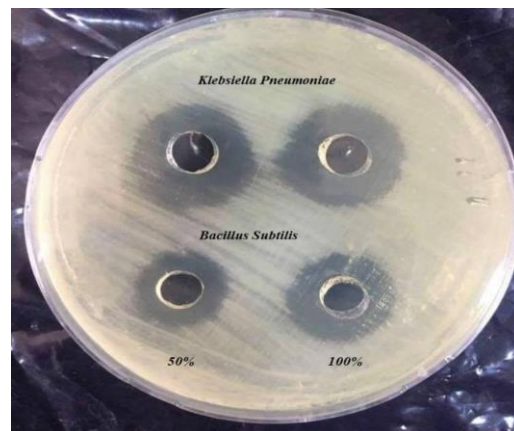


Figure (2): antimicrobial activity of ethanolic extract of *X. strumarium* on isolate of bacteria

In this study, the ethanolic extract of *X. strumarium* was used to inhibit bacterial growth. The results showed that the antibacterial activity expressed as inhibition zone, ranges from (0 to 20) mm for the application of Concentration of ethanolic extract (25,50,75,100%) suggesting a high antimicrobial activity. The antibacterial activity was expressed as diameters of inhibition. The *X. strumarium* extract, which showed a diameter of inhibition zone, was tested using the well diffusion. The results showed that *X. strumarium* extract is active against bacteria's, and ethanolic extract of *X. strumarium* have different inhibitor growth with different concentrations, in gram negative bacteria results there is higher inhibition zone it is due to the bacterial complex structure, but for gram positive bacteria the results show low inhibition zone. There are many factors that affect sensitivity of microbes to antimicrobial agent like size of inoculum, concentration of inoculum, diffusion rate of antimicrobial agent, concentration of antimicrobial agent, incubation time, PH of the medium and components of the medium. The study showed that *Staphylococcus aureus* and *Bacillus subtilis* were found to be most resistant followed by *Klebsiella pneumoniae*. Also the study determined that *Staphylococcus aureus* was the most bacterial resistant that produced the lowest rate of inhibition zone 9.5mm at (100%) of the concentrated oil. While the maximum inhibition zone with the same concentration was recorded by *P. aeregenosia* which was (16.2 mm) respectively. Previous study indicated that *Xanthium strumarium* have a good effect for many fungi like, *Candida albicans* and *Aspergillus niger*. In the other study recorded the extract of *Xanthium strumarium* has a good effect on *Echinococcus granulosus protoscolices* and *Salmonella*. (Sharifi-Rad *et al.*, 2015). The antibacterial activity may be possibly attributed to the presence of phenolic acids, flavonoids, tannins and triterpenoids in the methanol extract, as reported in literature (Talakai *et al.*, 1995).

## Conclusion

The recent study showed that *X. strumarium* ethanolic extract can be used as microbial growth inhibitor against both gram positive and negative bacteria. Gram positive bacteria were found to be more resistant than gram negative bacteria. Also the studies determine that *Pseudomonas aerogenos* was the most bacterial sensitive that produced the highest rate of inhibition zone, while the minimum inhibition zone with the same concentration was recorded by *Staphylococcus aureus*.

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