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ANTIBACTERIAL POTENTIAL OF LEAF EXTRACTS OF XANTHIUM STRUMARIUM L. AGAINST MULTI-DRUG RESISTANT E. COLI & STAPH. AUREUS.

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ABSTRACT

The antibacterial activity of leaf extract of Xanthium strumarium Lin. belonging to family Asteraceae was evaluated in-vitro against most common multi-drug resistant clinical isolate Escherichia coli and Staphylococcus aureus by disc diffusion and agar-well diffusion method. Two solvents chloroform and methanol were used for extraction of bioactive compound from fresh leaves. The yield (%) of crude extract was 5.4 % and 6.3 % respectively. Antimicrobial potential of leaf extract was determined by measuring the zone of inhibition. It was concluded from the results that methanolic as well as chloroform extracts of leaf of Xanthium strumarium were quite effective in inhibiting the growth of E. coli and Staphylococcus aureus which is a serious human pathogen of UTI and wound infection. Decoction – Hot aqueous extraction also supported the antibacterial potential of this plant. Result also revealed that chloroform extract has more antibacterial potential than methanolic extract against Staphylococcus aureus. Therefore, the leaf extracts of this plant can be selected for further investigation to determine their therapeutic potential.

Keywords: Antibacterial, Clinical Isolate, Crude Extract, Zone Of Inhibition

Introduction

Antibiotics have been the heart of the modern healthcare, since their invasion into medicine in the 1940s. Their role has really expanding to the great extent. Now, however, once-treatable infectious diseases are becoming a hard to cure. There is a continuous increase in antibiotic resistance and emerging threats globally. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and GI tract disturbance. This situation forced scientists to search for new alternative antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new, safer, cheaper, and effective therapeutic agents for the treatment of infectious diseases.

Over three-quarters of the world population dependent mainly plant extract for health care. More than 30 % of plant species of the world were used for medicinal purposes. It has been estimated that plant drug constitute about 25% of total drugs in developed countries i.e. USA and about 80 % in developing countries like India. In India thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times. Natural plants have been seen as a valuable source of medicinal agents with proven potential of treating infectious diseases and with lesser side effects compared to the synthetic drug agents. There are over a million types of extracts derived from plants which can be used for multiple purposes.

Recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine. Plant-based antimicrobials represent a vast untapped source of medicines and further exploration of plant antimicrobials needs to occur. Antimicrobials of plant origin have enormous therapeutic potential. Researchers have proved that plant extract can be used to cure many health ailments and it has lesser side effects compared to other forms of medications. There have been lots of intensive studies on extracts and biologically active compounds isolated from plant species used for natural therapies or herbal medicine in last decade. In past few years, antimicrobial activity of plant extracts has been increasingly reported by several researchers. (Parekh J., 2006; Nair, R. and Chanda, S. 2007; Bharath, G. and Farzin, P., 2011; Chanda, S. et. al., 2011; Iyer, V. et. al., 2011). Extraction and phytochemicals screening of different plant part shows antibacterial, antifungal and antioxidant activity (Venkata & et. al., 2010; Prashant Tiwari & et. al., 2011; Vaghasiya, Y. and et. al., 2011)

Xanthium strumarium Lin. is a tropical American, erect herbs belongs to *Asteraceae widely distributed* in India. Various parts especially fruit (Stuart B. P. et. al., 1981; Hsu F. L. Chen et. al., 2000), leaves (Kim Y. S., 2003; Yadav R. N., 2007) and root (S. Ishwarya and Singh M. K., 2010) of this plant species were found to possess useful medicinal properties. In addition, P. Srinivas et.al., 2011and Rehman Ullah et.al., 2015 reported, a good source of alkaloids, phenols, diterpenes, saponins, glycosides, and phytosterols in *X. strumarium*.

Considering this facts & figures, it can be claimed that plants are the valuable sources for new safer, cheaper therapeutic compound and should be a special attention in research to develop new therapeutic agents urgently required in near future to combat resistant and emerging

pathogens. The main aim of the present study was to evaluate and determine the antibacterial potential leaf extracts of *Xanthium strumarium* Lin. against most common multi-drug resistant clinical isolate *Escherichia coli* and *Staphylococcus aureus.*

Materials and Methods:

Plant Material

Fresh diseased-free leaves of *Xanthium strumarium* were collected locally from Vyara, District Tapi, Gujarat, India. The plant was identified with the help of flora of Gujarat and confirmed by Dr. T. G. Gohil & Dr. Minoo Parabia. The leaves of *Xanthium strumarium* were washed thoroughly under tap water and then dried in an oven at 55°C for 24 hours. The dried plant material was pulverized to fine powder & the powder was stored in air tight bottle in a dark at room temperature.

Extraction

The dried powder (5 gm) was soaked separately in 50 ml of chloroform and methanol in Erlenmeyer flask. The flasks were covered with aluminum foil and allowed to stand in a dark for 72 hrs for extraction. These extracts were filtered through Whatmann filter paper no. 1 and evaporated at 55°C in an oven to get dark greenish residue (crude extract), which was stored at 4°C prior to use. These crude extract was further dissolved in DMSO to prepare the stock solution of 100 mg/ml. Decoction (Hot aqueous extract) - 1 gm of dried powder and 16 ml of dist. Water boiled to reduce the content up to ¼ parts and stored at 4°C prior to use.

Sources of clinical isolates

The antibacterial activity of leaf extract of *Xanthium strumarium* was tested against to most common multi-drug resistant *Escherichia coli* and *Staphylococcus aureus.* These bacteria were isolated from urine and pus sample of a patient suffering from UTI & wound infection respectively and identified biochemically. These isolated pathogens were stored in nutrient agar slants at 4°C.

Antibiogram of clinical isolates

Antimicrobial sensitivity testing of clinical isolates was carried out by WHO recommended Kirby-Bauer NCCLS modified disc diffusion technique. Antimicrobial sensitivity testing of control strain (*Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923) was also carried out. The Muller Hinton agar plate was seeded with control strain (from NCL, Pune) and clinical isolates (standard inoculum - McFarland standard 0.5 spread uniformly on Muller Hinton agar plate by spread plate technique). Sterile disc of known concentration of antibiotics (Himedia Lab.) was placed at sufficient distance on a plate aseptically. Plates were kept at 4˚ C for one hour and then incubated at 37˚ C for 24 hours.

Screening the Antibacterial activity

Screening for antimicrobial properties of crude extract was tested by agar well diffusion method. A nutrient agar plate was seeded with clinical isolates (100 µl inoculum spread uniformly over a by spread plate technique). A sterile stainless steel borer (7 mm) was used to make a well in each plate. These wells were filled with 40 µl of crude extract. Then plates were kept at 4˚ C for 1 hour & incubated at 37˚ C for 24 hours. In disc diffusion method (Bayer et. al. 1997), a nutrient agar plate was seeded with clinical isolates. Sterile disc (Himedia Lab.) was loaded with 10 µl of crude extract and placed at sufficient distance on a plate aseptically. Plates were kept at 4˚ C for one hour and then incubated at 37˚ C for 24 hours. Different aliquots of crude extract were also tested by agar well diffusion method.

Results and Discussion:

5 gm dried powdered were extracted in chloroform and methanol, the percentage recovery of crude extract were 270 mg (5.4%) & 315 mg (6.3%) respectively. Antibiogram of clinical isolates showed that *E. coli* was resistant to chloramphenicol, ciprofloxacin, cefixime, doxycycline, norfloxacin & tetracycline (06 out of 13) and *Staphylococcus aureus* was resistant to amoxyclave, ampicillin, ciprofloxacin, norfloxacin, azithromycin & erythromycin (06 out of 15, Figure 1). The both crude extract showed good activity against tested clinical isolates which was shown in Table 1 and 2. Chloroform extract showed significant activity than methanol extract. Result showed that crude extract has more activity against *Staph. aureus* then *E. coli.* Result of different aliquots of crude extract tested by agar well diffusion method which was shown in Figure 2 and 3. It showed that crude extract has antibacterial potential against *Staphylococcus aureus* but no result against *E. coli.*

***Table 1. Antibacterial activity of chloroform & methanol extract of leaves of* Xanthium strumarium L by well diffusion method.**

Clinical isolates	*Diameter of zone of inhibition (mm)*		
	Chloroform extract	*Methanol extract*	*Hot aqueous extract*
E. coli	*13*	*11*	--
S. aureus	*28*	*15*	*16*

Note: * 8 mm well was loaded with 40 µl of crude extract (100mg/ml).

***Table 2. Antibacterial activity of chloroform & methanol extract of leaves of* Xanthium strumarium L by disc diffusion method.**

Clinical isolates	*Diameter of zone of inhibition (mm)*		
	Chloroform extract	*Methanol extract*	*Amikacin 30 µg/disc*
E. coli	--	--	*24*
S. aureus	*18*	--	*25*

Note:** 6 mm sterile disc was loaded with 10 µl of crude extract (100mg/ml).

Figure 1. One of the plate of antibiogram of S. aureus.

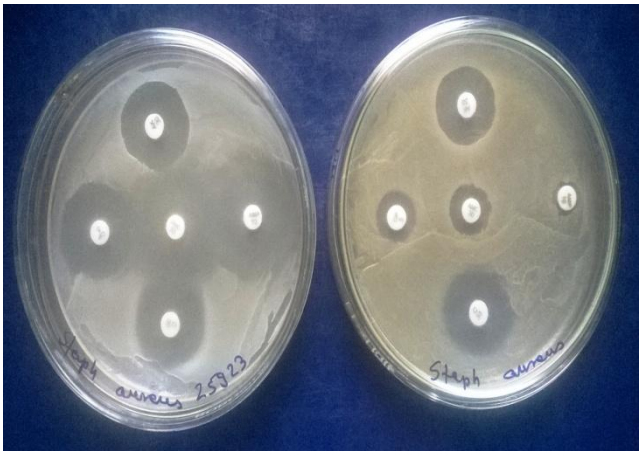
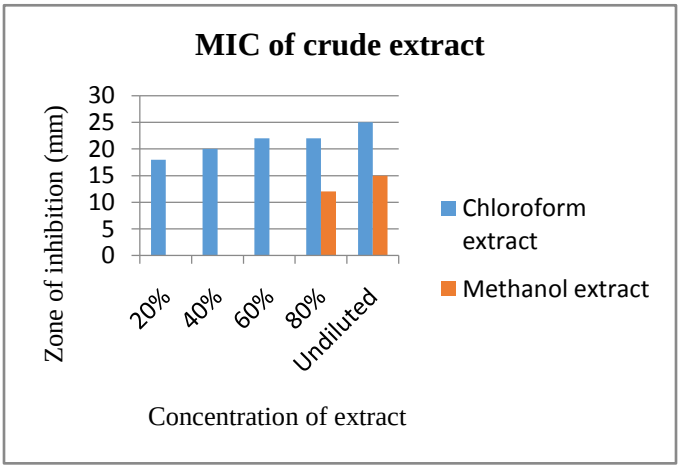


Figure 2. MIC of crude extract of leaves of *Xanthium strumarium L against S. aureus.*



Note: 8 mm well was loaded with 40 µl of 20%, 40%, 60%, 80% crude extracts (100mg/ml).

***Figure 3. MIC of crude extract* against S. aureus**

Conclusions:

It was concluded from the results that *Staphylococcus aureus* and *E. coli* are multi-drug resistant clinical isolates and chloroform as well as methanol extract of leaf of *Xanthium strumarium* were significant effect to inhibit the growth of multi drug resistant *Staphylococcus aureus* and *E. coli* which are considered as a serious human pathogen causing wound infection & UTI. Chloroform extract has more potential than methanol. Crude extract has significant antibacterial activity against gram +ve bacteria.

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