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Phytochemical, Antioxidant and Antibacterial Activity of Aqueous Extract of *Borassus Flabellifer (L.)*

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ABSTRACT

Objective: To analyse the phytochemicals responsible for free radical scavenging activities and antibacterial activity in aqueous extract of fruit of *Borassus flabellifer(L.)*

Method: Phytochemical analysis of *B. flabellifer* was performed. Antioxidant assay was carried out using DPPH. The antibacterial activity was tested against pathogenic bacteria *B. cereus, B. subtilis, S. aureus, P. aeruginosa* and *E. coli* using the standard method of agar disc diffusion on Muller Hinton agar.

Result: A zone of inhibition of 23mm diameter was observed against *S. aureus* when 30 µl of aqueous fruit extract was introduced. The extract showed zone of inhibition of about 24mm and 28mm in diameter against 20 µl and 30 µl of *B. subtilis*. To calculate %DPPH radical-scavenging activity, optical density (O.D) of the extract was measured. Phytochemical analysis of aqueous extract of fruit of *B. flabellifer (L.)* showed the presence of saponins, quinones, cardiac glycosides, terpenoids, phenols, steroids, coumarins and betacyanin.

Conclusion: *B.flabellifer (L.)* exhibited good inhibitory effect of medical relevance against facultative anaerobic bacteria owing to the rich phytochemicals. There was no work carried out earlier in the ripened fruit of *B. flabellifer(L.)* hence; this study was carried out to excavate the abundant therapeutic nature of mother earth.

Key words: Phytochemical, Antibacterial, Terpenoid, Disc diffusion, *Borassus flabellifer(L.).*

I. INTRODUCTION

The Palmae plant, *Borassus flabellifer(L.*) (Palmyra palm in English), is a native of tropical Asian countries It is grown casually as ornamental species or on plantations as perennial having woody stems exotic palm species. The fruit pulp has been used in traditional dishes and the sap, which was trapped from the flower part also used as a sweetener for diabetes. Literature study revealed that it has been used as antidote, anti-inflammatory, wound healing, anthelmintic activity, analgesic and antipyretic. The knowledge that was passed-on by our ancestors on the various medicinal aspects of such fruits are now proven and tested under *in vitro* conditions. The ripened fruit pulp has unique antioxidant and

antibacterial properties when compared to antibiotics due to the presence of phytochemicals such as saponins,alkaloids etc (1-5) .

II. MATERIALS & METHOD

2.1 Collection of the fruit

The fruit was washed, before peeling off the epidermic layer. The peeled fruit was then cut into pieces by using a sterile knife.

2.2 Preparation of the plant extract

Two grams of fruit pulp was added to 25ml of distilled water. The pulp is ground in pestle and mortar for 15 minutes. The extract is then transferred into a test tube and is incubated at 45^0c for further assay.

2.3 Phytochemical Screening from fruit extracts of *Borassus flabellifer(L.)*

The phytochemical screening of fruit pulp extract was assessed by standard method as described by **Savithramma *et al.,* (2011)** and **Selvaraj *et al.*, (2014)**. Phytochemical screening was carried out on aqueous fruit extract to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides, coumarins and steroids.

2.3.1 Test for Tannins

1 ml of the aqueous fruit extract, 1 ml of ferric chloride (5% FeCl3) was added. Formation of dark blue or greenish black indicates the presence of tannins.

2.3.2 Test for Saponins

Two ml aqueous fruit extract was added to two ml of distilled water and shaken in graduated cylinder for 15 min lengthwise, formation of 1cm layer of foam indicates the presence of saponins.

2.3.3 Test for Quinones

One ml of aqueous fruit extract was added to one ml of concentrated sulphuric acid (H2SO4) was added. Formation of red colour indicates the presence of Quinones.

2.3.4 Test for Flavonoids

Two ml of aqueous fruit extract was added to one ml of 2N Sodium Hydroxide (NaOH) Formation of yellow colour indicates the presence of flavonoids.

2.3.5 Test for Alkaloids

Two ml of aqueous fruit extract was added to two ml of concentrated Hydrochloric acid (HCl) was added. Then few drops of Mayer's reagent was added. Presence of green colour or white precipitate indicates the presence of alkaloids.

2.3.6 Test for Glycosides

Two ml of aqueous fruit extract was added to 3ml of chloroform and 1ml of 10% ammonium solution was added. Formation of pink colour indicates the presence of glycosides.

2.3.7 Test for Cardiac glycosides

0.5 ml of aqueous fruit extract was added to two ml of glacial acetic acid and few drops of 5 % ferric chloride was added. This was under layered with 1 ml of concentrated sulphuric acid. Formation of brown ring at interface indicates the presence of cardiac glycosides.

2.3.8 Test for Terpenoids

0.5 ml of aqueous fruit extract was added to two ml of chloroform along with concentrated Sulphuric acid. Formation of red brown colour at the interface indicates the presence of Terpenoids.

2.3.9 Test for Phenols

One ml of aqueous fruit extract was added to two ml of distilled water followed by few drops of 10 % ferric chloride. Formation of blue / green colour indicates the presence of phenols

2.4 Test for Steroids

0.5 ml of aqueous fruit extract was added to two ml of chloroform and 1 ml of concentrated Sulphuric acid (H2 SO4). Formation of reddish brown ring at interface indicates the presence of steroids.

2.4.1 Test for Coumarins

One ml of aqueous fruit extract was added to one ml of 10 % NaOH. Formation of yellow colour indicates the presence of coumarins.

2.4.2 Test for Anthocyanin and Betacyanin

Two ml of the aqueous fruit extract was added to one ml of 2N Sodium Hydroxide (NaOH) and heated for 5 min at 100 °C. Formation of bluish green colour indicates the presence of anthocyanin and formation of yellow colour indicates the presence of betacyanin.

III. ANTIOXIDANT ACTIVITY

The quantitative analysis of free radical scavenging activity of the sample on DPPH radical was estimated following the protocol by Brand-Williams et al. (6) 0.1ml of sample was added to 3.9 mL of DPPH (100 lM) (Sigma-Aldrich) . The absorbance was determined using a UV double beam spectra scan (Chemito, India) at 515 nm after incubation for 45 min. The 0.1 mL ethanol solution and 3.9 mL of DPPH solution were used as control and only ethanol was used as blank. The inhibitory percentage of DPPH was calculated according to the following

% DPPH radical-scavenging = (Absorbance of control - Absorbance of test x100

(Absorbance of control)

IV. Antibacterial activity in fruit extract of *Borassus flabellifer(L.)*

The aqueous fruit extract of *Borassus flabellifer* fruit was used as sample for antibacterial study using Kirby Bauer method. Different concentration (10, 20 and 30 µg/ml) of the concentrated fruit extract was tested for its antimicrobial strain such as *Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli.* The medium used for antibacterial study was Muller Hinton Agar (Himedia) and the bacterial cultures were maintained in Nutrient broth (Himedia). Antibacterial activity was measured using the standard method of disc diffusion on Muller Hinton agar as per *Erturk et al* (7). Then 0.1ml of each culture of bacteria was spread on agar plate surfaces. Paper disc (6mm in diameter 10, 20, 30µg/ml of different concentrations of aqueous fruit extract of *Borassus flabellifer (L.).* Inhibition diameters were measured after incubation for 24 - 48 hours at 37°C. Blanks of solvent only (processed in the same way), were also tested for antibacterial activity. The zone of inhibition in accordance with the increase in concentration was tested.

V. RESULTS

Preliminary phytochemical screening from aqueous extract of *B. Flabellifer(L.)*

The preliminary phytochemical screening from aqueous extract of *B. Flabellifer(L.)* revealed the presence of saponin, quinones, cardiac glycosides, terpenoids, phenols, steroids, coumarins, betacyanin (Table 1). The presence of these phytocompounds relates to the antibacterial activity of the extract against a few bacteria used in this study.

Table 1. Phytochemical screening of aqeuous extract of *B. flabellifer(L.)*

S.No	Phytochemical	Aqueous
	Compounds	
1	Tannins	-
2	Saponin	+

3	Quinones	+
4	Alkaloids	-
5	Flavonoids	-
6	Glycosides	-
7	Cardiac Glycosides	+
8	Terpenoids	+
9	Phenols	+
10	Steroids	+
11	Coumarins	+
12	Anthocyanin	-
13	Beta-cyanin	+

+ indicates presence of the phytochemical constituents;

- indicates asbsence of the phytochemical constituents.

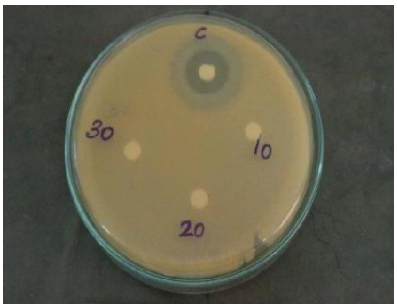
Antioxidant Activity of *Borassus flabellifer(L.)*

The antioxidant compound present in the aqueous *Borassus* extract liberates electrons which are accepted by DPPH, the violet color of the DPPH radical was reduced to yellow colored diphenylpicrylhydrazine radical which was measured colorimetrically. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers. The anti-oxidant activity of the extract *was* measured in a time interval of 5 minutes each (TABLE 2) until 30 minutes. Thus the percentage of radical scavenging of DPPH was calculated. Results obtained in this investigation indicate that aqueous extract of *Borassus flabellifer(L.)* is rich in many of the phytochemicals which contribute to highest antioxidant and reducing activities.

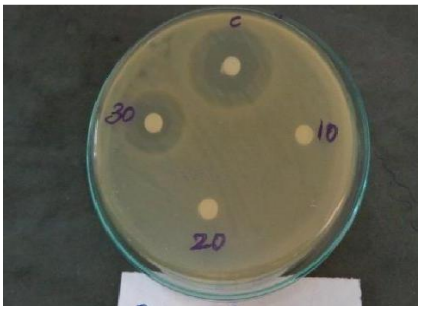
Table 2. Antioxidant Activity of *Borassus flabellifer(L.)*

Time taken (mins.)	Absorbance (OD)		Radical Scavenging %
	Control	Test Sample	
0	1.27	0.55	56.69
5	1.27	0.48	62.20
10	1.27	0.47	62.99
15	1.27	0.45	64.57
20	1.27	0.43	66.14
25	1.27	0.42	66.93
30	1.27	0.41	67.72

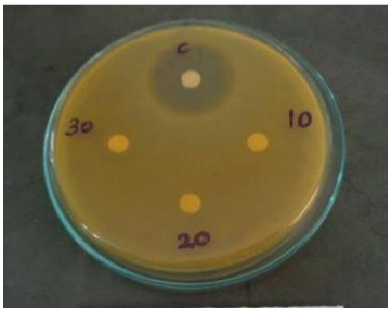
Antibacterial activity in fruit extract of *Borassus flabellifer(L.):*



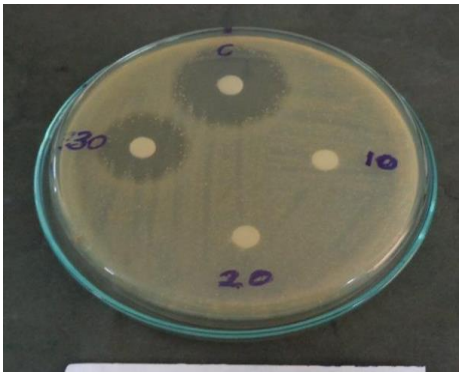
Pseudomonas



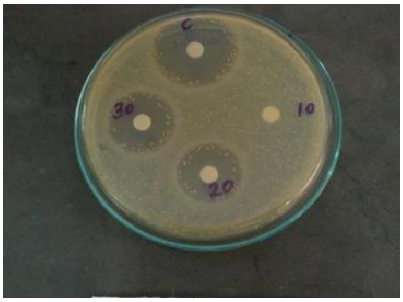
Bacillus



Escherichia coli



Staphylococcus aureus



Bacillus subtilis

VI. DISCUSSION

Different concentration of the aqueous extract of *Borassus flabellifer(L.)* (10,20,30 µg/ml) was added to wells on Mueller Hinton agar swabbed with five bacteria on different plates like *B. cereus, B.subtilis, S. aureus,P. aureus and E. coli.* The result revealed that the zone of inhibition of highest concentration of 30 *µg/ml was found* against *B.cereus, B.subtilis* and *S. aureus.* Also this extract was effective against *B.subtilis at 20µg/ml.* Azithromycin(15µg) was used as control. The bioactive compound present in the aqueous extract of *B. flabellifer* can further be utilized for its distinguishing property as a pharmaceutical agent to control the prevalence of diseases disturbing mankind.

Inspite of the fact that there is a wide range of current antibiotics available for treatment of bacterial infections, there are still some challenges to be met in microbial chemotherapy. Nature is the best remedy for all medical ailments; plants are the ultimate source of phytochemicals (8-10) combating infections and rich in antioxidants. Antioxidants present in plant leaves are a major sample source for phenolic flavanoids, which have gained particular interest because of their broad pharmacological activity having radical scavenging activity and reducing potential(11). Recent molecular studies of the enzymatic machinery in plants have made progressive understanding of complex pathways of plant natural product biosynthesis (12). Many researches around the world

emphasized on phytochemical analysis, their outcome is the development of new solutions to enumerable ubiquitous microbes such as Candida, antifungal activity in vitro against phytopathogenic fungi, Alternaria solani, Botrytis cinerea, Botrytis fabae, Fusarium oxysporum and Fusarium solani, pathogenic bacteria like Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa(12-13). In nature antioxidant sources are plenty like natural oils, berries, herbs, spices and teas which reduce the effects of free radicals, also called oxidative damage due to stress in the body . The exquisite effect of antioxidants in natural oils in flowers have also expressed their strong antibacterial effect against pathogenic Salmonella enteritidis and Mycobacterium smegmatis(14).

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