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IN VITRO EVALUATION OF ANTI-MICROBIAL ACTIVITY OF *ANNONA SQUAMOSA*

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ABSTRACT

Antimicrobial activity of leaves of *Annona squamosa* were studied during the course of this project. Several plants extracts were prepared using solvents such as ethanol, methanol, ethyl acetate, chloroform, hot water and cold water. Agar well diffusion method was used for the assessment of antimicrobial activity. 100% acetone leaf showed an average zone of inhibition of 14.83mm against *Staphylococcus aureus*, 15.67mm against *Escherichia coli* and 14.6mm against *Pseudomonas aeroginosa*. 100% chloroform leaf showed an average zone of 15.37mm against *Staphylococcus aureus* 13.0mm against *Escherichia coli* and 15.33mm against *Pseudomonas aeroginosa*. 100% chloroform leaf hot showed an average zone of 14.33mm against *Candida albicans*. 70% ethanol leaf hot showed an average zone of 14.23mm against *Candida albicans*. Tetracycline was used as a standard antibiotic against bacterial pathogens and Flucanozole was used against fungal pathogen throughout the project.

Keywords: Antimicrobial activity, Zone of inhibition, Agar well diffusion method

INTRODUCTION

Natural remedies from medicinal plants are found to be safe and effective. Many plant species have been used in folkloric medicine to treat various ailments. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries. Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties **(Vanitha, V., *et al.*, 2011)**.

Based to the assorted health organization surveys such as WHO, IHO, almost 70-80% populations living in the developing countries rely almost solely on conventional medicine for their basic health care needs. Investigation of the chemical constituents of the plants and pharmacological test may endow with us the basis for developing the progress of new agents. The importance of conventional medicines in solving the best of health problem solutions is invaluable on a global pharmaceutical market. Natural products have been a significant source of marketable medicines and drug source. **(Aamir, J., *et al.*, 2013)**.

In India, from ancient times, different parts of medicinal plants have been used to cure specific ailments. Today, there is widespread interest in drugs derived from plants. This interest primarily stems from the belief that green medicine is safe and dependable, compared with costly synthetic drugs that have adverse effects. Hence, there is need to screen medicinal plants for promising biological activity. Plants of the genus *Annona*, members of the Annonaceae family, are native to South and Central America. They are mostly trees, and produce compound fruits. *Annona squamosa* L., known as custard apple, is commonly found in deciduous forests, also cultivated in wild in various parts of India. Literatures of many research works prove that every parts of *Annona squamosa* possess medicinal property Roots are employed internally in depression of spirits and spinal diseases. Bark is known to be a powerful astringent. In Ayurveda, fruits are considered as a good tonic, enrich blood, used as expectorant, increases muscular strength, cooling, lessens burning sensation and tendency to biliousness, sedative to heart and relieves vomiting. **(Padhi, L.P., *et al.*, 2011)**

Annona squamosa has been named botanically from Jamaica. The leaves of the plants have been used as insecticide, anthelmintic, styptic, externally used as suppurant. Unripe and dried fruit work as anti-dysenteric. Bark is used as powerful astringent, anti-dysenteric and vermifuge. Root bark, leaves and stems gave isoquinoline alkaloids. Powdered seeds are used to kill head-lice and fleas but care should be taken that the powder does not come in contact with the eyes as this causes great pain. Two acetogenins, annoreticuin and isoannoreticuin, isolated from the leaves, were found to be selectively cytotoxic to certain human tumours. The leaves and stems also gave alkaloids dopamine, salsolinol and coclaurine. **(Saha, R., 2011).**

Taxonomy

Kingdom	**Plantae**
Order	**Magnoliales**

Family	Annonaceae
Genus	Annona
Species	Squamosa

Traditional uses

Food: Fruits are normally eaten fresh. The pulp can be used as a flavouring in ice cream. Between 50-80% of the fruit is edible. The vitamin C content is appreciable (35-42 mg/100 g) and slightly higher than in grapefruit. The nutrient value of thiamine, potassium and dietary fibre is also significant.

Medicine: Leaves, shoots, bark and roots have been reported to have medicinal properties. The unripe fruit is astringent, and the root is a drastic purgative, the seed of the plants is believed to have anti-fertility activity. The roots are used as a drastic purge. The leaves are used as a vermicide, for treating cancerous tumours and are applied to abscesses, insect bites and other skin complaints. Scrapings of root-bark are used for toothache. Powdered seeds are used to kill head-lice and fleas but care should be taken that the powder does not come in contact with the eyes as this causes great pain.

Fuel: The tree is a good source of firewood. Timber: The light yellow sapwood and brownish heartwood are soft, light in weight and weak. Poison: Green fruits, seeds and leaves have effective vermicidal and insecticidal properties. **(Saha, R., 2011)**

MATERIALS AND METHODS

SAMPLE COLLECTION

Fresh green leaves of *Annona Squamosa* plant were collected from Sarojini Nagar Area of Lucknow, Uttar Pradesh after proper investigation by experts from MRD Life Sciences, Lucknow.. The leaves were then washed and kept for drying in the sun. The dried leaves were then ground to powder using a mixer and grinder.

PATHOGENS

Four different pathogens namely *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeroginosa* (bacterial) and *Candida albicans* (fungal) available at MRD Life Sciences (P) Ltd., Lucknow, collected from IMTECH, Chandigarh, were sub cultured and used throughout the project work.

EXTRACT PREPARATION

5g of plant powder was mixed in 50ml organic solvent and kept in dark for 2-3 days at room temperature. After that the extracts were filtered through a wattman's filter paper in the bowls. Then the bowls were kept in hot air oven at 50°C till the extracts got dried. After that the extracts were dissolved in DMSO making the extract concentration 100 mg/ml. 8 Different extracts were prepared for each part used and the list is given in. for 48 hours. In order to solubilise the bioactive components in the solvents. After 48 hours shake the solution well and filter it using Whatman's filter

paper 1. Evaporate it at room temperature. Add DMSO in a conc. so as to make the final concentration of extract to be 100mg/ml.

Table 1: Different-Different samples used

LEAF (L)
100% Acetone (1L)
70% Ethanol (2L)
80% Methanol (3L)
100% Ethyl Acetate (4L)
100% Chloroform (5L)
Aqueous Room Temperature (6L)
100% Acetone Hot (7L)
70% Ethanol Hot (8L)
100% Chloroform Hot (9L)
Hot Extract (10L)

ANTIBIOGRAM ANALYSIS

Agar Well Diffusion method

Sterile Nutrient agar media(for bacteria) and Potato Dextrose Agar media (for fungus) was poured into sterile petri plates and allowed to solidify. A 20µl inoculums of pathogen was gently spread over the media. After 2-3 minutes wells of diameter 8mm were bored into the media using a sterile borer. To the wells 50 µl of the extracts and also same quantity of +ve control i.e. tetracycline (for bacteria) and fluconazole (for fungus) and –ve control DMSO respectively. Kept bacterial plates in incubator (37ºC) and fungal plates (28ºC) at room temperature for overnight incubation.

RESULTS AND DISCUSSION

Kirby bauers method of agar well diffusion was used to perform the antibiogram analysis of the different solvent extracts against the test pathogens. 100% acetone and 100% chloroform leaf extracts showed best results against bacteria and 70% ethanol hot leaf and 100% chloroform hot leaf showed best results against fungus.

Table 2: Antibiogram Analysis of various extracts against bacterial pathogens

S.no.	NAME OF EXTRACT USED	ZONE OF IHHIBITION(mm) (sample/antibiotic)			
		Staphylococcus aureus	*Escherichia coli*	*Pseudomonas aeroginosa*	DMSO
1	1L	15.5/16.6	16.5/20.3	12.0/13.7	0.0
2	2L	11.5/16.6	11.020.3	0.0/13.7	0.0
3	3L	0.0/17.0	0.0/22.0	0.0/15.0	0.0
4	4L	0.0/16.6	0.0/20.3	0.0/13.7	0.0
5	5L	17.0/18.5	15.5/25.0	15.5/15.0	0.0
6	6L	0.0/16.6	0.0/20.3	0.0/13.7	0.0
7	7L	14.0/11.0	0.0/20.5	0.0/15.0	0.0
8	8L	18.0/11.0	0.0/20.5	0.0/15.0	0.0
9	9L	0.0/11.0	15.0/20.5	0.0/15.0	0.0
10	10L	0.0/17.0	0.0/22.0	0.0/15.0	0.0

Table 3: Antibiogram Analysis of various extracts against fungal pathogen

S.no.	NAME OF EXTRACT USED	ZONE OF IHHIBITION(mm) (sample/antibiotic)	
		Candida albicans	DMSO
1	1L	16.0/20.0	0.0
2	2L	15.5/20.0	0.0
3	5L	0.0/20.0	0.0
4	7L	15.5/20.0	0.0
5	8L	16.0/20.0	0.0
6	9L	16.5/20.0	0.0

DISCUSSION

The extensive use of antibiotics and other medicines have been a trend in the modern day medication. But these medicines though cure us today, are also responsible for several adverse effects we face later. So there arises a need for the use of herbal medicines. The medicines from the arms of mother nature. And this study we have evaluated the antimicrobial activity of one such plant given by nature to us. *Annona squamosa* or custard apple was found to be a good source of antimicrobial compounds. Zones of 15.5mm, 16.5mm, 12mm were recorded for 100% acetone leaf extract against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeroginosa.* 100% chloroform leaf extract gave zone of 17.0mm, 15.5mm, 15.5mm against the respective pathogens. 70% ethanol hot leaf and 100% chloroform hot leaf gave zones of 16.0mm and 16.5mm against *Candida albicans.*

CONCLUSION

Summing up the whole work done throughout this project, we come up to a point where we can state that *Annona squamosa* plant leaves are a good source of antimicrobial compounds and can serve to be a good source of herbal drug. The yield of antimicrobial compounds can be enhanced by using more sophisticated procedures and can be tested in various other solvents. It has proven to be effective against both fungus and bacteria and can used at elevated temperatures. So we can conclude that drugs made out of *Annona squamosa* leaves would not be dependent on what conditions they are stored, this gives an extra adavantage in handling the drugs made out of this plant. Future aspects of my project work would be to, firstly lay emphasis on the specific compounds rendering it with the prolific properties it has shown.Secondly, to develop a more sophisticated protocol for the extraction of secondary metabolites from the plant materials.And would also like to explore the other latent magical properties that the plant holds within itself.

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