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Review Article

Evaluation of the Antibacterial Activity of *Xanthium strumarium* Leave Extracts Against Clinical Isolates of *Staphylococcus aureus* from the Burn Unit in Jibla University Hospital

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Abstract

The rise of antibiotic resistance has spurred a search for alternative antibacterial agents. *Xanthium strumarium* L. (*Asteraceae*) holds promise due to its phytochemical composition, including terpenoids, saponins, and tannins, which have been associated with antimicrobial properties. This study aimed to evaluate the antibacterial efficacy of *Xanthium strumarium* leaf extracts against 20 clinical isolates of *Staphylococcus aureus* obtained from the burn unit at Jibla Hospitals, Ibb, Yemen. The primary objective was to determine the potential of these extracts as effective antibacterial agents for combating *Staphylococcus aureus* infections. Ethanol and distilled water extracts were prepared from *Xanthium strumarium* leaves and tested at concentrations ranging from 0.5 µg/ml to 12.5 µg/ml. The agar-well diffusion method was employed to assess antibacterial activity, with the zone of inhibition (ZOI) serving as a measure of efficacy. Phytochemical screening was conducted to identify bioactive compounds present in the extracts. The ethanol extract exhibited superior antibacterial activity compared to the distilled water extract. Notably, at a concentration of 12.5 µg/ml, the ethanol extract produced a maximum inhibition zone of 30 mm against clinical isolates AS6 and AS11. In contrast, the distilled water extract showed a maximum inhibition zone of 28 mm against clinical isolate AS2 at the same concentration. The findings of this study highlight the significant bactericidal potential of *Xanthium strumarium* leaf extracts against *Staphylococcus aureus*. The presence of bioactive compounds in the extracts, coupled with their demonstrated antibacterial efficacy, suggests that further research into the development of novel antibacterial therapies utilizing *Xanthium strumarium* extracts is warranted.

Keywords: *Xanthium strumarium*; *Staphylococcus aureus*; Leaves; Ethanol; Distilled Water; Antibacterial Activity; Yemen

Introduction

Plant extracts or bioactive herbal compounds have been reported scientifically for their biological activities. Humans may be protected by phytochemicals from disease-causing pathogens [1] *Xanthium strumarium*, belonging to the Asteraceae family, includes more than 20 species worldwide, with three species and one varietas found in China [2]. The key compounds isolated from *X. strumarium* include xanthanol, isoxanthanol, hydroquinone, caffeyolquinic acids, alkaloids, and thiazinedione [3,4]. The plant may have some medicinal properties and has been used in traditional medicine in south Asia and traditional Chinese medicine. All parts of plant possess sedative, diaphoretic and diuretic properties. Extracts of whole plant parts especially leaves, roots, fruits and seeds have traditional medicinal value and used mainly for the treatment of poisonous insects bites, epileosy, leucoderma [5].

Researchers have proven that plant extracts can be used to cure many health ailments and have fewer side effects compared to other forms of medication [6]. They are utilized internally for the management of allergic rhinitis, sinusitis, mucus buildup, rheumatism, rheumatoid arthritis, constipation, diarrhea, lower back pain, leprosy, and itching of skin. So far, many phytochemical studies of *X. strumarium* have been conducted, isolating and identifying more than 170 compounds from this plant. Among them, sesquiterpenes and phenylpropanoids are the most abundant and major bioactive constituents, considered characteristic of this plant. In addition to the chemical compounds present in the fruits, those found in other parts of *X. strumarium*, such as the leaves, roots, and stems, have also been studied, along with their corresponding structures [7]. Antibiotic resistance has become a global challenge for human health, as it is linked to high rates of mortality and morbidity [8].

Staphylococcus aureus is a Gram-positive, spherical shaped microorganism, non-motile, non-spore former, and some strains are capsulated. The initial isolation was performed by Alexander Ogston during his investigation of septicemia and wound-infecting bacteria in 1880. Microscopic analysis of 88 pus samples uncovered the presence of Gram-positive cocci (*S. aureus*) [9,10]. The majority of *S. aureus* strains (94%) are markedly reluctant to penicillin and its derivatives due to the release of penicillinase enzyme [11,12]. Certain strains of *S. aureus* exhibit resistance to methicillin, a condition referred to as MRSA (Methicillin-Resistant *Staphylococcus aureus* [13].

Staphylococcus aureus is one of the pathogens capable of developing a broad spectrum of infections in humans. In addition to being present in hospitals, this bacterium is also found in the community and exhibits high resistance to antibiotics, which is continuously increasing. Resistance to the β-lactam antibiotic family is a significant concern, making the treatment of such infections difficult. Due to the increasing resistance and importance of this bacterium, new strategies are needed to control this pathogen [6].

The aim of this study is to evaluate the antibacterial potential of *Xanthium strumarium* L extracts against 20 clinical isolates of *Staphylococcus aureus* from the burn unit at Jibla hospitals and comparing the efficacy of ethanol and distilled water leaf extracts in inhibiting these isolates.

Methods

Plant material

Fresh and healthy leaves of *Xanthium strumarium* were collected during the summer. The stander parts of the plants were collected from Salabt Al-sidah in Ibb city. The plant was identified with the help of Botany department, University of Ibb.

Extraction

The leaves were thoroughly washed with running tap water 2-3 times and then finally rinsed with distilled water. Afterward, they were shade-dried for seven days and then dried in an oven at temperatures below 50ºC. The completely dried leaf samples were ground into a fine powder using an electric grinder and stored in a glass container in the dark [14].

A fixed weight of 25 g of the dried powdered leaves was soaked separately in 250 ml of ethanol and distilled water for 72 hours. Each mixture was stirred at 24-hour intervals using a sterile glass rod [15].

Afterward, the plant samples were squeezed and filtered using a triple-layered cotton cloth. The extracts were then evaporated in the dark in an oven at 40 °C for 2 hours [16].

Collection of microorganism sample

The microorganisms used in this study consisted of 20 clinical isolates of *Staphylococcus aureus*, obtained from the burn unit at Jiblah Hospital. The isolates were confirmed by the Bacteriological Laboratory staff at Jiblah University Hospital and preserved in glycerol for subsequent use.

Preparation of plant extract concentrations

Different concentrations of the plant leaf extract (0.5 µg/ml, 1 µg/ml, 1.5 µg/ml, 2.5 µg/ml, 5 µg/ml, 7.5 µg/ml, 10 µg/ml, 12.5 µg/ml) were prepared in DMSO using ethanol, distilled water extracts powder, and DMSO served as negative controls, while vancomycin (30 µg/ml) were used as positive controls [17].

Antibacterial test

The antibacterial activity was determined by the agar-well diffusion method using a cell suspension of about 1.5×10^6 CFU/mL obtained following 'Macfarland turbidity standard No. 0.5'. Standardization of the suspension concentration was achieved by adjusting the 'optical density' to 0.1 at 600nm (Shimadzu, UV-VIS Spectrophotometer). Holes of 6 mm diameter were then made on the Mueller Hinton agar plate (6mm thick) and filled with 100 µL of ethanolic and distilled water extract, followed by incubation at 37 ± 1 °C for 24 hrs. Zone of growth inhibition around the hole was then measured to evaluate antimicrobial activity. The procedure was repeated in triplicate and the mean diameter was recorded [18,19].

Statistical analysis

The One – way Analysis of Variance (ANOVA) was used to determine the significant differences between the parameters (inhibition zone of ethanol and distilled water) and the Tukey HSD test was done to compare the differences between different concentrations of ethanol and distilled water at $p < 0.05$ using statistical package SPSS version 15.

Results and Discussion

Yield of ethanol and distillated water extract of *Xanthium strumarium*

The yield of ethanol and distilled water extracts of *Xanthium strumarium* in this study showed that the ethanol extract yield was 10.4%, which is higher than the yields reported in previous studies: 8.8% by [20] and 6.3% by [21]. In the distilled water extract, the yield was 8.8%, while the yield with chloroform was 5.4%.

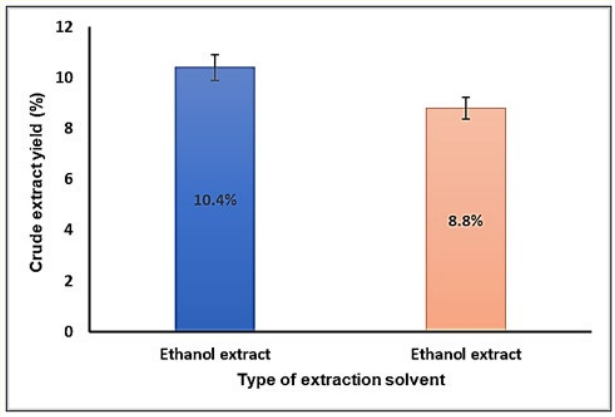

Figure 1: Yield of Ethanol and distilled water extracts of *Xanthium strumarium*.

Phytochemical screening qualitative test

A study indicated that phytochemical screening revealed active chemical constituents in the plant, including tannins, which appeared brownish-green or blackish. Saponins demonstrated stable foam persistence, while terpenoids exhibited a reddish-brown color. These findings are consistent with those reported by [22].

Antibacterial activity of *Xanthium strumarium*

The study demonstrated antibacterial activity of the extracts at different concentrations against most clinical isolates of *Staphylococcus aureus*, with the exception of clinical isolates numbered 4, 5, 8, 13, and 20, which showed no effect toward the crude ethanol extract. The results indicated that the ethanol crude leaf extract of *Xanthium strumarium* produced the highest inhibition zone of 30 mm against clinical isolates 6 and 11 at a concentration of 12.5 µg/ml, in contrast, no inhibition zones were observed for clinical isolates 4, 5, 8, 13, 16, 18, and 20 at all tested concentrations. These findings align with a study by [23], which reported lower inhibition zones of 7.2, 10.2, 10.8, and 9.4 mm at concentrations of 25, 50, and 75 µg/ml against *Staphylococcus aureus*, possibly due to the use of the Soxhlet extraction method in his research, Similarly, [22] reported inhibition zones of 25, 25, 26, 26, and 27 mm at concentrations of 50, 100, 150, 200, and 250 µg/ml, respectively, using methanol crude leaf extract against *Staphylococcus aureus* (ATCC 25923).

In another study by [24], no strains of *Staphylococcus aureus* showed resistance. Additionally, [21] reported inhibition zone diameters of 28 mm, 15 mm, and 16 mm for chloroform, methanol, and hot aqueous extracts at a concentration of 100 mg/ml, respectively, against *Staphylococcus aureus* (ATCC 25923) (Table 1).

Clinical isolates of bacteria	Concentration (µg/ml) Negative Positive								Control			
									Negative		Positive	
	0.5	1	1.5	2.5	5	7.5	10	12.5	DMSO	Ethanol	Van	P value
Staph1	11	13	15	15	16	20	18	22	0	0	12	0.001
Staph2	0	0	12	9	14	20	22	24	0	0	10	0.001
Staph3	0	0	0	0	12	14	14	18	0	0	19	0.001
Staph4	0	0	0	0	0	0	0	0	0	0	12	0.001
Staph5	0	0	0	0	0	0	0	0	0	0	12	0.001
Staph6	16	17	19	20	25	24	27	30	0	0	0	0.001
Staph7	20	19	8	12	18	20	22	24	0	0	12	0.001
Staph8	0	0	0	0	0	0	0	0	0	0	10	0.001
Staph9	17	18	14	16	17	10	14	17	0	0	0	0.001
Staph10	16	14	18	21	24	23	25	29	0	0	12	0.001
Staph11	0	0	20	20	22	26	29	30	0	0	14	0.001
Staph12	17	20	15	14	18	20	20	16	0	0	16	0.001
Staph13	0	0	0	0	0	0	0	0	0	0	12	0.001
Staph14	14	13	15	16	17	20	21	20	0	0	14	0.001
Staph15	0	0	0	0	0	0	0	18	0	0	12	0.001
Staph16	0	0	0	0	0	0	0	0	0	0	10	0.001
Staph17	11	13	9	10	14	17	20	21	0	0	20	0.001
Staph18	0	0	0	0	0	0	0	0	0	0	0	0.001
Staph19	0	0	0	0	0	0	0	0	0	0	12	0.001
Staph20	0	0	0	0	0	0	0	0	0	0	14	0.001
Staph: *Staphylococcus aureus,* Van: Vancomycin, DMSO:Dimethyl sulfoxide												

Table 1: Zone of Inhibition (ZOI) mm in Ethanol leaf extract of *Xanthium strumarium*.

Additionally, the table 2 show the clinical isolates numbered 4, 5, 6, 7, 8, 13, 16, 18, 19, and 20 exhibited no response to the crude distilled water extract. The distilled water crude leaf extract of *Xanthium strumarium* also exhibited a maximum inhibition zone of 28 mm against clinical isolate number 2 at a concentration of 12.5 µg/ml, with no inhibition zones observed for clinical isolates 4, 5, 6, 7, 8, 13, 16, 18, 19, and 20 at all concentrations. These results are consistent with those of [22], who reported inhibition zones of 0, 0, 0, 6, 6, and 0 mm at concentrations of 50, 100, 150, 200, and 250 µg/ml, respectively, using distilled water crude leaf extract against *Staphylococcus aureus*. The differences in results may stem from variations in solvent preparation (methanol vs. distilled water) as well as the use of standard strains of *Staphylococcus aureus*.

Clinical isolates of bacteria	Concentration (µg/ml) Negative Positive								Control			
									Negative		Positive	
	0.5	1	1.5	2.5	5	7.5	10	12.5	DMSO	Ethanol	Van	P value
Staph1	0	0	11	15	16	18	20	22	0	0	12	0.001
Staph2	0	0	14	17	21	23	20	28	0	0	10	0.001
Staph3	0	0	0	0	11	14	13	21	0	0	19	0.001
Staph4	0	0	0	0	0	0	0	0	0	0	12	0.001
Staph5	0	0	0	0	0	0	0	0	0	0	12	0.001
Staph6	0	0	0	0	0	0	0	0	0	0	0	0.001
Staph7	0	0	0	0	0	0	0	0	0	0	13	0.001
Staph8	0	0	0	0	0	0	0	0	0	0	10	0.001
Staph9	0	0	0	11	15	16	18	23	0	0	0	0.001
Staph10	0	0	0	6	13	12	14	15	0	0	12	0.001
Staph11	0	0	10	9	5	11	15	14	0	0	14	0.001
Staph12	0	0	14	16	17	22	24	21	0	0	16	0.001
Staph13	0	0	0	0	0	0	0	0	0	0	12	0.001
Staph14	0	0	8	12	17	18	22	22	0	0	14	0.001
Staph15	0	0	0	10	15	11	0	15	0	0	12	0.001
Staph16	0	0	0	0	0	0	0	0	0	0	10	0.001
Staph17	0	0	9	14	12	14	18	19	0	0	20	0.001
Staph18	0	0	0	0	0	0	0	0	0	0	0	0.001
Staph19	0	0	0	0	0	0	0	0	0	0	12	0.001
Staph20	0	0	0	0	0	0	0	0	0	0	14	0.001
Staph: *Staphylococcus aureus,* Van: Vancomycin, DMSO:Dimethyl sulfoxide												

Table 2: Zone of Inhibition (mm) in Distilled water leaf extract of *Xanthium strumarium*.

In one-way ANOVA statistical analysis, significant differences were seen in the antibacterial activity among the bacterial isolates at different extract concentrations *($p < 0.05$)*. Tukey's HSD post hoc tests further confirmed that the ethanol extracts of *Xanthium strumarium* had significantly greater antibacterial activities when compared with the distilled water extracts *($p < 0.05$)*.

Transparency declaration

The authors declare that there is no conflict of interest relevant to this work.

Author Contributions

RS was involved in all aspects of this article and is the guarantor for the data. Sample collection was done by TA, EA, AB, ZR, AA, MA YA and technicians stuff performed laboratory procedures. RS and SA analyses the data and wrote the manuscript. RS, S.M.A and AA reviewed the manuscript.

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