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# RESEARCH HIGHLIGHTS

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## COMPARATIVE STUDY ON ANTIMICROBIAL PROPERTY OF *ANNONA SQUAMOSA* L. LEAVES AND SEEDS

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### Abstract

*Annona squamosa* L. belongs to the family Annonaceae, commonly known as custard apple. A comparative antimicrobial activity of ethanolic leaves and seed soxhlet extracts of *A. squamosa* were evaluated against four fungi namely, *Candida* and two bacteria, *Escherichia coli* and *Staphylococcus using* agar well diffusion method. Maximum inhibition was found with 12mm/1.5g concentration of ethanolic leaves and seed crude extracts against all the tested organisms under investigation. The study suggests that the leaves and seed of *A.squamosa* are promising in the development of phytomedicine for antimicrobial properties.

**Key words :** *Annona squamosa*, A comparative antimicrobial activity, ethanol extracts.

### Introduction

The textile industry is one of the nation's oldest and dynamic segments of an entire manufacturing industry. Textile industries in India are the backbone of the national economy. It is the largest industry

in India occupying a vital role in the Indian economy and occupies a unique place in the country. It accounts for 14 percent of the total exports and is the second largest employment generator after agriculture. Due to increasing requirements on the finishing of textile fabrics, increasing use of technical textiles that have been processed by environmentally sound methods, new innovative production techniques are demanded (Hyde *et al*, 2007).

Cotton has been grown and used to make fabrics in ancient China, Egypt, India, Peru and Mexico and today is one of the most important and widely used fibers worldwide. It grows in any part of the world with temperature to hot climate, long growing seasons and adequate rain (Stauffer, 2004). One of the main advantages of cotton is its resistance to alkali solution and the mild alkali solution and mild alkalis like sodium carbonate have no action on cotton in the absence of air at low temperature. However, in the presence of oxygen or air, cellulose is formed with graduated tendering of cotton. The most commonly encountered natural fiber is

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cotton, but the widespread uses of white cotton fibers also has various characteristics microscopic features and appear flat and spiraled or twisted under the microscope. Their fibers can be dyed with a wide range of dyes and because of their convoluted structure; transfer easily during contact (Jilstone, *et al*, 2006).

Medicinal herbs are the local heritage with global importance. The world is endowed with a rich wealth of medication herbs. The variety and sheer number of plants with therapeutic properties due to the presence of various complex chemical substances of different composition, which are found as secondly plant metabolites, according to their composition, are grouped as alkaloids, glycosides, corticosteroids, essential oils etc. During the past decade, a dramatic increase in exports of medicinal herbs attests to worldwide interest in these products as well as in traditional health systems. The pharmaceutical industries have made massive investment on pharmacological, clinical and chemical researches. Efforts have been made to discover drugs that are still more potent. The benefits of these efforts would reach to the masses in future in farmer's initiate's commercial cultivation of medicinal herbs.

*Annona squamosa*. L commonly known as Tamil sitapalam, English sweets and custard apple, is a native of West Indies and is cultivated throughout India, mainly for its edible fruit. The young leaves of *A.squamosa* are used extensively for their

anti diabetic activity. The plant contains atropine alkaloids, carbon and linalool, limonene (Likcy, 2005). The green fruits, seeds and leaves have effective vermicial and insecticidal properties. In addition, the phytochemical isolated from *A.squamosa* have shown the antimalarial, antidiabetic, hepatoprotective, antitumor, antimicrobial, anti HIV-1 and wound healing activities. *Annona squamosa* Linn is a small evergreen tree is cultivated throughout India for its fruits different parts of *Annona squamosa* line are used in folkloric medicine for the treatment of various diseases (Suresh *et al*, 2006). The hot water extract of *A.squamosa leaves* has been reported to possess hypoglycemic and antidiabetic activity (Gupta *et al*, 2005). Antimicrobial and insecticidal properties of partially purified flavonoids from an aqueous extract of *A.squamosa* have been reported against *callosobruchus chinensis* (Maheswari, 2002).

Various medicinal plants have been used for years in daily life to treat diseases all over the world. Interest in medicinal plants reflects the recognition of the validity of many traditional claims regarding the value of natural products in healthcare. In the present day world most of them were conscious about the hygiene and cleanliness. Clothing and textile are a very suitable medium for the growth of the microorganisms. They can act as the carriers of some microorganisms such as pathogenic bacteria, odour generating bacteria and mould fungi. Microbial

infestation poses danger to both living matters. Therefore, in the textile and garment world, finishing plays a vital role for quality and value.

Finishing is the treatment of fabrics that produces finished textile goods. Finishing is one of the necessary aspects that gains good appearance and feel of the garment. Functional finishes are non-toxic and free in nature. The antimicrobial finish controls the growth of odor causing bacteria arising in the human body use of apparel and home textiles. Antimicrobial treatment is necessary for textile materials, in order to control microorganisms, to reduce odor from perspiration, to reduce the risk of cross infection being carried by the feet, to control the spread of diseases and determination of textile fabrics. The need for antimicrobial textile is increasing nowadays, to determine the effects of microorganisms on textiles as well human hygiene. So it is essential to control or inhibit the growth of these organisms on textile fabrics which turns out to be undesirable for the wearer as well as the textiles itself.

The natural and eco-friendly antimicrobial agents for textile applications are gaining interest in recent times (Jayakumar, 2011). Antibacterial activity of small ones, like, silver, zinc, copper and quaternary ammonium compounds are well documented. The silver impregnated textiles are used as wounds at high risk of infection, whereas linkages between biocide moieties and cellulose, are covalently formed on reactive sites (Eisner *et al*, 2003).

Antimicrobial finishes help to protect fabrics against mold, mildew and bacteria attacks. In addition to the finishes described here, several manufactured fibers are produced with antimicrobial agents incorporated into the fiber structure. These modified acetates, acrylics and olefins can provide durable protection against growth of microbes (Coiler and Tortora, 2001).

## Materials and Methods

### Collection of source

#### Preparation of *Annona squamosa* seed and Leaf

The fresh leaves and seeds of the plant *Annona Squamosa* samples collected from the local area of Coimbatore, Tamilnadu. The collected leaves and seeds were washed with running tap water and allowed to air dry. The plant materials were dried in shade for two or four weeks. Precaution was taken to avoid direct sun light otherwise it will destroy the active compounds of plant leaves and seeds. After the plant leaves were grained finely, stored in airtight cover. The green fruits, seeds and leaves have effective vermicides and insecticidal properties. In addition, the phytochemical isolated from *Annona Squamosa* have shown the antimalarial, antimicrobial properties and cytotoxic activity against the tumors.

#### Extraction of Active Compounds from *Annona squamosa* Seed and Leaf

##### Soxhlet extraction method

The leaves and seeds cotyledon materials were ground using a grinding

machine in the laboratory then 30g of shade-dried powder was weighed and extracted successively with ethanol in soxhlet extractor for 48h. The ethanol extracts were concentrated under reduced pressure and preserved in refrigerator in airtight bottle for further use. The dried powder samples extracted with ethanol (300 ml) using Soxhlet extractor was kept for 48 hours until complete extraction. After extraction, it was filtered and evaporated by rotary evaporator to give amorphous solid masses. The crude extracts was transferred into a separator funnel and finally extracted by different solvent.

#### **Determination of Suitable Solvent for Extraction**

The active compounds from the *Annona Squamosa* seed Fig.1 and leaf Fig.3 was extracted using various solvents such as ethanol, methanol, acetone and chloroform. For extraction, 10g powder of *Annona Squamosa* seed and leaf was taken in a conical flask and mixed with 100ml of ethanol. Then the flask was kept in soxhlet extractor at 100°C for one day. Next day the solution was filtered using filter paper stored at 4°C. The same procedure was followed for extraction of active compounds from *Annona Squamosa* seed and leaf using methanol, chloroform and acetone. After extraction, the solution was evaluated for antimicrobial activity by agar diffusion method. Then the solvent, which showed the maximum zone of inhibition, was selected for further finishing process. The activity showed the best result in the ethanol

medium. Therefore, ethanol was selected as the medium for further research.

#### **Selection of finishing**

Antimicrobial finishes have increased its importance in the recent years for several reasons. They serve the consumer by offering protection from the harmful effects of certain microbes. More commonly, the finish is designed to inhibit odour that may have been generated by the body, soils, contaminants or personal care products (Sivaramakrishnan, 2007). Antimicrobial are used on textiles to control bacteria, fungi, mould, mildew, yeast and the problems of fabric rotting, staining, unpleasant odour and health concerns ranging from simple discomfort to physical irritation, allergic sensitization, toxic responses, infection and disease that the presence of microbes can raise. Hence, these finishes minimize or control the action of microbes preventing the transfer and spread of disease and protects the delicate human skin. Medical application of encapsulation has centered on the delivery of drug treatment through clothing to patients. One such application involves the delivery of antimicrobial treatments to cut down the bugs causing the hospital super infection MRSA. The potential of microencapsulation for use in sportswear, underwear and work wear is soon recognized and now it is becoming a common treatment for fashion clothing. The consumers are now increasingly aware of the hygienic life style and there is a necessity and expectation for a wide

range of textile products finished with antimicrobial properties. Botanical research shows that some plant species exhibit extraordinary antimicrobial properties. Hence, antimicrobial finishing was selected for this present study.

#### Optimization of antimicrobial finish

The optimization of standard condition for antimicrobial finish was done for original and treated fabrics. Optimization for antimicrobial treatment was carried out for various parameters such as extract concentration time and temperature after giving the finish to the fabric. The samples were taken out, dried

and the antimicrobial activity was evaluated.

#### Concentration of herbal solution

The fabric was treated with three different concentrations of *Annona Squamosa* extract of seed and leaf. The material liquor ratio was 1:15. The concentrations were varied from 0.5g, 1.0g and 1.5g with constant temperature of 40°C respectively. After 1 hr, the samples were taken out, dried and its antimicrobial activity was evaluated. The concentration level of 0.5g and 1g did result to the activity but only in 1.5g level of concentration the activity was enriched enough so that it could be used for the study.

#### Seed extraction

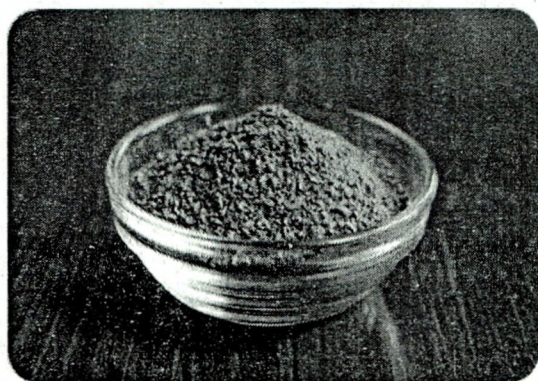


Fig.1

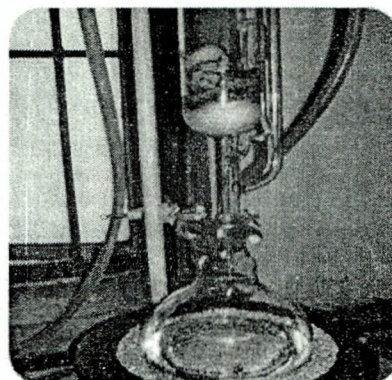


Fig.2

#### Leaf extraction

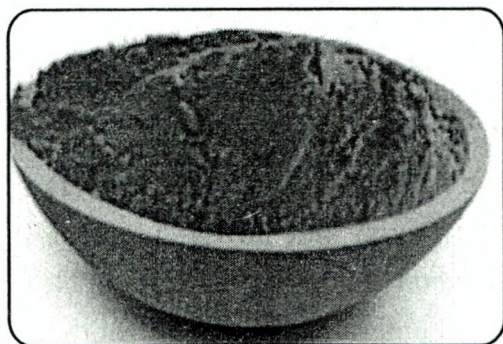


Fig.3

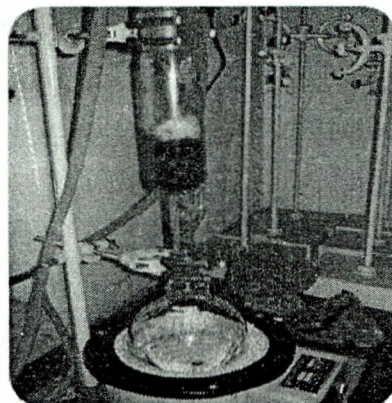


Fig.4

### Determination of optimum time for finish on fabric

The antimicrobial finish of cotton fabric treated with extract of *Annona Squamosa* seed and leaf was carried out at optimized concentration with constant temperature of 40°C for different time intervals like 1hr, 3hrs and 5hrs. After treatment at different time interval, the fabric is taken out and evaluated for antimicrobial activity. The time, which shows the maximum zone of incubation, was selected for further finishing process.

### Optimization parameters

Parameters	Levels
Concentration	0.5g, 1.0g and 1.5g of extract
Time	1hr, 3hrs and 5hrs
Temperature	40°C, 50°C and 60°C

### Application method

#### Padding mangle

The wet finishing treatment to increase the barrier effect of the fabric was carried out under the application of finishing agents by means of well-known padding method. This technique is related to coating and as has already been mentioned is actually a method of producing a coated fabric, if carried out repeatedly. Fabric "padding" or "dipping" terms for impregnation is universally used in fabric finishing. In order to obtain consistent chemical application, the nip pressure should be uniform across the fabric width. The solution level and temperature in the pad should be constant and the fabric speed should not vary throughout the application process (Schindler and Hauser, 2004).

The finishing solution was prepared by mixing the solvent extract (500 ml/l) with liquor ratio 1:80. The cotton fabric was placed in the finishing solution and kept there for 20 min. Then the fabric was taken out and padded in the padding mangle with 80 percent wet pick up to get an even distribution of finishing and was then air dried at room temperature. The wet pick up or percent expression is calculated as follows:

$$\text{Percentage expression} = \frac{W_1 - W_2}{W_2} \times 100$$

Where  $W_2$  = weight of the fabric after padding and  $W_1$  = weight of the fabric before padding (Kim, 2011).

## Results and Discussion

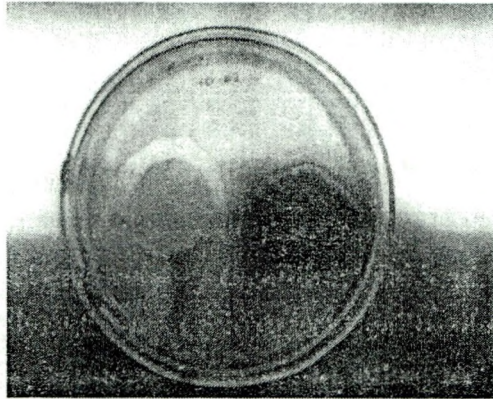
### Antimicrobial result

Organisms	Zone of Inhibition Crud extract (mm)	Zone of Inhibition in soxhlet extraction
E. coli	12 mm	8 mm
S. aurous	8 mm	7mm
Candida fungi	-	9 mm

Antimicrobial test was carried out in crude extract and soxhlet extract form. The test revealed that E.coli formation in crude and soxhlet extraction was 12mm and 8mm respectively, species of s.aurous in crude extract was 8 mm and then the soxhlet extract was 7mm. The same procedure was carried out for the fungi Candida to find out the zone of inhibition. The soxhlet extract shows 9 mm zone of inhibition.

### Antibacterial activity of crude extract

*E.coli*

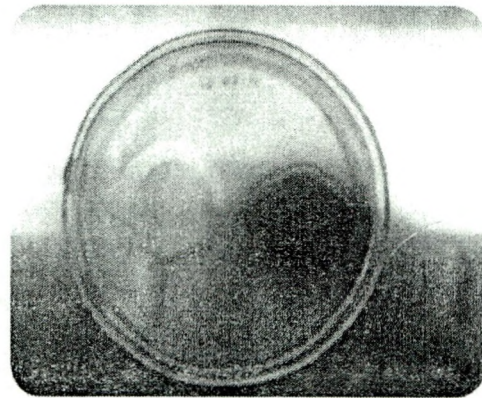


Seed

Leaves

Fig.5

*Staphylococcus*

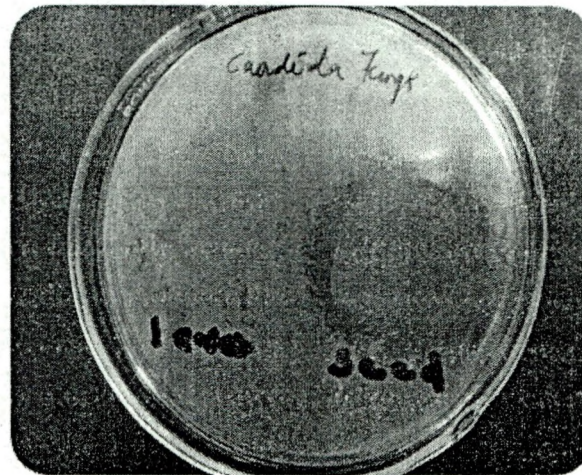


Leaves

Seed

Fig.6

*Candida fungi*



Leaves

Seed

Fig.7

### Conclusion

*Annona* is the best for antimicrobial agent from seed and leaves suitable extract. *Annona squamosa* seed extract have great potential for antimicrobial finish. The natural antimicrobial finish shows good microbes resistant, which are non-toxic, non-allergenic, non-skin irritant and also

eco-friendly. When applied to the cotton fabric. from this study it was concluded that, the antimicrobial activity is greater in *annona squamosa* seeds and it enhances excellent fabric properties, such as weight, strength, drape, stiffness and absorbency testes. Compared with leaves and seed extracts, seeds showed maximum antimicrobial activity.

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