Research Article

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Antimicrobial screening of Alfalfa(*Medicago sativa*) in various bacterial strains

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Abstract:

According to a recent report nearly 42 per cent Indian kids are malnourished and stunted. The aim of our project was to develop nutraceutical products using alfalfa as one of the components to overcome under nourishment particularly among women and children . The seeds of alfalfa (*Medicago sativa*) contain more nutritional property when compared with other leguminous seeds. The alfalfa seeds contains 18.9% of protein when compare with egg (13.1%), milk(3.3%),beef(16.5%). This paper reports the investigation results of anti microbial activity of alfalfa seed extract against five bacterial strains namely *Bacillus licheniformis*, *Pseudomonas aeruginosa, Lactococcus lactis, Klebsiella pneumonia*, *Bacillus cereus* using well diffusion method . . Inhibition zones were significantly different (P< 0.001) based on concentration of extract. The maximum inhibition was seen in 300µg/ml concentration of extract. The minimal inhibitory concentration(MIC) of alfalfa extract to *Bacillus licheniformis*, *Pseudomonas aeruginosa Lactococcus lactis, Klebsiella pneumonia*, *Bacillus cereus* were also determined The maximum amount of inhibition was observed in gram positive bacterial strains.

Keywords: Antimicrobial, Alfalfa, Under nutrition, Canavanine sulfate.

Introduction

As a social concern oriented and need of the hour development this project aims to strengthen the population of under nourished children in India who account for nearly 42 % as per the recent survey by the Nandi Foundation in their Hunger and Malnutrition (HUNGaMA) report[1]. Under nutrition is a consequence of consuming too few essential nutrients or excreting them more rapidly than they can be replaced**.** Alfalfa is a perennial plant which belongs to family-legume(Leguminoseae), it grows up to 2-3 feet tall, it has smooth and erect stem ,Leaves. .are pinnately trifoliate, Flowers are in racemes form and its purple-violet in colour, the flowering will be at June-August, seeds are spirally-coiled in form.it is also called as “Father of all Plant”. Until now alfalfa seeds are not used widely for human consumption for increasing the nutritional level because of the presence of canavanine sulfate. It is most commonly used for cattle and horse feed. By reducing the presence of canavanine sulfate by proper laboratory methods, we can use *Medicago sativa* (alfalfa) seeds for daily consumption as a good provider of nutrition

1.1. Health benefits of alfalfa sprouts*:*

Sprouted alfalfa seeds contains saponins, it is toxic to red blood cells only in vitro(outside of the body in a test tubes),but in human consumption it is harmless and it has many health beneficial properties like anti-inflammatory, immune-stimulating activity, anti-tumor activity. Saponins appear to be beneficial, being responsible for major part of cholesterol lowering effect of legumes. Saponins have a direct stimulatory effect on the immune system[2.] Saponins inhibit cancer cells in many ways. Saponins will fight against the fungal, microbial, viral infections. Presence of saponins in alfalfa sprouts will destroy tumor causing cells particularly it is more effective in lung and blood cancer. It gives more immunity for stomach**.** Alfalfa sprouts have about 8% saponins content according to commercial sprout growers. Interestingly, the sprouting increases the saponins content of the alfalfa sprouts 450% to the level of that in the alfalfa seed. Saponins bind with bile acids. Some large intestinal bacteria converts bile into highly carcinogenic substance where, bile that binds with saponins prevent the formation of toxin[3].

Materials and Methods:

The laboratory methods include sprouting, autoclaving, roasting and powdering. Sprouting is a method to reduce the canavanine sulfate from the seeds. After sprouting of seeds the sprouted seeds were autoclaved at 121˚C. Roasted and powdered and processed for further use. After powdering of sprouted alfalfa seeds the soxhlet extraction is done in order to get a concentrated extract. The extraction was done with the help of powder of alfalfa sprouts by using three successive solvents namely Chloroform, Petroleum ether, Ethanol. The recovery % of Petroleum ether, Ethanol and Chloroform were 12%, 8% and 14% respectively. With the help of different concentration of extract the antimicrobial studies are done to check the antimicrobial activity of alfalfa.

Results and Discussion:

Antimicrobial Activity:

Antimicrobial activities were checked using five bacterial strains namely *Bacillus licheniformis*, *Pseudomonas aeruginosa,Lactococcus lactis, Klebsiella pneumonia*, *Bacillus cereus* , Antimicrobial activity of three concentrations (50µg, 100µg, 200µg, 300µg) /ml of soxhlet extracts of alfalfa(*Medicago sativa*) were assessed by measuring inhibition zones, using well diffusion method[4]. Inhibition zones were significantly different (P< 0.001) based on concentration of extract. The maximum inhibition was seen in 300µg/ml concentration of extract. The minimal inhibitory concentration (MIC) were determined.MIC of alfalfa extract to *Bacillus licheniformis*, *Pseudomonas aeruginosa Lactococcus lactis, Klebsiella pneumonia*, *Bacillus cereus were 1.2,* 0.9 , 0.8, 0.7 and 1 cm/µg respectively. Alfalfa extract had better antimicrobial activity to *Bacillus licheniformis.* By using double dilution method and blood plate identification, the minimal inhibitory concentrations(MIC) were determined.MIC of alfalfa extract to *P.aeruginosa* and *S.dysgalactiae* were 0.674 and 1.347 mg/ml respectively.MIC of alfalfa extract to *E.coil* and *S.aureus* were both 2.695 mg/ml. Alfalfa extract had better antibacterial activity to *P.aeruginosa* and *S. dysgalactiae*. But the antibacterial effects on *E.coil* and *S.aureus* were ordinary[5-9]

Summary and conclusion:

The toxic substance canavanine sulfate from alfalfa seeds are removed by applying some laboratory process like sprouting, air drying, roasting and it is made as powder by grinding for human consumption for increasing the nutritional level. Alfalfa seeds are not to be taken by pregnant women it is restricted even though in the absence of canavanine sulfate. Thus the cost effective nutraceutical product containing alfalfa along with other cereals and pulses would serve the humanity to fight under nourishment in an easy and economical way and the alfalfa has good antimicrobial activity towards gram positive bacteria. The maximum inhibitory zone was observed in *Bacillus licheniformis* ,MIC of alfalfa extract on *Bacillus licheniformis* were 1.2 cm/µg Our future studies are to check the effectiveness of the nutraceutical product in animal models and human volunteers.

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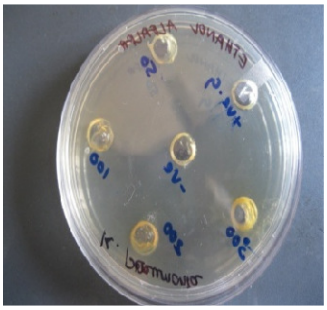
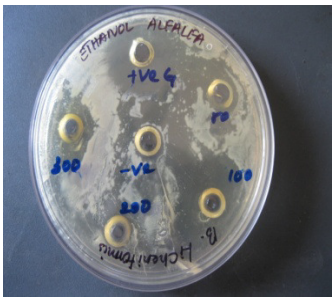
Antimicrobial Results

Lactococcus lactis

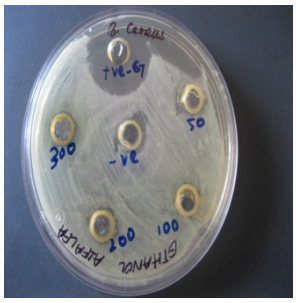


Klebsiella pneumonia



Bacillus licheniformis

Pseudomonas aeruginosa



Bacillus cereus

Table 1: Antimicrobial Activity (Inhibition zone in mm)

Bacterial strains used	C1 5mg/ ml	C2 10mg/ ml	C3 20mg/ml	C4 30mg/ml	Negative control Ethanol 0.1ml	Positive control Gentamycin 1mg/ml
Bacillus licheniormis	**10**	**7**	**7**	**12**	**10**	**17**
Pseudomonas aeruginosa	**8**	**7**	**6**	**9**	**7**	**15**
Lactococcus lactis	**4**	**4**	**5**	**8**	**6**	**12**
Klebsiella pneumonia	**3**	**3**	**4**	**7**	**5**	**12**
Bacillus cereus	**6**	**6**	**7**	**10**	**5**	**13**

References:

1. Citizen's Alliance ,Nandi Foundation, Mahindra & Mahindra et al . 2012 42 per cent Indian kids malnourished, stunted: report CNN-IBN Jan 10, 2012 at 04:58pm IST #Malnutrition report #Malnutrition #Manmohan Singh #HUNGaMA report 1524
2. Peter Cheeke,2008 www.sprout-as-medicin.com,
3. Sara Ding,copyright 2005-2011 www.juicing-for-health.com,
4. Marjorie Murphy Cowan* , Oct 1999,Plant Products as Antimicrobial Agents, Department of Microbiology, Miami University, Oxford, Ohio 45056, American Society for Microbiology.vol 12,pp 564-582.
5. Pinarosa Avato[1,*], Rossella Bucci[1], Aldo Tava[2], Cesare Vitali[1], Antonio Rosato[1], Zbigniew Bialy[3], Marian Jurzysta[3],"18 APR 2006 Antimicrobial activity of saponins from *Medicago* sp.: structure-activity relationship, African journal of Biotechnology,vol.10(55),pp.11433-11441.
6. Sarada L., Nandiwada[1], Gerry P. Schamberger, H. William Schafer, Francisco Diez- Gonzalez Department of Food Science and Nutrition, University of Minnesota, 1334 Eckles Avenue, St. Paul, MN 55108, USA, 15th June2005, Characterization of an E2-type colicin and its application to treat alfalfa seeds to reduce *Escherichia coli* O157:H7 , International journal of food microbiology,vol.93,Issue3.pp 267-279.
7. Steven Pao*, Mahmoud F. Khalid, and Aref kalantarivirginia, 2006, State University, Agricultural Research Station, P.O. Box 9061, Petersburg, VA 23806, USA, , Inhibiting the Growth of Bacillus cereus in Raw Sprouts and Cooked Rice using Red Clover Seeds, Internet Journal of Food Safety, Vol.8,pp.44-47.
8. Wyman-Simpson,C. L. , G. R. Waller, M. Jurzysta, J. K. Mcpherson and C. C. Young, 1991,Biological activity and chemical isolation of root saponins of six cultivars of alfalfa (Medicago sativa L), kluwer academic publisher.vol 135.pp.1,83-94.
9. Xiao-kang LU et al(Gansu Agricultural University,Lanzhou,Gansu 730000), 2008, Study on the Antibacterial Activity of Alfalfa Extract, Journal of Anhui agricultural science,vol 23,pp.31-47