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***Research Paper***

# Antimicrobial finishing of fabric with *Pongamia pinnata* leaves extract

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■ **ABSTRACT :** Antibacterial textile production has become increasingly prominent for hygienic and medical applications. Therefore, to reduce the growth of bacteria various antibacterial compounds have been used for all types of textiles. In the current study, an eco friendly natural antibacterial extract has been prepared from Pongamia pinnata plant leaves for textile finishing application. After knowing the phytochemical properties, extracts were selected and were tested on cotton fabric by the method of direct application. *Pongamia pinnata* leaves extract treated samples showed better antibacterial and antifungal properties. Therefore, *Pongamia pinnata* tree leaves could be a potential source of active antimicrobial agents.

■ **KEY WORDS:** Antimicrobial finishing, *Pongamia pinnata* leaves, Phytochemical properties

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Cotton textiles in contact with the human body offer an ideal environment for microbial growth. Microbial infestations possess danger to both living and non-living matters. Obnoxious smell from the inner garment, spread of diseases, staining and degradation of textiles are some of the detrimental effects of microbes. The consumers are now increasingly aware of the hygienic life style and there is a necessity and expectation of a wide range of textile products finished with a range of textile antimicrobial properties under different trade names for textile industry. Majority of such products are synthetic based and may not be environment friendly. Their compliance to the regulations imposed by international bodies, such as EPU is essential. Textile industry continuously searches for new technologies in order to accomplish the consumers´ demands. Especially in recent years, new developments allowed the production of functional and smart textiles which are capable of sensing changes in environmental conditions or body functions and responding to these changes. Likewise, consumers' attitude towards hygiene and active lifestyle has created a rapidly increasing market for a wide range of textile products finished with antimicrobial properties, which in turn has stimulated intensive research and development. There is a measureless resource of natural antimicrobial peptides which can be exploited for imparting antimicrobial properties to textile substrates. The main advantage of antimicrobial substances is that they are small molecules that can be impregnated or covalently bound to textiles in a very effective and homogeneous deposition.

There are many natural/herbal products which show antimicrobial properties. Extracts from different parts of diverse species of plants like root, flower, leaves, seeds etc. exhibit antibacterial properties. Many of the plants contain compounds like phenolic, terpenoids, flavonoids, alkaloids, polypeptide, polyacetylenes, etc. which are acting as antibacterial. Some of them act as bactericides and some act as bacteriostatic. For a long period of times plant have been valuable sources of natural products for maintaining human health, especially in the last decade with more intensive studies for natural therapies.

*Pongamia pinnata* is a medium-sized evergreen tree 15-25 m high, with straight or crooked trunk 50-80 cm or more in diameter and broad crown of spreading or drooping branches. Bark is grey-brown, smooth or faintly vertically fissured. Branchlets are hairless with pale stipule scars. Leaves are alternate, imparipinnate with long slender leafstalk, hairless, pinkish-red when young, glossy dark green above and dull green with prominent veins beneath when mature. Leaflets are 5-9, paired except at end, short stalked, ovate elliptical or oblong, obtuse-acuminate at apex, rounded to cuneate at base, not toothed at the edges, slightly thickened. Flower clusters at base of and shorter than leaves, to 15 cm long, slender, drooping. Flowers are 2-4 together, short-stalked, pea-shaped, 15-18 mm long. Pods borne in quantities, smooth, oblique oblong to ellipsoid, flattened but slightly swollen, slightly curved with short, curved point (beaked), brown, thick-walled, thick leathery to sub woody, hard, indehiscent, 1-2 seeded, short stalked. Seeds are compressed ovoid or elliptical, bean-like with a brittle coat long, flattened, dark brown, oily.

Nutritive Value of *Pongamia pinnata* tree leaves Karanj has nutritive value of major dry season feed resources available for Goats in Bhilwara and Udaipur Districts. Leaves are used as fodder. The composition (% dry basis) is CP (Crude protein) 10 to 13, NDF (neutral detergent fibre) 35 to 41, ash 6, EE (ether extract) 3 to 5, lignin 11, TP (Total Phenols) 3. It has medium to high fermentability, and it appeared to be very deficient in fermentable protein, but may be a source of by-pass protein (Wood, Matthewman, Badve, and Conroy).

The objective of present investigation is to find out the phytochemical constituent of *Pongamia pinnata* tree leaves. And to assess the total bacterial and total microbial count after finishing the fabric with *Pongamia pinnata* tree leaves extract.

## ■ RESEARCH METHODS

### Selection of plant and collection of leaves:

*Pongamia pinnata* tree leaves were selected on the basis of their medicinal properties against as reported in various literatures. *Pongamia pinnata* tree leaves were collected from fully grown plant from University campus of Udaipur city. The sample was authenticated for its botanical identity by Botanist. Plant material was kept for drying, away from direct sunlight, temperature was maintained at below 45°C (shade dried) for about two weeks. The dried material was crushed in an electric grinder and uniformly stored in an air tight container.

### Sample extraction for photochemical screening:

Powdered *Pongamia pinnata* tree leaves (2 g) were added in 25 ml solution of Ethanol (70%), Methanol (70%) Acetone (100%) and aqueous solution (100%) in separate beakers. The mixture was left for 24 hours and after that the extract was centrifuged and measured. The residual extract was subsequently mixed with 25 ml. of the respective solvent and same process was repeated for next 24 hours. The final extract was filtered, labeled and stored in refrigerator for experiments.

### Phytochemical screening:

The extract was tested for the presence of some active chemical compounds such as alkaloids,

flavonoids, phenolic, tannins, saponins and terpenoids. The analysis was conducted as per universal standard methodology as follows.

**Alkaloids (Mayer's test) :**

1.36g of mercuric chloride dissolved in 60 ml and 5 g of potassium iodide were dissolved in 10 ml of distilled water, respectively. These two solvents were mixed and diluted to l00 ml using distilled water. To one ml of acidic aqueous solution of samples few drops of reagent was added. Formation of white precipitate showed the presence of alkaloids.

**Flavonoids:**

In a test tube containing 0.5 ml of extract of the samples, 5 to10 drops of diluted HCI and small amount of Zn and Mg were added and the solution was boiled for few minutes. Appearance of reddish pink or dirty brown colour indicated the presence of flavonoids.

**Glycosides:**

A small amount of alcoholic extract of samples was dissolved in 1 ml water and then aqueous sodium hydroxide was added. Formation of a yellow colour indicated the presence of glycosides.

**Steroids (Salkowski's test) :**

About 100mg of dried extract was dissolved in 2ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish brown colour at the interface was an indicative of the presence of steroidal ring.

**Saponins:**

A drop of sodium bi-carbonate was added in a test tube containing about 50 ml of an aqueous extract of sample. The mixture was shaken vigorously and kept for 3min. A honey comb like froth was formed and it showed the presence of saponins.

**Resins:**

To 2 ml of chloroform *or* ethanolic extract 5 to 10 ml of acetic anhydrite was added and dissolved by gentle heating. After cooling, 0.5 ml of $H_2SO_4$ was added. Bright purple colour was produced. It indicated the presence of resins.

**Phenols (Ferric chloride test):**

To 1ml of alcoholic solution of sample, 2ml of distilled/water followed by a few drops of 10 per cent aqueous ferric chloride solution were added. Formation of blue or green colour indicated the presence of phenols.

**Tanins (Lead acetate test) :**

In a test tube containing about 5 ml of an aqueous extract, a few drops of 1 per cent solution of lead acetate was added. Formation of a yellow or red precipitate indicated the presence of tannins.

**$FeCl_3$ test:**

A 2 ml filtrate (200mg of plant material in 10ml distilled water, filtered), and 2ml of $FeCI_3$ were mixed. A blue or black precipitate indicated the presence of tannins

**Terpenoid:**

2 ml of chloroform and 1ml of cone. $H_2SO_4$ was added to 1 mg of extract and observed for reddish brown colour that indicated the presence of terpenoid

**Finishing of cotton fabric for antimicrobial process with *Pongamia pinnata* tree leaves :**

***Preparation of grey cotton fabric :***

– Desizing : The grey cotton fabric was desized and scoured with alkali and soap solution. The bath composition for desizing and scouring process is as follows-

- NaOH- 2 g/lit.
- $Na_2CO_3$- 2 g/lit.
- Soap- 1g/lit.
- Weight of grey fabric- 1 g
- Time for heating- 1 hour
- Temperature of the bath – 100° C

After desizing and scouring process the fabric is swilled with water to remove alkali and soap residuals.

– Bleaching: The fabric was bleached with suitable bleaching agent to get a white fabric with experimental conditions as follows-

- $Na_2SiO_3$ – 2g/lit.
- NaoH – 2 g/lit.
- $Na_2CO_3$ – 2g/lit.
- $H_2O_2$ – 12 ml/lit.
- Weight of grey fabric – 5 g
- Time for bleaching – 1 hr.

– Temperature of the bath- 80° C

The scoured cotton fabric is bleached with $H_2O_2$ to obtain pure fabric free from all impurities.

**Preparation of *Pongamia pinnata* tree leaves extract:**

Finishing solution was prepared with dried *Pongamia pinnata* tree leaves extract (25%,50%,75% and 100%) by adding water and 10 per cent acetone. A fixing agent like $CuSO_4$ was added and the whole solution was stirred well. Now this clear solution was ready for fixing the organic compounds to the fibre. The composition of the finishing bath is as follows-

– Weight of the fabric- 5 g.

– Concentration of the *Pongamia pinnata* tree leaves Extract- 25 per cent, 50 per cent, 75 per cent and 100 per cent

– Acetone- 10 per cent

– Copper Sulphate (mordant)- .05 per cent

**Finishing process:**

The finished cotton fabric was dipped in extract for an hour and the material was washed and dried.

**Determination of antimicrobial activity by using agar plate:**

Potato Dextrose agar was prepared and sterilized at 121° C for 15 minutes. Petri plates were autoclaved in hot air oven at 121°C for 45 minutes. Treated Fabric (1x2 cm) sample and five treated fibre strands (2 cm) were placed in Petri plates; Staphylococcus Aureus (Gram positive) bacteria was used to test the effectiveness of the antibacterial activity. 20 ml of Potato Dextrose Agar was poured in Petri plates and were allowed to solidify and incubated at 30 °C for 70 hours, and it were covered, marked and placed in upside down position. Similar procedure was carried out for untreated samples of fabric and fibres also. After 70 hours observations were compared and this process was repeated five times for accuracy and average colony count is mentioned in Table 2 for assessment.

**Preparation of PDA and MPH agar :**

The potato dextrose agar (PDA) was prepared by using standard composition and method, the composition used is as follows for preparing 1 lit. Agar solution.

| | | |
|---|---|---|
| Peeled potato | - | 200 g |
| Distilled water | - | 1000 ml. |
| Agar | - | 15 g |
| Dextrose | - | 20 g |
| pH | - | 5.6 |

This composition was prepared in conical flask and covered with cotton plug and wrapped with silver foil. MPH Agar solution was procured from chemical store and prepare according to directions *i.e.* 25 g powder was added in 1 liter distilled water. Both Agar Solutions were autoclaved for 1 hr. by maintaining 15 psi pressure at 121° C for 15 minutes.

LFR (Laminar Floor Room) was prepared with UV rays for 10 min. and wiped with spirit, Sterilized conditions were maintained and all glass wares and other articles needed during the experiment were also autoclaved. After that PDA, MPH agar solution was poured separately in Petri dishes having herbal finished fine samples.

After jellification of Agar solutions; Pouring, Streaking, Spreading techniques were used to apply E-coli culture with the help of inoculation loop, L- shape spreader, respectively. Marked Petri dishes were kept in incubator at 37° C, assessment was done after 24 hr., 48 hr., 52 hr. and after seven days also. Similarly, to evaluate the growth of aerobic microbes' lactose broth with e - coli culture was used.

## ■ RESEARCH FINDINGS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under following heads :

**Determination of active ingredients in crude extract of *Pongamia pinnata* tree leaves :**

Table 1 describes the phytochemical screening of *Pongamia pinnata* tree leaves, and the presence of phytoconstituents is mentioned in tabulated form for Methanol, Ethanol, Acetone and Aqueous solution.

Table 1 describes the phytochemical screening of *Pongamia pinnata* tree leaves, and the presence of phytoconstituents is mentioned in tabulated form for Methanol, Ethanol, Acetone and Aqueous solution. It is observed from table that presence of Tannins was observed in all extracts, in Methanol it was high whereas saponins shows high presence with acetone and aqueous solution. In chloroform extract flavonoids, tannin and glycosides showed presence.

These classes (alkaloids, saponins, tannins, anthraquinones and flavonoids) of compounds are

**Table 1 : Phyto chemical constituents of *Pongamia pinnata* tree leaves**

| Phyto chemical constituents | | Methanol | Ethanol | Acetone | Aqueous |
|---|---|---|---|---|---|
| Flavo | Ammonia test | ++ | + | _ | ++ |
| noids | Sodium hydroxide test | +++ | + | _ | ++ |
| Alkaloids | | + | - | - | + |
| Glycosides | | ++ | ++ | + | ++ |
| Steroids | | ++ | - | - | ++ |
| Phenols | | ++ | + | ++ | ++ |
| Terpenoids | | +++ | ++ | + | ++ |
| Saponins | | - | - | + | + |
| Tannins | $FeCl_3$ test | ++ | + | ++ | ++ |
| | Lead acetate | +++ | +++ | ++ | ++ |
| | Cardiac glycosides | + | + | - | ++ |

+++ Highly present, ++ Moderately present, +Present – Absent

**Table 2 : Total microbes count (TMC) and total bactria count (TBC) of controlled and *Pongamia pinnata* tree leaves extract treated fabric**

| Extract / Count | TMC | | | | TBC | | | |
|---|---|---|---|---|---|---|---|---|
| | 25% | 50% | 75% | 100% | 25% | 50% | 75% | 100% |
| Control | | ±1300 | | | | ± 4 | | |
| Methanol(1) | ±290 | ±10 | ±3 | ±2 | ±4 | ±4 | ±2 | ±2 |
| Ethanol(2) | ±150 | ±25 | ±5 | ±2 | ±3 | ±2 | ±2 | ±2 |
| Acetone(3) | ±300 | ±17 | ±10 | ±2 | ±4 | ±4 | ±2 | ±2 |

BG- Bacterial Growth, 1=less than 25%, 2=less than 50%, 3= less than 75%, 4= 100% fungal growth,

known to have activity against several pathogens and therefore aid the antimicrobial activities of *Pongamia pinnata* tree leaves and suggest their traditional use for the treatment of various illness. In all the extracts, tannins were present resulting in the inhibition of cell protein synthesis as it forms irreversible complexes with proline rich protein. In research done by the phytochemical analysis showed the presence of alkaloid, saponins, tannins, terpenoids, steroids, glycosides, phenols and flavonoids.

**Assessment of antimicrobial activity** :

It is observed that treated samples with *Pongamia pinnata* tree leaves extract provide less favorable conditions for Microbes and Bacteria growth than Controlled sample. Among the various extract treated fabric Ethanol treated fabric showed more microbial growth than other fabric. The table clearly indicates that *Pongamia pinnata* tree leaves extract finished fabric showed less microbial and bacterial count as compared to controlled sample. It is observed from the table that TBC is moreover same for *Pongamia pinnata* tree leaves extract prepared with Methanol, Ethanol, Acetone and Chloroform. On the other hand TMC was observed different for each extract media. In control sample it was approximately 1300 and in treated sample it came down to 2 with Methanol, Ethanol and chloroform media extract

Researches stated that plant extracts exhibiting diameters of zones of inhibition larger than 10 mm are considered active. Therefore, the extract is a better antimicrobial agent for various pathogenic fungus and bacteria. Therefore, *Pongamia pinnata* tree leaves could be a potential source of active antimicrobial agents.

**Conclusion:**

It may be concluded that extract of *Pongamia pinnata* found to have all the phytochemical such as alkaloids, saponins, terpenoids, phenolic acids and tannins confirmed by chemical tests. The extract was applied on the cotton fabric. The results revealed that *Pongamia pinnata* leaves extract finished fabric showed less microbial and bacterial count as compared to controlled sample. Study concluded that the antimicrobial activity showed by the plant leaves was due to the presence of phytochemical.

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