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ANALYSIS OF ANTIMICROBIAL PROPERTIES OF DIFFERENT TYPES OF SEEDS AGAINST PATHOGENIC MICROORGANISMS FOR HERBAL DRUGS FORMULATION

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ABSTRACT

Antimicrobial assay showed promising evidence for the antibacterial and antifungal effect of seeds of flax, pinenuts (chilgoza), lotus, pumpkin and figs against three bacterial and two fungal pathogens. The plant extracts have great potential as antibacterial compounds against enteric pathogens and they can be used in the treatment of various infections. It is hoped that this study would lead to the founding of some compounds that could be used to formulate new and more potent natural herbal drugs. The study shows that the Ayurveda compound Vachea is also a great potent natural drug as its exhibits largely antimicrobial activity.

Key words: Antibacterial, Pathogens, Herbal drugs, Infections, Phytochemicals

INTRODUCTION

Plants have long provided manhood with a foundation of medicinal agents, with natural metabolites, once serving as the source of all herbal drugs **[1].** Dependence on plants as the source of medicine is prevalent in developing countries where traditional medicine plays a major role in health care **[2, 3].** The rural population of a country is more disposed to traditional ways of treatment because of its easy availability and cheaper cost **[4].** Herbal therapy, although still an unrecorded science, it is well recognized in some cultures and traditions, and has become a way of life in almost 80% of the people in rural areas, especially those in Asia, Latin America and Africa **[5].** Plants, which form the strength of traditional medicine, have in the last few eras been the subject of very strong pharmacological studies. This has been taken about by the acknowledgement of the value of medicinal plants as potential sources of new compounds of therapeutic value and as sources of lead compounds in drug development. Besides, there also exists a very large market of minimally processed medicinal plant parts especially in Europe and America, which are usually dispensed as over-the-counter medication **[6].** Medicinal higher plants have been used extensively as a source for numerous active constituents for treating human diseases and they, as well, have high contain of therapeutic value **[7].** Despite the great progress in human medicine, infectious disease caused by bacteria, viruses and fungi are still a serious threat to public health. The worldwide increase in multidrug resistance of pathogenic bacteria has led to an urgent need for identifying alternative strategies to counter bacterial infection. The latest research has been focused on identifying the potential antimicrobial agent from the natural resources. Infectious disease caused by bacteria, viruses and fungi are still a serious problem in public health. It is estimated that over 90% of *Staphylococci, pneumococci* and *entercocci* are resistant to antibiotics. There has been reported the increasing prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA), β-lactam and macrolide resistant *pneumococci*, and glycopeptide-resistant *enterococci* **[8].** The antimicrobial activity of plant extracts has been known for many years, as plants are known to produce useful antimicrobial phytochemicals **[9].**

Seeds have been the objects of many different types of research. They are quantitatively most important parts of human diet (pulses, cereals) so nutrionists as well as molecular biologists and biochemists demanded detailed knowledge of their chemical composition, biochemical, molecular mechanisms and antimicrobial activity involved in reserve deposition. Different seeds like flax seeds, pine nuts seeds (chilgoza), lotus seeds, pumpkin seeds and fig seeds were used to determine antibacterial and antifungal activities against different pathogens. Two Ayurvedic compounds Vacha and Lodhra, were also used for determining antibacterial and antifungal activity.

Material Methods

Clinical bacterial strains

The bacterial strains used in the study are S. *typhi, K. pneumoniae,B. subtilis.* The fungal strains used are A. *niger* and *C albicans.*

Collection of seeds

Seeds like flax seeds, pine nuts (chilgoza) seeds, lotus seeds, fig seeds and pumpkin seeds as well as ayurvedic compounds Vacha and Lodhra were purchased from local market Bareilly (UP), India.

Seeds extract

Bioactive compounds from various seeds were obtained by dissolving the above mention seeds in solvents like Distilled water, Petroleum ether, Acetone and Ethyl acetate and keeping the seeds in solvents for 48 hours. The liquid mixture was then filtered by using filter paper and the residue was then dried in an oven at 50˚C for 1-2 days. The dried seeds extract was scraped and dimethyl sulphoxide was added for preservation and it was stored at 4˚C prior extraction **[10, 11].**

Broth Dilution Assay

In this method gradual increasing volumes of seeds extract and bacterial cultures were added to the test tubes to know the inhibitory concentration. The tubes were incubated at 37˚C for 18-24 hours. The tubes were examined for visible turbidity and optical density of cultures was determined at 620 nm using nutrient broth as control. The lowest value of MIC of the tube was recorded as result **[12, 13].**

Antibiotic sensitivity test

Antimicrobial susceptibility testing was done using the well diffusion method to detect the presence of anti-bacterial or anti-fungal activities of the plant samples **[14, 15].** All the media plates were prepared with nutrient agar(in case of antibacterial test) and potato dextrose agar (PDA).After agar solidification a sterile swab was used to evenly distribute bacterial or fungal culture over the appropriate medium as stated previously. Wellswere then created and a pipette was used to place 50 µl of the crude seeds extract of flax seeds, pinenuts, lotus seed, pumpkin seeds, fig seeds and lodhra as well as vacha. The seedsextracts are taken as positive control against pathogens such as K. *pneumoniae*, B. *subtilis*, S. *typhi*, A. *niger* and *C. albicans.* The plates were incubated at 37˚C for 24 hours after which they were examined for inhibition zones **[16, 17, 18].**

Results

Collection of seeds

The respective seeds were collected from the local shops in Bareilly.

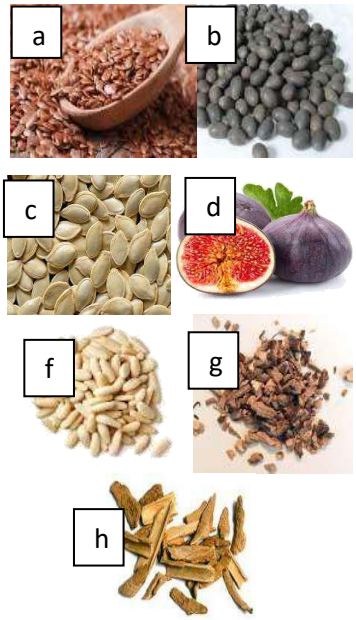

Figure 1: The seeds of a:*Linumusitatissimum L.* (Flax seeds) b: *Nelumbonucifera* (lotus seeds) c: Cucurbitamoschata (pumpkin seeds) d: *Ficuscarica* L. (Fig seeds) e: Pine nuts (chilgoza) f: *Acoruscalamus*L. (Vacha) g: Lodhra.

Antimicrobial screening

Table 1: Antimicrobial screening of tetracycline and Fluconazole as positive control against bacterial and fungal pathogens.

Pathogens	Zone of Inhibition (mm)	
	Tetracycline	Fluconazole
St	14	-
Kp	20	-
Bs	18	-
An	-	20
Ca	-	13

St: *S. typhi, Kp: K. pneumoniae, Bs: B. subtilis, An: A. niger, Ca: C. albicans*

Antibacterial activity of seeds extracts against bacterial strains

From the below data it is clear that the seeds extract taken against different bacteria cultures shows antibacterial property by the formation of zone of inhibition. In pinenuts seeds extract partial zones of inhibition were observed whereas in lotus seeds and fig seeds prominent zones of inhibition were observed. Pumpkin seeds also showed inhibitory effect against different bacterial cultures. The ayurvedic compound Lodra exhibited no such antibacterial activity as no zones of inhibition were observed; whereas in Vacha prominent zones of inhibition were observed for acetone and ethyl acetate. No zones formation was seen in water and petroleum ether in Vacha.

Table 2: Antibacterial screening of seeds extracts against bacterial cultures.

Seeds	Pathogen	Zone of inhibition (mm)			
		W	A	P	E
Flax	*St*	10	16	14	12
	Kp	9	15	15	13
	Bs	13	13	16	12
Pine nuts	*St*	10	11	9	9
	Kp	11	11	10	8
	Bs	11	11	11	11
Lotus	*St*	17	15	12	11
	Kp	16	17	13	11
	Bs	15	15	11	0
Pumpkin	*St*	0	13	15	14
	Kp	0	15	16	12
	Bs	0	15	16	12
Figs	*St*	17	14	0	0
	Kp	16	13	0	0
	Bs	17	14	0	0
Lodhra	*St*	0	0	0	0
	Kp	0	0	0	0
	Bs	0	0	0	0
Vacha	*St*	0	19	0	18
	Kp	0	20	0	18
	Bs	0	19	0	18

St: *S. typhi, Kp: K. pneumoniae, Bs: B. subtilis, W: water, A: acetone, Pe: petroleum ether, Ea: ethyl acetate.*

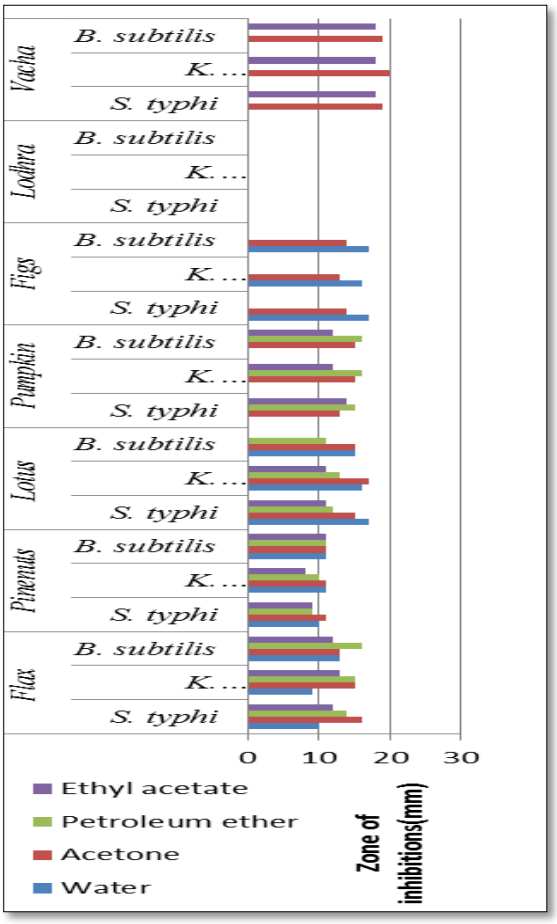

Figure 2: Graphical representation of antibacterial screening of seeds extracts against bacterial pathogens.

Antifungal activity of seeds extracts against fungal strains

The seeds extract were also tested against differentfungal strains to determine their antifungal activity. Most of the seeds extract exhibited antifungal activity like in fig seeds andlotus seeds; the zones of inhibition were observed. Pinenuts seeds extract again showed partial zones of inhibition Antifungal activity was less observed in pumpkin seeds for water and ethyl acetate. No zones formation was observed in flax seeds for water. The ayurvedic compound Lodhra showed no zone formation against fungal strains i.e the fungal strains *A .niger* and *C. albicans* were resistant against Lodhra.

Table 3: Antifungal screening of seeds extracts against fungal cultures

Seed	Pathogens	Zone of inhibition (mm)			
		W	A	Pe	Ea
Flax	*An*	0	15	16	17
	Ca	11	13	16	14
Pine nuts	*An*	0	0	12	13
	Ca	0	13	12	12
Lotus	*An*	0	20	14	16
	Ca	0	23	16	0
Pumpkin	*An*	0	18	20	0
	Ca	0	0	16	0
Figs	*An*	13	18	13	14
	Ca	12	23	12	18
Lodhra	*An*	0	0	0	0
	Ca	0	0	0	0
Vacha	*An*	14	21	0	16
	Ca	15	23	15	18

Ca: C albicans, An: A. niger, W: water, A: acetone, Pe: petroleum ether, Ea: ethyl acetate.

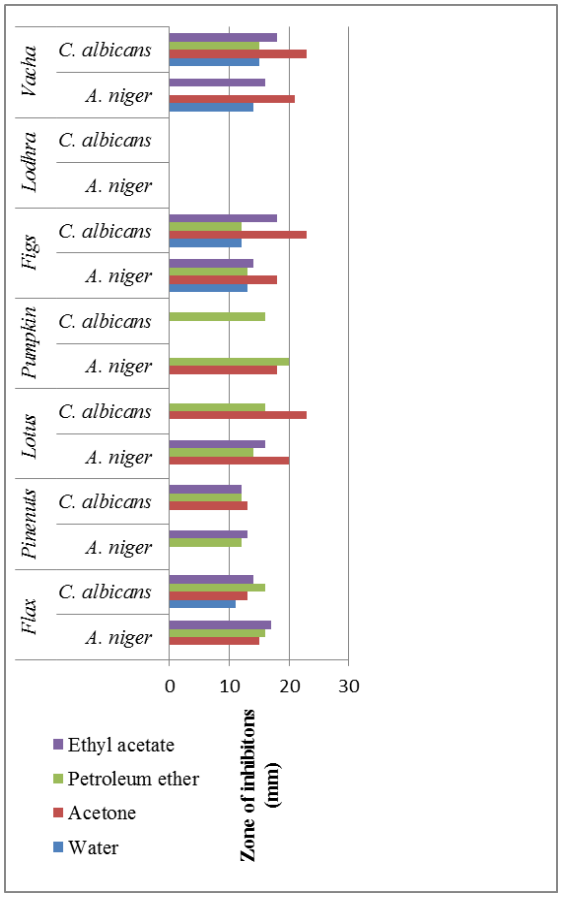

Figure 3: Above graph represents the antifungal analysis of seeds extracts against fungal pathogens.

Broth dilution assay

After antimicrobial activity of seeds extracts against pathogens, on the basis of zone of inhibition, few extracts founds best against respective pathogens only, hence the MIC tests were performed for those extracts to calculate the dosage. The minimum inhibitory concentration (MIC) values were determined by using a modified macro broth dilution technique.

Table 4: Minimum Inhibitory Concentration values of seeds extracts against pathogens.

Pathogens	Seeds extracts (with solvents)	MIC (mg/ml)
St	Fig (water)	21
Bs	Lotus (acetone)	18
Bs	Vacha (acetone)	29
An	Pumpkin (acetone)	35

*An: A. niger,*St: *S. typhi, Bs: B. subtilis.*

Discussion

Antibacterial are the agents which can effectively treat the infections caused by the various strains of bacteria. The frequency of life threatening infections caused by the pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in developing countries.

The antimicrobial properties have been examined by a number of studies globally and many of them have been used as therapeutic substitutes because of their medicinal properties. The practice of alternative and complementary medicine is now on the rise in developing countries in reply to World Health Organization directives and in this, the folk medicine is playing a key role to treat infections. Plants are the cheapest and safest alternative sources of antimicrobials **[19].**Plants and their derivatives have been used widely throughout history to treat medical difficulties. For examining the antimicrobial activity, many studies have been performed, in extraction and screening of active metabolites of these products for their antimicrobial activity.Today most antibiotics are making pathogenic organisms resistant. To overcome this huge problem, the discovery of new active compounds against new targets is a matter of determination. Thus phytochemicals and seeds extracts could become a promising natural antibacterial agent with potential applications in pharmaceutical industry for controlling the pathogenic bacteria **[20].** If plant extracts are to be used for medicinal purposes, the issues of safety and toxicity should be monitored.

Conclusion

The result of the antibacterial assay showed promising evidence for the antibacterial effect of seeds of flax seeds, pine nuts seeds (chilgoza,), lotus seeds, pumpkin seeds and fig seeds. The plant extracts have great potential as antibacterial compounds against enteric pathogens and they can be used in the treatment of various infectious. It is expected that this study would lead to the formation of some new compounds that could be used to formulate new and more potent antimicrobial drugs of natural origin. The study also shows that the ayurvedic compound Vacha is also a great potent natural drug as it exhibits largely antimicrobial activity.

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