In-vitro antioxidant and antibacterial activities of *Xanthium strumarium* L. extracts on methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*

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ABSTRACT

Background and Aims: The excessive and repeated use of antibiotics in medicine has led to the development of antibiotic-resistant microbial strains, including *Staphylococcus aureus* whose emergence of antibiotic-resistant strains has reduced the number of antibiotics available to treat clinical infections caused by this bacterium. In this study, antioxidant and antimicrobial activities of methanolic extract of *Xanthium strumarium* L. leaves were evaluated on methicillin-susceptible and methicillin-resistant *Staphylococcus aureus* (MRSA) spp.

Materials and Methods: Antiradical and antioxidant activities *X. strumarium* L. leaf extract were evaluated based on its ability to scavenge the synthetic 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and by the paired diene method, respectively, whereas the antimicrobial activity was assayed by the disc diffusion method.

Statistical Analysis: Data were subjected to analysis of variance following an entirely random design to determine the least significant difference at $P < 0.05$ using SPSS v. 11.5.

Results and Conclusions: The IC_{50} values of the extract were 0.02 mg/mL and 0.09 mg/mL for the antioxidant and DPPH-scavenging capacity, respectively. *X. strumarium* extract affected both methicillin-sensitive *Staphylococcus aureus* and MRSA, though antibacterial activity was more effective on methicillin-susceptible *S. aureus* spp. The antibacterial and antioxidant activities exhibited by the methanol extract may justify the traditional use of this plant as a folk remedy worldwide.

KEY WORDS: 1,1-diphenyl-2-picrylhydrazyl, antioxidant activity, methicillin-resistant *Staphylococcus aureus*, methicillin-sensitive *Staphylococcus aureus*, *Staphylococcus aureus*, *Xanthium strumarium L*

INTRODUCTION

Many medicinal plants are considered as important natural remedies for the treatment of various diseases. The excessive and repeated use of some synthetic drugs in modern medicine has led to the development of antibiotic-resistant microbial strains, including *Staphylococcus aureus*.[1] The emergence of antibiotic-resistant strains of this bacterium reduces the number of antibiotics available to treat clinical infections caused by this pathogen.[2] *S. aureus* is a highly variable pathogen with considerable impact on human health. It is responsible for a wide range of hospital and community-acquired infections globally, from skin infections and food poisoning to life-threatening conditions such as toxic-shock syndrome, endocarditis, pneumonia, bacteremia, and osteomyelitis.[3,4]

Xanthium strumarium L. is an annual plant belonging to the family *Asteraceae*. In Iran, *X. strumarium* is available between August and September. In many countries, different plant parts, especially fruit and root, are used as remedies. Various parts of this plant species were found to possess useful medicinal properties such as antitrypanosomal,[5] diuretic,[6] hypoglycemic,[7]

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anthelmintic,[8] antifungal,[9] antileishmanial,[9] antiulcerogenic,[10] and anti-inflammatory[11,12] activities, it is also known to inhibit proliferation of human cancer cells *in vitro*[13] and to exert a neuroprotective activity on the central nervous system.[14]

The chemical constituents of *X. strumarium* include phenolic compounds such as ferulic acids, chlorogenic acids and thiazolidinediones;[15] caffeic acid, 1,3,5-tri-*O*-caffeoyl quinic acid and 1,5-di-*O*-caffeoyl quinic acid;[16] isoprenoids as β-sitosterol and strumasterol;[17] monoterpene and sesquiterpene hydrocarbons;[17] xanthanolide sesquiterpene lactones[18] and triterpenoid saponins.[19] In addition, Srinivas *et al*.[20] reported, high levels of alkaloids, phenolic acids, and diterpenes and significant concentrations of saponins, glycosides, fixed oils, and phytosterols in *X. strumarium*.[20] The main aim of the present study was to carry out *in vitro* tests on *X. strumarium* from Iran by to assess the antioxidant and antimicrobial activities of methanolic leaf extracts.

MATERIALS AND METHODS

Plant material and extract

Leaves of *X. strumarium* were collected between August and September 2012 from the area of Hamun Lake of Zabol (31° 1′ 43″ N, 61° 30′ 4″ E), Sistan and Baluchestan Province, Iran. The plant was taxonomically identified by a botanist at the herbarium of Department of Botany, Shahid Beheshti University, Iran. 20 g of dried leaves were and extracted in 200 mL 85% methanol using a shaker water bath for 24 h at 25°C. After filtration with Whatman No. 1 filter paper, filtrate was concentrated by a rotary evaporator at 50°C for 30 min., to remove solvent from the extract. Solid extract was dissolved in 20 mL of distilled water. This working solution was used for all tests in this study.

Antioxidant activity

Antioxidant activity was determined by the paired diene method.[21] The antioxidant activity measured represents the capacity of the plant extract to inhibit the peroxidation of linoleic acid, in which the double bond is changed to a paired diene. Each extract sample (0.01-30 mg/mL) in methanol (100 μL) was blended with 3 mL of 10 mM linoleic acid (Sigma Chemical Co., St. Louis, MO, USA) to form an emulsion in 0.2 M sodium phosphate buffer (pH 6.6) in test tubes, and then placed in the dark at 37°C to stimulate oxidation. After incubation for 17 h, 7 mL of 70% methanol in deionized water was added, and the absorbance of the mixture was measured at 234 nm against a blank in a Hitachi U-2001 spectrophotometer (Tokyo, Japan). Antioxidant activity was measured as follows:

Antioxidant activity (%) = [(ΔA_{234} of control − ΔA_{234} of sample)/ΔA_{234} of control] ×100.

IC_{50} value (mg/mL) is the efficient concentration at which the antioxidant capacity was inhibited by 50%, and was gained by interpolation from linear regression analysis Analyses were repeated 3 times (technical replicates). α-tocopherol, butylated hydroxyanisole (BHA) and ascorbic acid (Sigma-Aldrich, USA) were used as standard controls.

Scavenging ability on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals

The scavenging ability on the synthetic (DPPH, Sigma) free radical, determined according to Shimada *et al.*,[22] is the capability of the extract to respond rapidly with DPPH radicals and to scavenge most DPPH radical molecules. The test was repeated 3 times. α-tocopherol, BHA and ascorbic acid (Sigma-Aldrich, USA) were used as standards. A volume of 5 mL of the methanolic extract (0.5-40 mg/mL) was mixed with 1 mL of methanolic solution containing DPPH radicals, resulting in a final concentration of 0.2 mM DPPH. The mixture was shaken vigorously, left to stand for 40 min., in the dark, and the absorbance was read at 517 nm against a blank. The scavenging ability was determined as follows:

Scavenging ability (%) = [(ΔA_{517} of control − ΔA_{517} of sample)/ΔA_{517} of control] ×100.

IC_{50} value (mg/mL) is the efficient concentration at which the antioxidant activity was inhibited by 50% and DPPH radicals were scavenged by 50%, and was gained by interpolation from linear regression analysis.

Bacterial isolates

The *S. aureus* strains used in this study were clinical isolates from patients with *S. aureus* infections, obtained from the Microbiological Laboratory of the Central Hospital in Zabol, Iran. Isolated were identified by biochemical (catalase, coagulase and DNase) and molecular tests. Isolated methicillin-resistant *Staphylococcus aureus* (MRSA) were identified by screening tests on Mueller-Hinton agar (MHA, Torlak, Berlin, Germany) complemented with 5% NaCl and 1 mg/mL oxacillin-impregnated disc.[23] The two strains used in this study were ATTC 25923 (MRSA) and PTCC 1341 (MSSA).

Disc-diffusion assay

Antimicrobial tests were carried out by the disc diffusion method using 100 µL of bacteria suspension (containing 2.0×10^8 CFU/mL of bacteria) dispersed on MHA in sterilized Petri dishes (60 mm in diameter). To the discs (6 mm in diameter, HI Media Laboratories Pvt. Ltd., Mumbai, India) placed on the inoculated agar, 50, 100, 200, and 300 µL of leaf extracts were added. The inoculated plates were maintained at 4°C for 2 h and later incubated at 37°C for 24 h. Antimicrobial activity was determined by measuring the zone of inhibition (mm) against the test bacterial (MRSA and MSSA) strains.

Statistical analysis

The extract was prepared in triplicate for antioxidant and antibacterial tests. Data were subjected to analysis of variance following an entirely random design to determine the least significant difference at $P < 0.05$, using statistical software package (SPSS, version 11.5, IBM Corporation, NY, USA). All results are expressed as mean ± standard deviation.

RESULTS

The results on antioxidant and antiradical activities of the tested extract are summarized in Table 1. The levels of both antioxidant and DPPH radical scavenging capacities are inversely correlated with their IC_{50} values. The IC_{50} values of antioxidant activity were 0.04, 0.06, 3.12, and 0.02 mg/mL for α-tocopherol, BHA, ascorbic acid and *X. strumarium* leaf extract, respectively. For the radical scavenging capacity, IC_{50} values were 1.01, 0.27, 4.01, and 0.09 mg/mL for α-tocopherol, BHA, ascorbic acid and *X. strumarium* extract, respectively. In both assays, activity of *X. strumarium* methanol extract was significantly higher than that of the three tested reference compounds ($P < 0.05$) [Table 1]. The results of antibacterial activity of the leaf extract are shown in Figures 1 and 2. Our result showed that inhibition zones for MSSA (PTCC 1341) bacteria were 12.11 ± 0.12 (f), 16.08 ± 0.14 (d), 23.06 ± 0.04 (b), and 26.00 ± 0.00 (a) mm at concentrations of 50, 10, 200, and 300 µL of plant extract, respectively ($P < 0.05$) [Figure 1]. The inhibition zones for MSSA isolates were 11.01 ± 0.03 (g), 14.03 ± 0.00 (e), 18.06 ± 0.14 (c), and 26.0.1 ± 0.02 (a) mm, at concentrations of 50, 10, 200, and 300 µL of plant extract, respectively ($P < 0.05$) [Figure 1]. Inhibition zones relative to MRSA (ATTC 25923) strain were 8.11 ± 0.00 (e), 12.11 ± 0.11 (c), 15.01 ± 0.00 (b), and 18.4 ± 0.07 (a) mm at concentrations of 50, 10, 200, and 300 µL of plant extracts, respectively ($P < 0.05$) [Figure 2]. Inhibition areas obtained for MRSA isolates were 6.4 ± 0.2 (f), 8.12 ± 0.01 (d), 12.2 ± 0.5 (c), and 17.8 ± 0.9 (b) mm, at concentrations of 50, 10, 200, and 300 µL of plant extracts, respectively ($P < 0.05$) [Figure 2]. Our results showed a dose-response correlation between the plant extract concentration and the inhibition of bacterial growth.

Table 1: IC_{50} values (mg/mL) of the *X. strumarium* leaf extract in two tests: Paired diene method and DPPH radical scavenging assay

Samples	Antioxidant activity	DPPH scavenging capacity
X. strumarium extract	0.02±0.00[d]	0.09±0.01[d]
α-tocopherol	0.04±0.04[c]	1.01±0.01[b]
BHA	0.06±0.00[b]	0.27±0.00[c]
Ascorbic acid	3.12±0.00[a]	4.01±0.00[a]

Results are mean±SD of three replicates; means with different letters within a column are significantly different ($P<0.05$; LSD). BHA: Butylated hydroxyanisole, *X. strumarium: Xanthium strumarium*, DPPH: 1,1-diphenyl-2-picrylhydrazyl, SD: Standard deviation, LSD: Least significant difference test at $P<0.05$

DISCUSSION

According to the European Antimicrobial Surveillance System, MRSA represents currently a huge burden for many healthcare institutions and it is by far the most significant antibiotic-resistant acquired pathogen worldwide.

In previous studies, ethanol extracts from leafs of *Eremophila alternifolia* (Myoporaceae), *Eremophila duttonii* R.Br. (Myoporaceae), *Amyema quandong* (Lindl.) Tiegh. (Loranthaceae) and from the stem base of *Lepidosperma viscidum* R.Br. (Cyperaceae), traditional Australian medicinal plants, showed antibacterial activity against MRSA.[24] Essential oils of *Thymus vulgaris* L. (Lamiaceae), *Eucalyptus globulus* Labill. (Myrtaceae)[25] and *Sinapis*

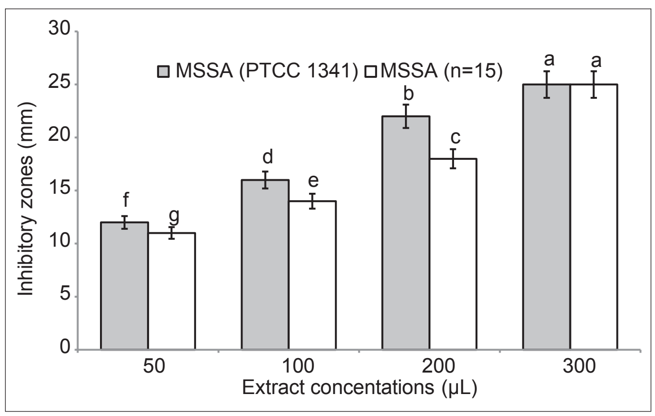

Figure 1: Antibacterial activity of *Xanthium strumarium* leaf extract against methicillin-sensitive *Staphylococcus aureus* (MSSA) standard (PTCC 1341) and the clinical isolate MSSA (n = 15) measured as diameter of the zone of inhibition (mm). Different letters indicate significant differences according to the least significant difference test at $P < 0.05$. All results are expressed as mean ± standard deviation

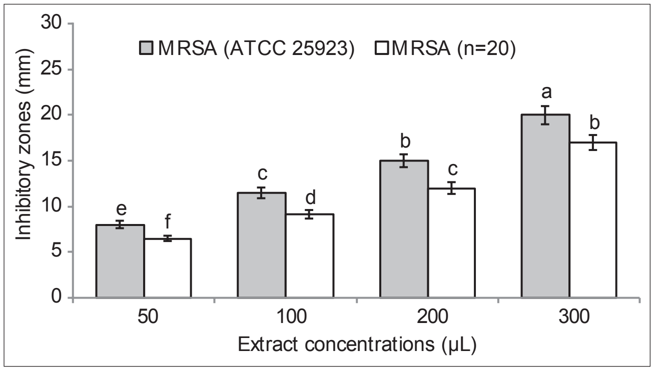

Figure 2: Antibacterial activity of *Xanthium strumarium* leaf extracts against methicillin-resistant *Staphylococcus aureus* (MRSA) standard (ATTC 25923) and the clinical isolate MRSA (n = 20) measured as diameter of the zone of inhibition (mm). Different letters indicate significant differences according to the least significant difference test at $P < 0.05$. All results are expressed as mean ± standard deviation

arvensis L. (Brassicaceae)[26] were also effective against clinical isolates of MRSA in disc diffusion assay. More recently, antimicrobial activity of essential oil of *Daucus crinitus* was reported with the same method, even if resistance or sensitivity to methicillin of *S. aureus* strains used were not specified by authors.[27] As regards *X. strumarium*, a methanol leaf extract exhibited a significant inhibitory activity on the growth of *S. aureus*,[28] and similar results were reported on chloroform and ethanol fractions from *X. strumarium* leaves.[29] Again, in both studies, the sensitivity to methicillin of the isolates was not specified.[28,29] Interestingly, chlorhexidine gluconate (1% and 4%) exerted a high biocide activity on both MSSA and MRSA.[30]

Our results showed that the maximum concentration of the extract (300 μL) was inhibitory both against MSSA and MRSA strains, with the highest inhibition zones of 25 mm and 20 mm, respectively. The inhibitory activity of the plant extract against MSSA was higher than against MRSA, i.e. in other words, *X. strumarium* exerted a higher antimicrobial effect on MSSA than on MRSA. Antibacterial activity of the methanol extract of *X. strumarium* leaves was previously reported by Srinivas *et al*.[20] and ascribed to the main components, alkaloids, phenolic acids, and saponins, phytochemicals with well-known antimicrobial properties.[30]

1,1-diphenyl-2-picrylhydrazyl assay is a sensitive method widely used to assess the free radical scavenging activity of plant extracts or isolated phytochemicals. DPPH is a stable free radical which accepts an electron or hydrogen radical to turn into a stable diamagnetic molecule.[31,32] This test possesses many advantages compared with other methods, such as good stability, sensitivity, feasibility, and handiness.[33] Antioxidant activity was expressed as the IC_{50} (mg/mL), which is the effective concentration at which the antioxidant activity was inhibited by 50%, gained by interpolation from linear regression analysis. Very recently, Kamboj *et al*.[34] have reported that, among all different organs of *X. strumarium*, leaf ethanol extract, with high levels of phenolics and the highest amount of flavonoids, showed the highest antioxidant activity. Finally, the relevant antioxidant and antiradical capacities of *X. strumarium* suggest a potential use for the prevention and treatment of diseases correlated with oxidative stress.

CONCLUSION

The antibacterial activity may be possibly attributed to the presence of phenolic acids, flavonoids, tannins and triterpinoids in the methanol extract, as reported in literature. The antibacterial and antioxidant activities exhibited by the methanol extract may justify the traditional use of this plant as folk remedy worldwide. *X. strumarium* has emerged as a relevant medicinal plant by virtue of its documented biological properties and possible applications.

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