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ANTIMICROBIAL ACTIVITY OF STEM BARK OF *MEDICAGO SATIVA* BY AGAR WELL DIFFUSION METHOD

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ABSTRACT

This paper reports the investigation results of anti-microbial activity of alfalfa stem bark extract against four bacterial strains namely *Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureas, E.coli* using agar well diffusion method. Inhibition zones were significantly different based on concentration of extract. The maximum inhibition was seen in 300µg/ml concentration of extract. First we prepared plant extracts using standard procedure and we have seen the anti-microbial activity by using minimal inhibitory concentration (MIC) and the maximum amount of inhibition was observed in gram positive bacterial strains.

Key Words: Agar well diffusion method, Anti-microbial activity, *Medicago sativa*.

INTRODUCTION

An antimicrobial agent that kills microorganisms or inhibits their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. For example, antibacterial agents are used against bacteria and antifungal are used against fungi. They can also be classified according to their function. Antimicrobial agents that kill microbes are called *microbicidal* those that merely inhibit their growth are called micro biostatic. Antiseptics help reduce infection during surgery. Disinfectants such as bleach are nonselective antimicrobials. They kill a wide range of microbes and are valuable for cleaning inanimate surfaces to prevent the spread of illness, but they are generally not medicinal (Fay PK and Duke WB, 1977).

Chemistry of Medicago sativa

Dried alfalfa leaves are ground and sold as tablets or powder for use as nutritional supplements. Leaf tablets are rich in protein, calcium, trace minerals, carotene, vitamins E and K, and numerous water-soluble vitamins. A steroidal saponin fraction composed of several factors (soyasapogenols, hederagenin, medicagenic acid) is believed to play a role in the hypocholesterolemic and hemolytic activity of the leaves and sprouts (Masaoka Y *et al*., 1990).

Saponin glycosides of alfalfa

Saponins (2–3%) that on hydrolysis yield the aglycones medicagenic acid, soyasapogenols A, B, C, D, and E, and hederagenin and the glycones glucose, arabinose, xylose, rhamnose, galactose, and glucuronic acid; sterols (β-sitosterol, α-spinasterol, stigmasterol, cycloartenol, and campesterol, with β-sitosterol.

MATERIALS AND METHODS

Bacterial Cultures

Escherichia Coli, Staphylococcus Aureus, Pseudomonas Aureus, Bacillus were obtained from Dept. of Microbiology, Osmania University, Hyderabad.

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Media employed

Nutrient Agar The bacteriostatic property of the compounds was tested by agar well-diffusion method.

Preparation of Antibacterial Solution

All the compounds were dissolved in sterile distilled water. Proper drug controls were used.

Compound was taken at concentrations of 100, 200, and 300 µg/ml for testing antibacterial activity (Dzubenko, N.N.,). The compound diffused into the medium produced a concentration gradient. After the incubation period, the zones of inhibition were measured in mm (Al-Saadawi IS and Rice EL, 1982; Al-Juboory BK, 1978; Blum U *et al*., 1985).

Test cultures

Following common standard strains were used for screening of antibacterial activities:

- *Staphlococcus aureus,*
- *Bacillus subtillus,*
- *Escherichia coli,*
- *Pseudomonas aeruginosa*.

Inoculum preparation

The inoculum was standardized at 1x 10^6 CFU/ml comparing with turbidity standard (0.5 MacFarland tube)

Swabs preparation

A supply of cotton wool swabs on wooden applicator sticks was prepared to spread the culture. They were sterilized in tins, culture tubes, or on paper, either in the autoclave or by dry heat (Dzubenko NN and Petrenko NI, 1971; Shirata A *et al*., 1983).

Experimental procedure

1) The plates were inoculated by dipping a sterile swab into inoculums. Excess inoculum was removed by pressing and rotating the swab firmly against the side of the tube, above the level of the liquid.
2) The swab was streaked all over the surface of the medium three times, rotating the plate through an angle of 60^o after each application. Finally the swab was passed round the edge of the agar surface.
3) Ditch the bore in plate. Add the extracted compound solution into the well.
4) The plates were placed in an incubator at 37^oC within 30 minutes of preparation for bacteria.
5) After 24 hrs incubation for bacteria, the diameter of zone (including the diameter disc) was measured and recorded in mm. The measurements were taken with a ruler, from the bottom of the plate, without opening the lid (Abdul-Rahman AAS and Al-Naib FAG, 1986).

RESULTS

The plates were incubated overnight at 37^0C. Antimicrobial activity was determined by measuring the diameter of zone of inhibition around the colonies (Blum, U, 1985). For each bacterial strain, controls were maintained where pure solvents were used instead of the extract.

Antimicrobial Activity against alfalfa extract (Inhibition zone in mm)

Agar well diffusion method:

All the extracts were dissolved in DMSO (1%) to get a concentration of 250µg/ml.

↓

Agar plates were used for the study using well plate method.

↓

The microorganisms were inoculated on the agar medium by spread plate technique.

↓

Four wells were bored in each plate and 100 µl of the extract samples were added in the well

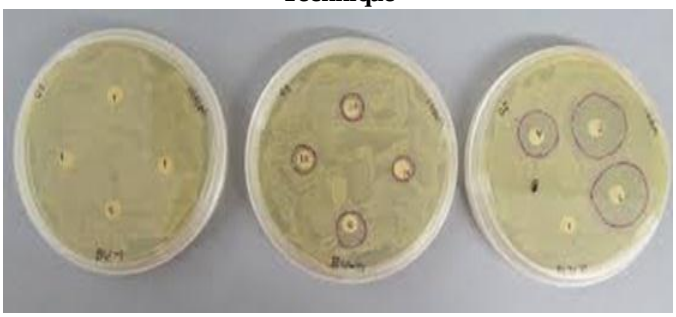
↓

The inoculated plates were incubated at 37°C for 24 h.

↓

Antimicrobial activity measured by the zone of inhibition against the test Organisms Ciprofloxacin at the concentration of 1mg/ml (50 µl/well) was used as standard.

Fig 1. Showing agar well diffusion method Agar Diffusion Technique



Staphlococcus aureus, Escherichia coli, Bacillus subtillus

Fig 2. Zone of Inhibition of various bacterial cultures

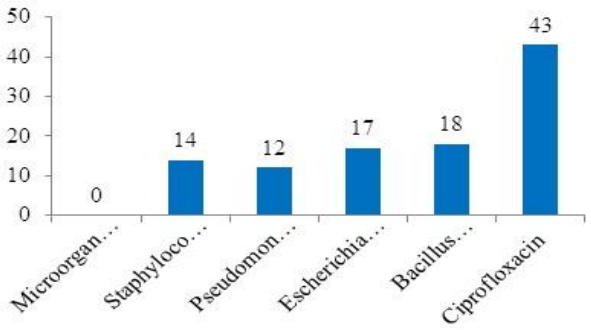


Table 1. Graph showing the Zone of Inhibition of various bacterial cultures

Drugs	Microorganism	Zone of inhibition (mm)
Methanolic extract of stem bark of *Medicago sativa* 300 µg/ml	*Staphylococcus aureus*	**14**
	Pseudomonas aureus	**12**
	Escherichia coli	**17**
	Bacillus subtillis	**18**
***Ciprofloxacin* 50 µg/ml**	*Staphylococcus aureus*	**43**
	Pseudomonas aureus	
	Escherichia coli	
	Bacillus subtillis	

CONCLUSION

Our studies showed that the alfalfa has good antimicrobial activity towards gram positive bacteria. The maximum inhibitory zone was observed in *Bacillus subtilis and E.coli* MIC of alfalfa extraction on *Bacillus subtilis* were 18 mm/µg and *E.coli* was 17 mm/ug. Our future studies are to check for the effectiveness of the alfalfa stem bark as nutraceutical product in animal models and human volunteers.

REFERENCES

Abdul-Rahman AAS and Al-Naib FAG. The effects of Bermuda grass (*Cynodondac-tylon* L. Pers.) on germination and seedling growth of cotton and three weed species.*J. Agric. Water Resour. Res.* 1986; 5:115–127.

Al-Juboory BK. *Imerata cylindrica* (L.) Beauv. Ecotypes in Iraq, some physiological aspects, and control by herbicides. Ph.D Dissertation. University of Baghdad, 1978.

Al-Saadawi IS, and Rice EL. Allelopathic effects of *Polygonium avicular* L. 1. Vegetational patterning. *J. Chem. Ecol.* 1982; 8: 993–1009.

Blum U, Dalton BR and Shann JR. Effects of various mixtures of ferulic acid and some of its microbial metabolic products on cucumber leaf expansion and dry matter in nutrient culture. *J. Chem. Ecol.* 1985; 11: 619–641.

Dzubenko NN and Petrenko NI. On biochemical interaction of cultivated plants and weeds, Grodzinsky AM (ed.). Physiological-Biochemical Interactions in Phy-tocenosis, Naukova Dumka, Kiev (in Russian), 1971: 2: 60–66.

Fay PK and Duke WB. An assessment of allelopathic potential in *Avena* germplasm.*Weed Sci.* 1977; 25: 224–228.

Masaoka Y, Kojima M, Sugihara S, Koshino M, Koshino H and Ichihara. 14th *Int. Ntn. Congr. of Soil Sci.* 1990; PIII-235-236.

Shirata A, Takahashi K, Takasugi M, Nagao S, Ichikawa S and Ueno S. *Bull. of Sericultural Exp. stn.* 1983; 28: 793–806.