

***Borassus flabellifer* Seed Coat: An *In Vitro* Exploration of its Antibacterial and Antifungal Properties with Preliminary Phytochemical Screening**

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Natarajan *et al.*: Antimicrobial Properties of *Borassus flabellifer* Seed Coat Extract

This study focuses on the analysis of phytochemical constituents, antibacterial activity, and antifungal activity of ethanolic extract isolated from the seed coat of *Borassus flabellifer*. The ethanolic extract of *Borassus flabellifer* seed coat was prepared by cold extraction technique using sterile filter paper. The extract was subjected to qualitative phytochemical analysis preliminarily. Antibacterial activity against gram-positive strains of *Bacillus cereus* and *Streptococcus mutans*, gram-negative strains of *Pseudomonas aeruginosa* and *Salmonella typhi*, and antifungal activity against strains of *Candida albicans* and *Aspergillus flavus* were assessed using agar well diffusion method by observing the diameter of zone of inhibition. The qualitative phytochemical analysis of ethanolic extract of *Borassus flabellifer* seed coat revealed the presence of phytoconstituents like tannins, saponins, flavonoids, alkaloids, phenol. It was found to have exhibited good antibacterial effect against gram-positive strains of *Bacillus cereus* (14-21 mm) and *Streptococcus mutans* (10-14 mm) at all concentrations but not exceeding standard antibiotic (16-26 mm) and also exhibited average antibacterial effect against *Pseudomonas aeruginosa* (12-16 mm) but ineffective against *Salmonella typhi*. There was limited antifungal activity against *Candida albicans* (14 mm) and nil antifungal activity against *Aspergillus flavus*. This study concludes that the seed coat of *Borassus flabellifer* has significant antibacterial activity against several human pathogens. The phytochemicals present are positively associated with antimicrobial properties. Hence, it can be used for treating a variety of infectious conditions.

Key words: Antibacterial, antifungal, *Borassus flabellifer*, phytochemical, seedcoat extract

Since prehistoric times, numerous medicinal plants have been unveiled and are being used for treating several illnesses. Plants have different phytochemical substances that are found to be pharmacologically active and have the potential to be effective as drugs. The use of plant products in drug research has always resulted in the creation of potential compounds that are useful in treating a variety of ailments. Plant products are preferred over synthetic drugs as they are readily available, much cheaper and also cause minimal serious adverse effects. Moreover, antibiotic resistance has become a major public threat leading to longer hospital stays, higher healthcare costs, and increased mortality. Hence, the development of drugs from plant products has become a prime necessity in recent days. *Borassus flabellifer* (Asian palmyra palm) commonly referred to as Ice apple, Nungu (Tamil), Tale hannu (Kannada),

Tadgola (Hindi), most prevalent in areas of South India, Malaysia, Thailand, Cambodia, Myanmar, and Srilanka^[1]. *Borassus* is the genus name and *flabellifer* is the species name belonging to the family, Arecaceae. Palmyra palm is a slow-growing, perennial, dioecious tree with a single stem that reaches 30 m in height and has big fan shaped leaves. The lifespan of the woody tree is estimated to be around 100 y^[2]. It takes nearly 12 y-20 y to get mature enough to bear its first flower. Different parts of this woody tree were found to have different pharmacological actions which help us in formulating a novel drug. The young roots

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and the male inflorescence of the palm tree were reported to possess anti-inflammatory activity^[2]. The palm fruit was found to have antioxidant and antimicrobial properties. Similarly, the seed was found to have antioxidant property^[3], antidiabetic property and also help in hydration. The sap obtained was reported to act as a diuretic, laxative, and anti-parasitic agent^[1]. Toddy which is produced by fermenting the palm sap was found to treat gastric ulcers^[1]. Several parts of this woody tree have been used by our ancestors and are being used in Ayurvedic medicines due to its enormous health benefits. It's a common practice to eat only the scrumptious, fleshy palatable seed, and the seed coat (skin) is usually discarded (fig. 1). Hence, the present study is focused on investigating the less explored discarded seed coat of *Borassus flabellifer* for its phytochemical constituents present, its antibacterial and antifungal properties *in vitro*.

MATERIALS AND METHODS

Plant material collection:

The tender seeds of *Borassus flabellifer* were procured from the palm grove situated in the southern part of Tamilnadu during the summer. The seeds were washed under running water for 2 min and the seed coat was peeled with the help of a sterile knife. The peeled seed coat was dried under shade for 5 d. The shade dried peels were powdered and stored in an airtight container at room temperature for later use.

Plant extract preparation:

The plant extract was prepared using ethanol as solvent by cold extraction technique. About 10 g of powdered seed coat was made to get soaked in 100 ml of ethanol and allowed to stand for 72 h. Followed by, filtering it using a sterile filter paper.

Then, the filtrate was collected and incubated at room temperature for evaporation. Finally, the ethanolic extract of *Borassus flabellifer* seed coat was stored in a sterile capped container under refrigeration at 4° for further studies.

Qualitative phytochemical screening:

The ethanolic extract of *Borassus flabellifer* seed coat was subjected to qualitative screening of phytochemical constituents present in it by diluting 10 mg of crude extract in 10 ml of ethanol according to the standard protocols^[4-6] and modified methods^[7-10].

Ferric chloride test-test for tannins:^[4]

1 ml of seed coat extract was mixed with a few drops of 0.1 % ferric chloride. The presence of gallic tannins and catechol tannins was determined by looking for blackish blue and blackish green colours respectively.

Frothing test-test for saponins:^[5]

3 ml of seed coat extract were added to 10 ml of distilled water and strongly shaken for 5 min. Later, it was permitted to stand for 30 min. The presence of saponins is indicated by frothing or foaming appearance.

Shinoda test-test for flavonoids:^[7]

A few drops of magnesium chloride and concentrated hydrochloric acid were added to 1 ml of seed coat extract and observed after 5 min. The presence of flavonoids is confirmed by observing red to pink colour.

Dragendorff test-test for alkaloids:^[6]

Few drops of Dragendorff reagent were added to 2 ml of seed coat extract. The presence of alkaloids is indicated by orange-red precipitate.



Fig. 1: Parts of *Borassus flabellifer* fruit, seeds with and without seedcoat

Bradford protein assay-test for proteins:^[4]

Few drops of Bradford's reagent were mixed with 2 ml of seed coat extract and observed for colour shift from brown to blue indicating the existence of proteins.

Salkowski's test-test for steroids:^[8]

3 drops of 10 % concentrated sulphuric acid were added to 5 ml of seed coat extract and observed for red colour which indicates the activity of steroids.

Borntrager's reaction-test for anthraquinones:^[9]

10 ml of 10 % ammonia solution was mixed with 1 ml of seed coat extract and strongly mixed for 30 s. The activity of anthraquinones is confirmed by observing pink, violet or red colour.

Salkowski's test-test for terpenoids:^[10]

2 ml of chloroform was added along with little drops of concentrated sulphuric acid to 1 ml of seed coat extract. The formation of a reddish-brown ring at the interface confirms the activity of terpenoids.

Keller kiliani test-test for cardiac glycosides:^[8]

1 ml of glacial acetic acid, 2 % ferric chloride solution in drops followed by 1 ml of concentrated sulphuric acid were added to 5 ml of seed coat extract. The formation of brown colour ring confirms the existence of cardiac glycosides.

Ferric chloride test-test for phenols:^[9]

A few drops of 5 % ferric chloride were added to 1 ml of seed coat extract and noticed for dark green or bluish-black colour indicating the activity of phenols.

Microorganism strains used for analysing antibacterial activity:

The antibacterial activity of *Borassus flabellifer* seed coat extract was analysed using gram-positive bacterial strains of *Bacillus cereus* and *Streptococcus mutans* and gram-negative bacterial strains of *Salmonella typhi* and *Pseudomonas aeruginosa*.

Antibacterial properties of *Borassus flabellifer* seed coat extract:

The antibacterial activity was evaluated by the agar well diffusion method on Mueller Hinton

Agar (MHA) medium^[11]. Active cultures of the aforementioned microorganisms were prepared from stock cultures which were maintained at 4° on the nutrient agar slant. Test tubes with nutrient broth for bacterial culture were incubated at 37° for 24 h and kept ready. A loop of cells from stock cultures was added to test tubes of nutrient broth and sub-cultured overnight. 10 mg of crude seed coat extract was diluted with 10 ml of ethanolic solvent to prepare the stock solution. 125 µl of stock solution was diluted in 875 µl of ethanolic solvent to formulate 125 µg/ml of ethanolic seed coat extract concentration. Similarly, other concentrations from 250 µg, 500 µg to 1000 µg were prepared.

The sterile MHA medium was poured into sterile petri plates and permitted to solidify for 1 h. Following solidification, the inoculums were made to spread on the plates with the help of a swab with cultured bacterial suspension. Wells of 6 mm size were made using sterile cork borer and seed coat extract was added in it. Streptomycin was added to one of the wells since it was used as a positive control. Similarly, Dimethyl Sulfoxide (DMSO) was used as a negative control. The plates were incubated at 37° for 24 h. Then, the diameter of the zone of inhibition was measured in mm using a HiMedia zone reader to estimate the microbial growth^[11]. Various concentrations of ethanolic seed coat extract (125 µg, 250 µg, 500 µg, 1000 µg) in respective wells of the inoculated petri plates were tested for each microorganism to analyse the zone of inhibition in accordance with the increasing concentration of seed coat extract.

Microorganisms used for analysing antifungal activity:

The antifungal activity of *Borassus flabellifer* seed coat extract was analysed using *Candida albicans* and *Aspergillus flavus*.

Antifungal properties of *Borassus flabellifer* seed coat extract:

The antifungal activity was evaluated by agar well diffusion method on Potato Dextrose Agar (PDA) medium^[12]. Active cultures of the aforementioned microorganisms were prepared from stock cultures which were maintained at 4° on slant of nutrient agar. Test tubes of nutrient broth for bacterial culture were incubated at 37° for 24 h and kept ready. The overnight nutrient broth was

sub-cultured by transferring a loop of cells from stock cultures to test tubes of nutrient broth. 10 mg of crude seed coat extract was diluted with 10 ml of ethanolic solvent to prepare the stock solution. 125 µl of stock solution was diluted in 875 µl of ethanolic solvent to formulate 125 µg/ml of ethanolic seed coat extract concentration. Similarly, other concentrations from 250 µg, 500 µg to 1000 µg were prepared.

The sterile PDA medium was poured into sterile petri plates and permitted to solidify for 1 h. Following solidification, the inoculums were made to spread on the plates with the help of a swab with microbial suspension. Wells of 6 mm size were made using sterile cork borer and seed coat extract was added in it. Ketoconazole was added to one of the wells since it was used as a positive control. Similarly, DMSO was used as a negative control. The plates were incubated at 37° for 24 h.

Then, the diameter of the zone of inhibition was measured in millimetres (mm) using a HiMedia zone reader to estimate the microbial growth^[12]. Various concentrations of ethanolic seed coat extract from 125 µg, 250 µg, 500 µg to 1000 µg in respective wells of inoculated petri plates were tested for each microorganism to analyse the zone

of inhibition in accordance with the increasing concentration of seed coat extract.

RESULTS AND DISCUSSION

The screening of phytochemicals present in seed coat extract is considered the preliminary step to identify the bioactive substances present which might be possibly positively associated with several medicinal properties like antioxidant property, anti-inflammatory property, antimicrobial property, and anti-cancer property. The phytochemicals present in *Borassus flabellifer* seed coat extract are listed in Table 1. Agar well diffusion method was employed to study the antibacterial activity (fig. 2) of *Borassus flabellifer* seed coat extract against gram-positive strains of *Bacillus cereus* and *Streptococcus mutans* and gram-negative strains of *Salmonella typhi* and *Pseudomonas aeruginosa* using MHA medium. As depicted in fig. 3, the seed coat extract exhibits strong antibacterial activity against *Bacillus cereus* with 14-21 mm zone of inhibition as the concentration of the extract increases. There is considerate antibacterial activity against *Pseudomonas aeruginosa* with 12-16 mm zone of inhibition at higher concentrations of seed coat extract.

TABLE 1: PHYTOCHEMICALS IN *Borassus flabellifer* SEEDCOAT EXTRACT

S. no	Phytochemicals	Seed coat extract
1	Tannins	+
2	Saponins	+
3	Flavonoids	+
4	Alkaloids	+
5	Proteins	+
6	Steroids	-
7	Quinones	-
8	Terpenoids	-
9	Cardiac glycosides	+
10	Phenols	+

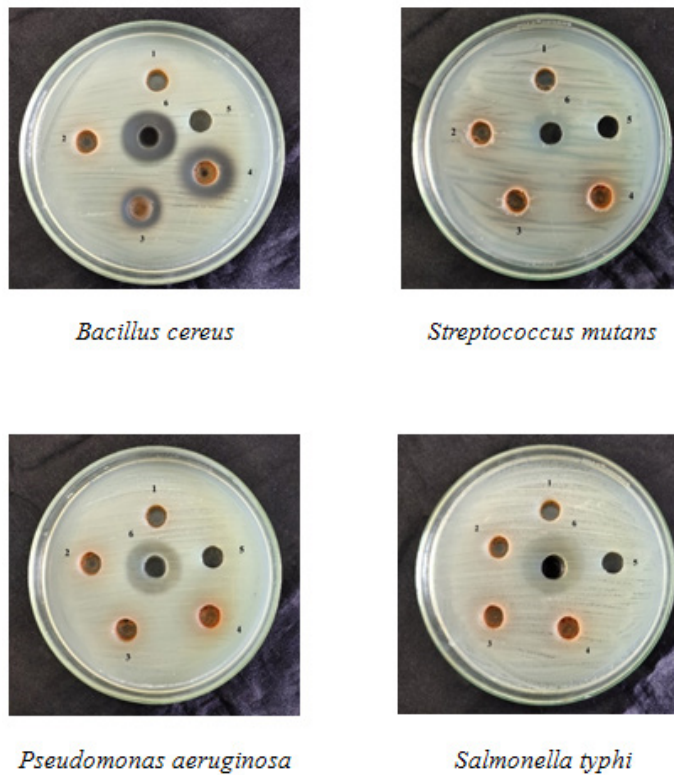


Fig. 2: Microorganisms exhibiting antibacterial property of *Borassus flabellifer*

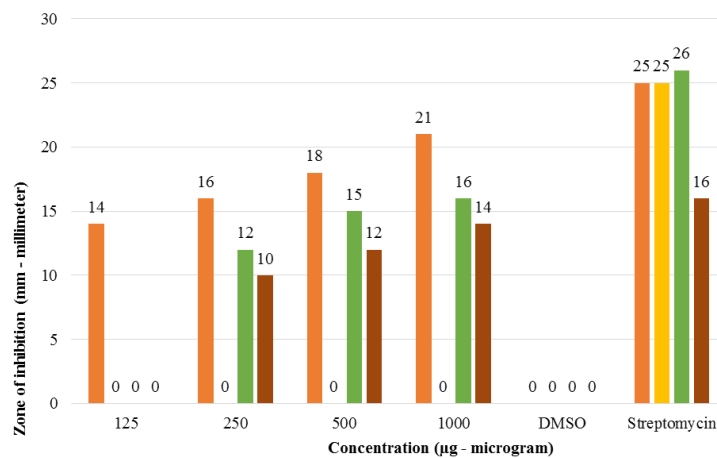


Fig. 3: Antibacterial activity of *Borassus flabellifer* seedcoat extract

Note: (■): *B.cereus*; (■): *S.typhi*; (■): *P.aeruginosa* and (■): *S.mutans*

Limited antibacterial activity was observed against *Streptococcus mutans* with 10-14 mm zone of inhibition and it was noticed that seed coat extract was ineffective against *Salmonella typhi* even at its maximum amount concentration. Using PDA, the antifungal (fig. 4), activity of seed coat extract was evaluated using agar diffusion method against strains of *Candida albicans* and *Aspergillus flavus*. As shown in the fig. 5, there was minimal activity of seed coat extract against *Candida albicans* with 14 mm zone of inhibition exhibited at the maximum concentration. Also, no antifungal

activity was noticed even at the maximum of seed coat extract concentration against *Aspergillus flavus*. Although we have a wide class of modern medicines available for treating different bacterial infections, most of them come with adverse effects like anaphylactic reactions, gastro-intestinal disturbances, and toxicity along with their therapeutic value as chemicals are the source of many drugs. So, always there is an unending search for medicines from plants to minimise the adverse effects. Nature is the ultimate source available to combat several medical ailments.

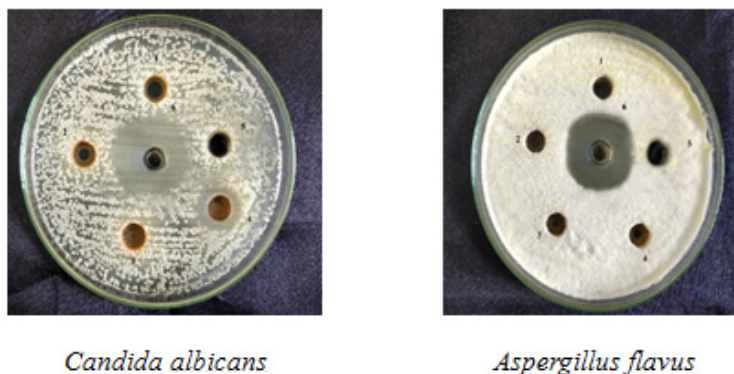


Fig. 4: Microorganisms exhibiting antifungal property of *Borassus flabellifer*

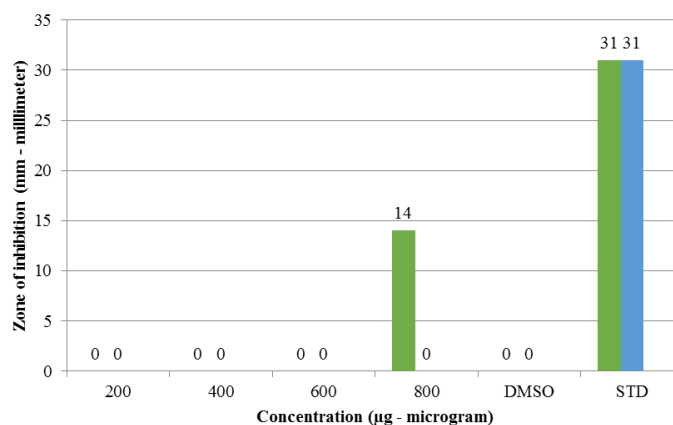


Fig. 5: Antifungal activity of *Borassus flabellifer* seedcoat extract

Note: (■): *C. albicans* and (■): *A. flavus*

The screening of phytochemicals present in seed coat extract is considered to be the preliminary step, to identify the bioactive substances present in it which might be possibly positively associated with several medicinal properties like antioxidant property, anti-inflammatory property, antimicrobial property, anticancer property, anti-diabetic property. The presence of phytochemical constituents like tannins, saponins, alkaloids, and flavonoids has been reported already to be partly responsible for the antimicrobial activity^[13]. Our study revealed the presence of tannins, saponins, alkaloids, flavonoids, proteins, cardiac glycosides, and phenols which are attributed to the antimicrobial properties of the seed coat extract. 2 gram-positive strains and 2 gram-negative strains were selected for analysing in the study to test them against different varied medical conditions. *Bacillus cereus*, gram-positive strain is known to cause food poisoning^[14]. *Streptococcus mutans*, gram-positive strain colonises the dental surface resulting in dental caries^[15]. *Pseudomonas aeruginosa*, gram-negative organism causes hospital-acquired infections like pneumonia,

urinary tract infections. It always remains a concern to treat due to its resistance^[16]. *Salmonella typhi*, gram-negative strain is typically associated with typhoid fever^[17].

Candida albicans was selected as it is a widespread fungal infection affecting people of all ages causing oral thrush, genital infections like vulvovaginal candidiasis. It is also a normal commensal of our human body. Infection occurs when the fungus overgrows due to weakened immune system^[18]. *Aspergillus flavus*, a common opportunistic pathogen causing aspergillosis, allergic reactions^[19]. As the study implies, the seed coat extract can be tried effectively against food poisoning cases caused by *Bacillus cereus*, respiratory infections caused by *Pseudomonas aeruginosa*. A study done by Rani *et al.*^[10] reported that the aqueous fruit extract of *Borassus flabellifer* revealed zone of inhibition against pathogens like *Bacillus cereus*, *Bacillus subtilis*, and *Staphylococcus aureus* at the concentration of 30 µg/ml using azithromycin as control. In our study, pathogens like *Bacillus cereus*, *Streptococcus mutans*, *Salmonella typhi*, and *Pseudomonas aeruginosa* were analysed using

Streptomycin as a positive control. It was reported by Jamkhande *et al.*^[12] that the methanolic leaves extract of *Borassus flabellifer* had a significant antibacterial effect against *Bacillus subtilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* where ciprofloxacin was used as standard. The same extract had nil effect against *Aspergillus niger* as our study concluded^[12].

A study done by Sahni *et al.*^[2] had observed that the methanolic dried root extract of *Borassus flabellifer* revealed zero activity against gram positive *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and gram-negative *Proteus mirabilis*, *Escherichia coli*.

Sakande *et al.*^[20] revealed that there was significant antifungal activity of dichloromethane-methanolic extract of *Borassus aethiopum* male inflorescences against *Trichophyton rubrum*, *Trichophyton interdigitale*, *Microsporum langeronii* and *Epidermophyton floccosum*. On the contrary, it was observed to have weak antibacterial activity against *Escherichia coli*, *Enterobacter cloacae*, *Staphylococcus aureus*. Ketoconazole and amoxicillin were used as standards respectively.

According to the study results of Duddukuri *et al.*^[21] significant and consistent antibacterial effect of *Borassus flabellifer*'s methanolic seed coat extract was distinguished against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*. Our ethanolic seed coat extract expressed significant antibacterial activity against *Bacillus cereus*, *Streptococcus mutans*, *Salmonella typhi*, and *Pseudomonas aeruginosa* as the concentration of the seed coat extract increased.

Our current exploration on the ethanolic seed coat extract of *Borassus flabellifer* has revealed prominent antibacterial activity against *Bacillus cereus*, *Streptococcus mutans* (gram-positive strains) and *Salmonella typhi*, *Pseudomonas aeruginosa* (gram-negative strains). But, has only limited antifungal activity against *Candida albicans*. The emergence of antibiotic-resistant strains and their hazardous side effects has revived the search for novel antimicrobial agents from natural plant sources. The need of plant products as pharmacological agents is increasing day by day due to the increased cost of the available antibiotics and various serious adverse effects caused. Hence, natural antibiotics are preferred over other synthetic available drugs for a

healthier quality of life. The present study paves the way for the potential utilization of *Borassus flabellifer* seed coat in pharmaceutical industries as a pertinent antibacterial agent against several infectious ailments like enteric fever, urinary tract infections, upper respiratory tract infections, food poisoning. Further *in vitro*, *in vivo* and clinical studies are necessary to provide more scientific evidence on consuming *Borassus flabellifer* seed as a whole along with the seed coat therapeutically to utilise the great medicinal benefits.

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Conflict of interests:

The authors declare no conflict of interest.

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