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ORIGINAL ARTICLE

Biological activities of leaves of ethnomedicinal plant, *Borassus flabellifer* Linn. (Palmyra palm): An antibacterial, antifungal and antioxidant evaluation

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Abstract Plants contain a variety of phytochemicals that have the ability to exert effect on human body. Among them *Borassus flabellifer* Linn. is a medicinally important plant. In traditional medicine different parts of plants are being used for their medicinal properties. The methanol extract was obtained from powdered leaves and further fractions were prepared. Antimicrobial potential was investigated using eight pathogenic strains of bacteria and fungi by agar well diffusion method. Broth dilution method was employed to MIC and MMC of active samples and MIC index value was determined. ME was subjected to preliminary phytochemical analysis; and 1,1-diphenyl-2-picrylhydrazyl (DPPH) and hydrogen peroxide (H_2O_2) radical scavenging activity. Phytochemical screening revealed the presence of several phytochemicals. The ME showed dose dependent radical scavenging activity as evidenced by IC_{50} values for DPPH (40.19 μg/ml) and H_2O_2 (30.92 μg/ml) radicals. The inhibition zones and MIC values for bacterial strains were in the range of 10–16 mm and 50–70 μg/ml, respectively. All the samples showed an inhibitory effect on fungal strains with inhibition zone (10–17 mm) and MFC (50–70 μg/ml). Samples exhibited diverse patterns of antibacterial and antifungal effects. Among the tested samples, methanol extract and acetone fraction (AF) had potent antibacterial and antifungal activities. These results lead to the conclusion that the plant has a broad spectrum antimicrobial and antioxidant activity and could be a potential option for treating various infectious diseases. The strong antioxidant property of methanol extract might be employed in the development of natural antioxidants for agro-food and pharmaceutical industries.

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1. Introduction

The progression of improvement and dissemination of the knowledge about the plants and its traditional medicinal uses has become one of the basis for the cure of general ailments from the midst of primordial epoch. *Borassus flabellifer* Linn. is high in stature and distinctly differ as male and female one with a sturdy trunk and is unbranched. It is generally cultivated in most of the regions of India, Bangladesh, Burma, Sri Lanka, Malaysia and tropical Africa. The people residing in these regions are mostly dependent upon vegetation around them for the treatment of small ailments such as cuts and wound focusing on the medicinal importance of plant parts.[1,2] *Borassus flabellifer* Linn. is commonly known as Palmyra palm which is immensely distributed in the tropical regions of the Asian continent. Appreciable noteworthy economic value to the local population is aided by the *Borassus flabellifer* Linn.[2] It belongs to the family Palmae and the sub-family Boracidae. *Borassus aethiopum* Mart., *Borassus flabellifer* Linn. and *Borassus sundaicus* Becc. are the three most economically important species of Borassus.[3]

The plant mainly contains gums, albuminoids, fats, steroidal glycosides, and carbohydrate like sucrose. It also contains spirostane type steroids like borassosides and dioscin.[4] Seed coat extract of the *Borassus flabellifer* Linn. has been reported to possess antimicrobial activity.[5] Male inflorescence shows a significant anti-inflammatory activity.[6] Different parts of the *Borassus flabellifer* Linn. plant have been reported to comprise biological activities and pharmacological functions, including anthelmintic, diuretic[7], antioxidant[8] and antibacterial activities of the fruits, wound healing[9], immunomodulatory[10], and antimalarial.[11]

Fundamental parameter in domineering and sustaining human life is the biochemical reactions which take place within the organelle and cells of the body.[12] The chemical constituent of the plant produces free radicals that regulate biochemical processes by acting as an antioxidizing agent.[13] Many studies have shown a close relationship between a highly nutritious diet, maintenance of good health and reduction in the risk of chronic diseases. Besides nutrients such as carbohydrates, protein, and fibers another class of essential substances that has been studied in the last few years is antioxidant compounds which are present at low concentrations and can help to prevent cell damage such as cancers, inflammations, aging and atherosclerosis caused by free radicals throughout the body.[14] Many studies revealed that synthetic antioxidants produce toxic effects like carcinogenesis and liver toxicity.[13] Microbial infection is a one of the major motives responsible to evoke oxidative reactions which intern lead to cell injury.[15–17] Although many antimicrobials have been effectively used but remarkable resilience and the emergence of resistance are major problems.[18] It is known that leaves of *Borassus flabellifer* Linn. are rich in an abundant number of phytochemicals.[4] Several antimicrobial herbs like *Borassus flabellifer* Linn. have not revealed for all their facets so there is surge to reveal their medicinal properties. Therefore, the aim of this study was to investigate the antioxidant activity of leaf extract and antimicrobial efficacy of extract and fractions against most common human pathogenic strains.

2. Material and methods

2.1. Chemicals and reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH), nutrient broth (NB), potato dextrose broth (PDB), bacteriological agar and antibiotic disk (amoxicillin, ciprofloxacin and griseofulvin) were supplied by Himedia (Mumbai, India). Hydrogen peroxide (H_2O_2), sodium hydroxide and potassium dihydrogen phosphate were purchased from Rankem (India). Ascorbic acid was obtained from Oxford laboratory, India. All the reagents were of analytical grade purity and obtained from Rankem (India).

2.2. Collection and authentication of plant material

The *Borassus flabellifer* Linn. leaves were collected from Kurnapalli village of Nizamabad district of Andhra Pradesh state, India and was authenticated by Botanist, Dr. Gachande B.D., Associate Professor of Botany Department, N.E.S. Science College, Nanded, India. The voucher specimens were deposited at herbarium of School of Pharmacy, S.R.T.M. University, Nanded, Maharashtra, India.

2.3. Extraction and fractionations

The collected leaves were converted into small pieces and shade dried for 7 days at room temperature, and then powdered using a grinder (coarse powder by sieve No. 10, manual). The dried powder material (250 g) was Soxhlet extracted with 1 L methanol for 8 h at 64 °C. After extraction the solvent was evaporated and concentrated by rotary evaporation (Superfit, India). The obtained methanol extract was then mixed with 150 ml of distilled water and sequentially partitioned (3×; three times) using separatory funnel with an equal volume of pet. ether, n-butanol, chloroform and acetone solvents, respectively. Solvents were selected on solubility and polarity of phytochemicals. The fractions were later concentrated under reduced pressure in a rotary evaporator, and weighed and a percentage yield of 5%, 13.5%, 12% and 12.5% for pet. ether, n-butanol, chloroform and acetone fractions, respectively was obtained. The extracts were stored at 10 °C, protected from light and used within one week.

2.4. Qualitative phytochemical investigation

The methanol extract of *Borassus flabellifer* Linn. was subjected to phytochemical screening using standard procedures.[19] Extract was primarily intended for the phytochemical analysis and detection of major chemical constituents.

2.5. Antimicrobial assay

2.5.1. Microbial strains and culture media preparation

The methanol extract (ME) and fractions n-butanol (NF), chloroform (CF) and acetone (AF) of *Borassus flabellifer* Linn. were individually tested against a set of eight strains of bacteria (four gram positive and four gram negative) and fungi. Microbial strains were provided by School of Life Science, Swami Ramanand Teerth Marathwada University, Vishnupuri, Nanded, Maharashtra, India. Bacteria strains used include