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Study of Antibacterial and Antifungal Potential of Leaf Extract of *Xanthium Strumarium*

Mohanish V. Wankhade

Department of Botany, Indira Gandhi Kala Mahavidyalaya, Ralegaon

Abstract: ***For the study of antibacterial and antifungal activities, leaf extract of xanthium was prepared in 100% aceton. Three bacteria, Staphylococcus aureus (ATCC No. 6838), Bacillus subtilis (ATCC No. 6633), and Pseudomonas aeruginosa (ATCC No. 15442), as well as two fungal strains, Candida albicans (ATCC No. 14053) and Aspergillus niger (ATCC No. 11414), were the subjects of an antimicrobial study using varying concentrations of Xanthium leaf extract (5 mg and 10 mg). The agar well plate method was used to determine the antimicrobial screening. The antimicrobial activity is estimated by comparing the zone of inhibition of growth of sensitive micro-organisms produced by known concentration of the isolated substance or extract or synthetic compound to be examined against a reference substance. Among the tested bacteria strains, Bacillus subtilis was the most sensitive to leaf extract of Xanthium while S.aureus and P. aeruginosa showed the least response. While studying the antifungal effect indicate that leaf extract of Xanthium may have moderate efficacy.***

Keywords: Antibacterial, Antifungal, Xanthium, ATCC, acetone, Zone of Inhibition

1. Introduction

Xanthium strumarium L. (rough cocklebur), is a species of annual plants belonging to the Asteraceae family. It is found to be problematic in agricultural field. Plant is also found to be dominating in roadsides and open dry pastures (Tiwari *et al*., 2005) Two new thiazinediones along with five known compounds were isolated from the fruits of *Xanthium strumarium* L. The structures of the two new compounds namely 7-hydroxymethyl-8,8-dimethyl-4,8-dihydrobenzol [1,4] thiazine-3,5-dione-11-*O*-*β*-D-glucopyranoside and 2-hydroxy-7-hydroxymethyl-8,8-dimethyl-4,8-dihydrobenzol [1,4] thiazine-3,5-dione-11-*O*-*β*-D-glucopyranoside, the five known compounds viz .xanthiazone , chlorogenic acid, ferulic acid , formononetin , and ononin were identified (Thin Han et al.,2006). The chemical composition of the essential oil (EO) from fresh cocklebur (*Xanthium strumarium* L.) leaves was investigated by GC-MS. The main compounds in the EO were *cis*-β-guaiene (34.2%), limonene (20.3%), borneol (11.6%), bornyl acetate (4.5%), β-cubebene (3.8%), sabinene (3.6%), phytol (3.1%), β-selinene (2.8%), camphene (2.2%), α-cubebene (2.4%), β-caryophyllene (1.9%), α-pinene (1.8%) and xanthinin (1.04%). (Javed Sharifi-Rad et al. (2015). Xanthatin and xanthinosin, 2 sesquiterpene lactones isolated from the burs of *Xanthiun strumarium* L. (cocklebur), showed moderate to high in vitro cytotoxic activity in the human cancer cell lines WiDr ATCC (colon), MDA-MB-231 ATCC (breast), and NCI-417 (lung) (Irving Ramirez-Erosa et al. 2007).

However, there were no antibacterial activities of acetonic leaf extract of *X.strumarium* against *Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa* and antifungal activities against *Candida albicans* and *Aspergillus niger* has been reported.

2. Material and Methods

Plant collection and preparation crude extract

Healthy plant leaves of Xanthium strumarium were collected from Ralegaon region in month of February 2025 from their natural habitat. Leaves were washed and dried in sunlight for 3 days. The dried leaves (30 gm) were grind and then macerate in 100% acetone and and left on a shaker for two consecutive days Then the extract was filtered and evaporated to dryness under reduced pressure using a Rotavapor at 45^0 C.

Collection of Microorganism

Three bacterial strains *Staphylococcus aureus* (ATCC No. 6838), *Bacillus subtilis* (ATCC No. 6633), *and Pseudomonas aeruginosa* (ATCC No. 15442), *as well as two* fungal strains*, Candida albicans* (ATCC No. 14053*) and Aspergillus niger* (ATCC No. 11414), were selected.

Stock culture: *Staphylococcus aureus* ATCC No-6538 *Bacillus Subtilis* ATCC No-6633

***Pseudomonas Aeruginosa* ATCC No-1544**

Had streaked a loopful of suspension of ATCC. 6538, ATCC-6633ATCC-1544 on two slants of pre-incubated nutrient agar. Incubated the slants at 30-35°C for 24 hours in an incubator. After incubation, picked up the growth from the incubated slant and inoculated it in 3 ml of saline solution, vortexing to prepare a uniform suspension. Adjusted the O.D. of the culture to approximately 60-70 % OD at 530 nm using sterile saline and a calorimeter. After adjusting the O.D., we stored the test organism in refrigeration at 60-70C.

Stock culture: *Candida albicans* ATCC No-14053 and *Aspergilus Niger* ATCC No-11414

Had streaked a loopful of suspension of ATCC.14053, ATCC-11414 on two slants of pre-incubated nutrient agar. Incubated the slants at 30-35°C for 24 hours in an incubator After incubation, picked up the growth from incubated slant and inoculated in 3 ml of saline solution and vortexing to prepare the uniform suspension. Adjusted the O.D. of the culture to approx. 60-70 % OD at 530 nm using sterile saline and calorimeter. After adjusting O.D., we stored the test organism in refrigeration at 2-80C

Antibacterial and antifungal test analysis

The agar well (Cup plate) diffusion method was used to test inhibition growth of bacteria and fungi. 15-20 ml of the inoculated nutrient agar was distributed five different sterile petri dishes having different strains of *Bacillus,S.aureu, Pseudomonas, A.niger* and *Candida*. The agar was left to set and in each of these plates, 3-4 mm in diameter, were cut using a sterile cork borer No. 6 and the agar discs were removed. Alternate cups in each plate were filled with 100 µl DMSO, 1mg solution A and labelled as control and standard, different concentration of extracts at 5mg and 10mg using microliter-pipette. The dishes left standing for 15-20 min. at 2-8°C. Then Incubated them for about 24-48 hours at the temperature 30-35°C for bacteria and 20-25°C for yeast and mould and zone of inhibitions were recorded.

3. Results and Discussion

The results of antibacterial and antifungal activity are given in table 1 and 2.

Table 1: Zone of inhibition of extract against different bacteria

S. No.	Sample	Concentration	Zone of inhibition (in mm)		
			S.aureus	*Bacillus*	*Pseudomonas*
1	Control		-		
2	Standard Streptomycin	1mg/ml	30	32	28
3	Sample 473 (Plant extract)	5mg/ml	05	07	06
		10mg/ml	08	17	09

The antibacterial evaluation of the sample 473 was conducted against *Staphylococcus aureus*, *Bacillus*, and *Pseudomonas*, with Streptomycin serving as the standard and control showing no activity. The standard drug Streptomycin (1 mg/ml) exhibited strong antibacterial activity with zones of inhibition measuring 30 mm for *S. aureus*, 32 mm for *Bacillus*, and 28 mm for *Pseudomonas*, confirming its effectiveness as a reference antibiotic. In comparison, the sample 473 showed dose-dependent antibacterial activity. At 5 mg/ml, the inhibition zones were 5 mm (*S. aureus*), 7 mm (*Bacillus*), and 6 mm (*Pseudomonas*). At an increased concentration of 10 mg/ml, the activity improved, with inhibition zones of 8 mm, 17 mm, and 9 mm against the respective bacteria. Among the tested strains, *Bacillus* was the most sensitive to sample 473, while *S. aureus* showed the least response. Although sample exhibited weaker activity compared to the standard, its dose-responsive behaviour indicates potential antibacterial properties, particularly against *Bacillus*.

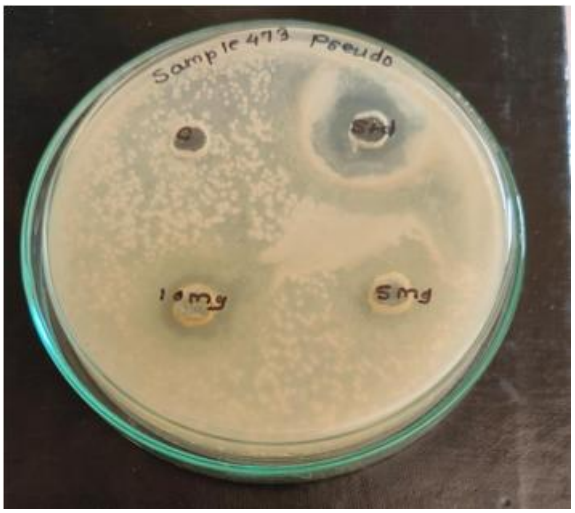
Table 1: Zone of inhibition of extract against different fungi

S. No.	Sample	Concentration	Zone of inhibition (in mm)	
1	Control		-	
2	Standard Streptomycin	1mg/ml	30	30
3	Sample 473 (Plant extract)	5mg/ml	06	04
		10mg/ml	08	07

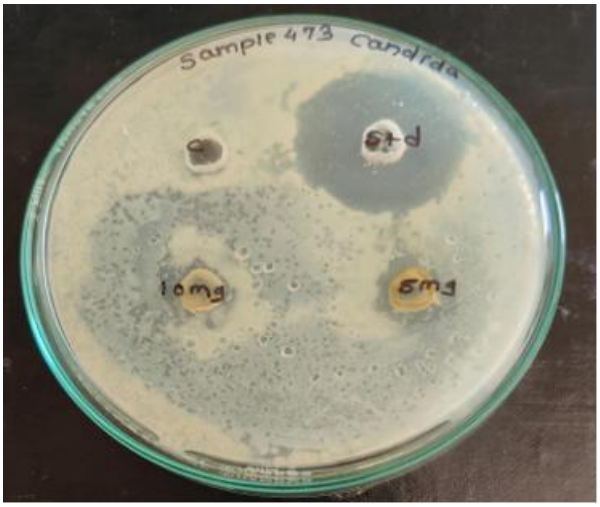
The antifungal activity of the sample was evaluated against *Candida albicans* and *Aspergillus niger*, using Fluconazole as the standard reference drug. The control showed no zone of inhibition, confirming the absence of inherent antifungal activity. Fluconazole (1 mg/ml) demonstrated strong antifungal efficacy, producing inhibition zones of 30 mm against *Candida albicans* and 20 mm against *A. niger*. In comparison, the sample 473 exhibited mild, concentration-dependent antifungal activity. At 5 mg/ml, sample 473 showed zones of 6 mm against *Candida albicans* and 4 mm against *A. niger*, which increased to 8 mm and 7 mm respectively at 10 mg/ml. While the antifungal effect of sample 473 was significantly lower than that of Fluconazole, the observed increase in activity with concentration suggests potential antifungal properties. These findings indicate that sample 473 may have moderate efficacy.

(a)

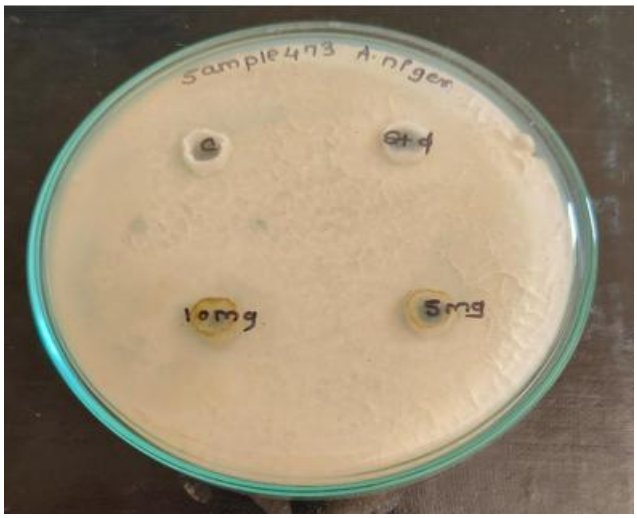
(b)



(c)



(d)



(e)

Figure 1: Antimicrobial activity of methanolic leaf extract of *Xanthium strumarium* against a) *S.aureus* b) *Bacillus* c) *Psuedomonas* d) *Candida albicans* e) *A.niger*

4. Conclusion

From the present study, it is concluded that, theacetonic leaf extract *of Xanthium strumarium* did not show promising efficacy against bacteria and fungi. It was also found that, increasing the concentration of the leaf extract resulted in moderate efficacy. It can also be suggested that, the concentration of the leaf extract should have been increased for higher efficacy.

5. Future Scope

This research will help to study the antibacterial and antifungal activities of cocklebur. In addition to the strains of bacteria and fungi studied, we can also experiment on other strains. Researcher can increase the amount of concentration of leaf extract to study high efficacy in near future.

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