Research Article

Phytochemical Screening, *in vitro* Antioxidant and Anti-inflammatory activity of Freeze-dried *Borassus flabellifer* L. Seed Powder

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ABSTRACT

Palmyra palm tree, native to Tamil Nadu bears seasonal summer fruits (Nungu) with numerous health benefits. Preserving the fruit throughout the year is essential to enjoy its goodness and health benefits. With this focus, the aim of the present study was to evaluate the phytochemical profile, *in vitro* antioxidant and anti-inflammatory activity of *Borassus flabellifer* seed and seed coat in the freeze-dried form. Aqueous seed powder extract was used in this study. Phytochemicals were quantified using standard methods. Bioactive compounds were identified using GC-MS. Radical scavenging potential was evaluated using DPPH, Hydrogen peroxide and Nitric oxide radical scavenging assays. Anti-inflammatory potential was evaluated using Albumin denaturation assay. Statistical significance of mean values was tested using One-way ANOVA followed by Tukey's test and IC_{50} value was calculated by non-linear regression analysis using GraphPad. Saponins, tannins, terpenoids, steroids, glycosides, flavonoids, alkaloids and phenols were present in the sample. The aqueous seed powder extract exhibited potent radical scavenging activity in a dose dependent manner and the IC_{50} value of DPPH, Hydrogen peroxide and Nitric oxide radical scavenging assay was 415.91, 1080.19 and 13.489 µg/mL respectively. The aqueous extract also exhibited potent protein denaturation inhibitory activity with an IC_{50} value of 1174.9 µg/mL. Results indicate that the phytochemical compounds present in the seed powder could have largely contributed to its good antioxidant and anti-inflammatory activity.

Key words: Antioxidant, Anti-inflammatory, Freeze-dried, *Borassus flabellifer*, Phytochemicals.

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INTRODUCTION

From early historical times, easily available and widely consumed foods such as fruits and vegetables have occupied a very important part of the human diet. They are known to be storehouses of dietary fiber, vitamins, especially vitamin A and C, minerals, particularly electrolytes, phytochemicals, more importantly antioxidants.[1] *Borassus flabellifer* L. widely referred to as Nungu in Tamil and Palmyra fruit in English, is a well-known tropical fruit that is abundant in carotenoids, vitamins and minerals[2] and is widely available throughout Tamil Nadu. Literature states that, despite the nutritional richness of this fruit, it is underutilized for its health potential.[3] The Palmyra tree bears fibrous fruits within which are 3 fleshy, tender edible seed portions. Each tender seed-like portion has a thin outer covering bearing a yellowish-brown color which is often discarded before consumption.[4] Some of the reported health benefits of *Borassus flabellifer* include its ability to serve as an analgesic, antidote, wound healing, antipyretic, anthelmintic and anti-inflammatory agent.[5,6] Seasonal fruits should be processed and preserved for long-term use owing to their tremendous nutritional properties. Adopting natural methods of preservation will enhance the storage period and

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promote claimed health benefits. One such method which enables postharvest usage of *Borassus flabellifer* fruits is drying technique which is widely preferred and adopted, as it results in a food product with low moisture content, reduced microbial spoilage, extended shelf life and thereby retention of nutrient quality.[2,7] In addition, freeze-drying of foods also result in food products that are lesser in weight and volume, thereby enabling easy transport.[8] As not many studies have focused on the freeze-dried form of *Borassus flabellifer* seed and with the above said, the aim of the present study was to identify the phytochemical compounds present in *Borassus flabellifer* seed powder obtained through freeze-drying and assess its *in vitro* antioxidant and anti-inflammatory potential.

MATERIALS AND METHODS

Procurement of materials: *Borassus flabellifer* fruits were procured from a local market in Madurai city, Tamil Nadu during summer in the month of May. Semi-matured fruits were selected because of lower water content. All reagents used in the study were purchased from Sigma-Aldrich, acids and chemicals were purchased from Research Laboratory Corporations, Pune.

Preparation of extract: The tender edible seed and seed coat found within the fibrous fruits were used in the present study. The fruit seeds along with their seed coats were washed, pulped in a mixer grinder and freeze-dried using a Lyophilizer (LSI 30). Freezing of pulp took place at -20°C and drying at 65°C. The freeze-drying process was completed in 16 hr and the sample was stored in vacuum pouches at 4°C until commencement of analysis. The extract was prepared by dissolving 200mg extract in 20mL distilled water to arrive at a concentration of 10 mg/mL.

Phytochemical screening: The sample was subjected to qualitative analysis to screen for presence of phytochemicals such as saponins, tannins, terpenoids, steroids, glycosides, flavonoids, alkaloids and phenols using standard techniques.[9]

Estimation of Total Alkaloid content: 1g of sample was placed in a 100mL beaker containing 10% acetic acid in ethanol and incubated for 4 hr at room temperature. It was filtered with Whatman filter paper and the filtrate was concentrated on a water bath. Concentrated ammonium hydroxide was added until alkaloid precipitation was complete. The precipitate was collected, washed with dilute ammonia solution, dried and weighed. The percentage alkaloid was calculated by difference.[9,10]

% alkaloid= W_2-W_1/ Wt. of sample *100

Where, W_1 = Weight of empty filter paper, W_2 = Weight of filter paper + Alkaloid, Wt. = Weight of sample taken

Estimation of Total Tannin content: The method adopted by Sowmya S. *et al.*[11] was followed with minor modifications. Different concentrations (50-450µL) of tannic acid aliquots were pipetted out into a series of test tubes. Stock solution of sample was prepared by dissolving 1mg/mL of the freeze-dried seed powder in distilled water. In another test tube, 1mL of sample solution was taken and volume of all tubes were made up to 3mL with distilled water. 20% Na_2CO_3 and Folin-Ciocalteu reagent was added and incubated at room temperature for 30 min. The samples were read at 700 nm with reagent blank using a colorimeter. The TTC of the sample was determined using the tannic acid standard curve and the result is expressed as mg of tannic acid equivalent (TAE) per 100g of extract.

Estimation of Total Phenol content: Folin-Ciocalteu reagent method was used to estimate the total phenolic content of the sample.[12] About 2.5, 5, 10, 15, 20µg/mL of standard Gallic acid aliquots was pipetted out into series of test tubes. Test solution was prepared by dissolving 1mg/mL of the freeze-dried seed powder in distilled water. To another tube 100mg/mL of test solution was taken and all tubes were diluted with distilled water followed by the addition of 20% Na_2CO_3 and Folin-Ciocalteu reagent and kept undisturbed at room temperature for 30 min. The reagent blank was prepared without test and samples were read at 700 nm. The TPC is expressed as mg of gallic acid equivalent per 100g of extract.

Estimation of Total Flavonoid content: Flavonoid content was determined according to the calorimetric assay[13] with slight modifications. 1mL of sample was diluted with 200µL distilled water followed by the addition of 150µL $NaNO_3$ (5%) solution. This mixture was incubated for 5 min after which 150µL of 10% aqueous $AlCl_3$ was added and left to stand for 6 min. Following this, 2mL of 4% NaOH was added and the volume was made up to 5 mL by adding distilled water. The mixture was shaken well and left to rest for 15 min at room temperature. The absorbance was read at 510 nm and the TFC was calculated as quercetin equivalents using a calibration curve.

Gas Chromatography – Mass Spectrometry (GC-MS): 50g of sample was dissolved in 1mL GC grade Ethyl acetate and vortexed. Following this, the sample was filtered through a 0.45µ filter cartridge and injected into the GC-MS instrument (Agilent Technologies 7890B GC and 5977B MSD). The GC-MS analysis was carried out under the following conditions: Column used was DB-5ms (30m ×250µm ×0.25µm) with

Helium as the carrier gas at a flow rate of 1.0 mL/min. The oven temperature was set at 60°C–325°C. The injection temperature was set to 280°C, the injection volume was 3.0 µL in the spitless mode. The transfer line temperature was maintained at 280°C and the total run time was 41.5 min.

Components identification: The National Institute of Standards and Technology (NIST) has a wide database comprising more than 62,000 patterns and is used to interpret the mass spectrum of GC-MS.[14] The analyzed samples chromatogram of the freeze-dried *Borassus flabellifer* seed sample was integrated by qualitative analysis and compared with the NIST library.

Antioxidant activity: The absorbance value of antioxidant assays was measured using a UV visible Spectrophotometer (UV1800 Shimadzu, Japan).

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay: DPPH scavenging activity of the sample was assessed according to the method of Blois MS.[15] with slight modifications. Different concentrations (200, 400, 600, 800, 1000µg/mL) of the extract was pipetted out into a series of test tubes and stock solution of DPPH (1mM) was prepared in methanol. The volume of all tubes were made up to 1.0 mL with distilled water followed by the addition of 2 mL DPPH and kept undisturbed at room temperature in darkness for 10 min. Color change from purple (DPPH) to yellow (diphenylpicrylhydrazine) indicated the radical scavenging activity of the sample through donation of a hydrogen atom. Ascorbic acid was used as standard and the absorbance was measured at 520nm. Results are expressed as percentage inhibition of DPPH free radical.

Hydrogen peroxide radical scavenging assay: The ability of the aqueous extract to scavenge hydrogen peroxide was studied, following the method of Ruch *et al.*[16] with slight modifications. About 100, 200, 400, 600, and 800µg/mL of test solution aliquots was pipetted out into a series of test tubes and the volumes of all tubes were made up to 1.0 mL with distilled water followed by the addition of 40mM hydrogen peroxide and kept undisturbed at room temperature for 10 min. Butylated hydroxytoluene was used as the standard. The scavenging activity of the freeze-dried extract was assessed by measuring the disappearance of H_2O_2 at 230nm wavelength. Results are expressed as percentage inhibition of peroxide free radical.

Nitric Oxide Scavenging activity: The method of Marcocci *et al.*[17] with minor modifications, was followed to study the nitric oxide scavenging ability of the sample. 3 mL of reaction mixture containing 10 mm sodium nitroprusside in phosphate buffer saline and different concentrations of the extract (100-500µg/mL) was incubated at 28°C for 150 min. After incubation, 1.5 mL of the reaction mixture was removed and 1.5 mL of Griess reagent (1% sulphanilamide, 2% orthophosphoric acid, 0.1% Napthylethyline diamine hydrochloride) was added. Vitamin C was used as the standard. The absorbance of chromophore (purple azo dye) formed during the diazotization of nitrite ions with sulphanilamide and subsequent coupling with naphthylethylene-diaminedihydrochloride was measured at 546nm. Results are expressed as percentage inhibition of nitric oxide free radical.

Anti-inflammatory activity

Albumin denaturation assay: The anti-inflammatory activity of the sample was evaluated using inhibition of albumin denaturation method of Mizushima[18] and Sakat.[19] To different concentrations of the sample, 1% bovine serum albumin was added and the pH was adjusted to 6.3 using 1N HCl. This mixture was pre-incubated for 10-15 min followed by heating for 20 min. The resulting solution was cooled down to room temperature and turbidity was measured at 700nm using a UV visible Spectrophotometer (UV1800 Shimadzu, Japan). Acetyl salicylic acid was taken as positive control. Results are expressed as percentage inhibition of protein denaturation.

Statistical Analysis: SPSS software version 7 was used to analyze the study data. All assays were performed in triplicates and results are expressed as mean ± SD. Mean values were tested for significance using one-way ANOVA followed by Tukey's test. IC_{50} value was calculated by non-linear regression analysis using GraphPad.

RESULTS

Phytochemical screening: Qualitative analysis of the sample indicated the presence of biologically active compounds such as saponins, tannins, terpenoids, steroids, glycosides, flavonoids, alkaloids and phenols. Upon quantification of phytonutrients, the total tannin content of the sample was 5.80 mg TAE/100g, the total phenol content was 5.06 mg GAE/100g and the total flavonoid content was 5.1 mg QE/100g seed powder extract while the total alkaloid content was below detectable levels.

Identification of active compounds using GC-MS analysis: The GC-MS analysis of the aqueous seed powder extract revealed the presence of various phytoconstituents having pharmacological significance.[20-25] (Table 1). Some of the compounds identified

were 1 – Heptatriacotanol, 1-Monolinoleoylglycerol trimethylsilyl, Phenol, 2,4-bis(1,1-dimethylethyl)- The chromatogram of the freeze-dried sample is presented below (Figure 1).

Antioxidant activity: Free radicals are oxygen reactive species which when produced in the body, can lead to oxidative damage associated with membrane damage, aging, heart disease and cancer. Determination of antioxidant activity of plant extracts is crucial due to their ability to scavenge or deactivate these free radicals that are produced as a result of metabolic processes in the body.[26] In the present study, the free radical scavenging activity of *Borassus flabellifer* seed powder was evaluated using DPPH, H_2O_2 and NO radical scavenging assays.

Percentage inhibition exhibited by sample and standard at different concentrations for DPPH, H_2O_2 and NO radical scavenging assays are illustrated in Tables 2-4. Table 2 shows that, at lower sample concentrations the *Borassus flabellifer* seed powder had greater ability to scavenge DPPH free radical when compared to the standard. However, Tables 3 and 4 indicate that, the standards had a significantly stronger ($p<0.05$) radical scavenging ability than the seed powder at all concentrations. Maximum DPPH radical scavenging activity of the sample and standard ascorbic acid was 63.76 ± 0.69% at 1000µg/mL and 92.36 ± 0.27% at 1000µg/mL respectively. Maximum H_2O_2 radical scavenging activity of the sample was 37.13 ± 0.68% at 800µg/mL while the standard butylated hydroxytoluene exhibited maximum radical inhibition of 96.66 ± 0.08% at 800µg/mL. Similarly, maximum NO radical scavenging activity of the sample and standard vitamin C was 20.15 ±1.03% at 500µg/mL and 94.45 ± 0.05% at 500µg/mL respectively.

Results indicate that, although the radical scavenging activity of the standards were higher than the sample, the sample had the ability to scavenge all three free radicals. Tables 2-4 illustrate that the radical scavenging ability of the sample is dose dependent and it significantly increased ($p<0.05$) with increase in concentration. The free radical scavenging activity of the sample can be ranked in order of NO > DPPH > H_2O_2 based on IC_{50} value.

Anti-inflammatory activity: Table 5 represents the anti-inflammatory activity of the *Borassus flabellifer* seed powder extract and indicates that, the inhibition ability significantly increased ($p<0.05$) with increase in concentration of the sample. Maximum protein denaturation inhibition occurred at 800µg/mL concentration (41.04%) and the IC_{50} value was 1174.9 µg/mL.

DISCUSSION

Tropical fruits are widely cultivated and consumed throughout the world. These fruits are rich sources of bioactive phytochemicals among which, polyphenols are widely researched due to their ability to exert biological activity such as antioxidant and anti-inflammatory properties against obesity related oxidative stress and chronic inflammation.[27] Among the many tropical fruits such as banana, litchi, mango, papaya, passion

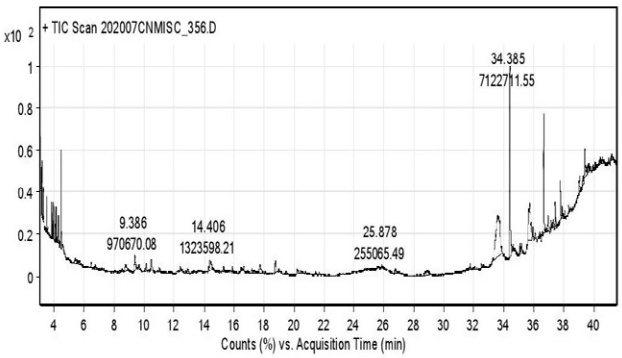

Figure 1: GC-MS chromatogram of freeze-dried *Borassus flabellifer* seed powder.

Table 1: Bioactive compounds present in aqueous extract of freeze-dried *Borassus flabellifer* seed powder (GC-MS analysis).

MF	MW(g/mol)	RT	Compound name	Pharmacological activity
$C_9H_9F_3O_2$	206.16	35.074	Phen-1,4-diol, 2,3- dimethyl-5-trifluoromethyl	Antioxidant, antithrombotic and anti-tuberculosis activity.[20]
$C_{37}H_{76}O$	537	35.717	1-Heptatriacotanol	Antioxidant, anti-inflammatory hypocholesterolemic, antimicrobial, and anticancer properties.[21,22]
$C_{27}H_{54}O_4Si_2$	498.9	36.059	1-Monolinoleoylglycerol trimethylsilyl	Antimicrobial, antioxidant, anti-inflammatory, antiarthritic, antiasthma and diuretic activity.[23]
$C_{14}H_{22}O$	206.32	10.486	Phenol, 2,4-bis(1,1-dimethylethyl)-	Antioxidant and antibacterial properties.[24]
$C_{22}H_{46}O_3Si$	386.7	34.385	Hexadecanoic acid, 3-[(trimethylsilyl) oxy]propyl ester	Antioxidant and antimicrobial activity.[25]

Table 2: DPPH radical scavenging activity of freeze-dried *Borassus flabellifer* seed powder.

S. No	Concentration (µg/mL)	% inhibition of free radicals	
		***Borassus flabellifer* seed powder**	**Ascorbic acid**
1	10	41.67 ± 0.50 [a, *]	12.89 ± 0.02[a]
2	50	46.89 ± 0.65 [a, *]	29.00 ± 0.04[b]
3	100	55.16 ± 0.66[b, *]	41.02 ± 0.04[c]
4	500	61.26 ± 0.80[c]	69.35 ± 0.41[d]
5	1000	63.76 ± 0.69[c]	92.36 ± 0.27[e, *]

Mean ±Standard deviation of three independent estimations
[a-e] Values followed by the different superscripts are significantly different within the column
[*]Values are significantly different within the row, $p<0.05$

Table 3: Hydrogen peroxide radical scavenging activity of freeze-dried *Borassus flabellifer* seed powder.

S. No	Concentration (µg/mL)	% inhibition of free radicals	
		***Borassus flabellifer* seed powder**	**Butylated hydroxytoluene**
1	100	0[a]	48.39 ± 0.15[a]
2	200	3.01 ± 1.02[b]	63.12 ± 0.08[b, *]
3	400	12.41 ± 1.00[c]	71.30 ± 0.04[c, *]
4	600	26.20 ± 0.83[d]	81.12 ± 0.09[d, *]
5	800	37.13 ± 0.68[e]	96.66 ± 0.08[e, *]

Mean ±Standard deviation of three independent estimations
[a-e] Values followed by the different superscripts are significantly different within the column
[*]Values are significantly different within the row, $p<0.05$

Table 4: Nitric oxide radical scavenging activity of freeze-dried *Borassus flabellifer* seed powder.

S. No	Concentration (µg/mL)	% inhibition of free radicals	
		***Borassus flabellifer* seed powder**	**Vitamin C**
1	100	7.82 ± 1.48[a]	59.47 ± 0.04[a, *]
2	200	10.70 ± 0.81[b]	65.00 ± 0.05[b, *]
3	300	12.26 ± 1.06[c]	76.15 ± 0.15[c, *]
4	400	12.26 ± 1.09[c]	83.74 ± 0.03[d, *]
5	500	20.15 ± 1.03[d]	94.45 ± 0.05[e, *]

Mean ±Standard deviation of three independent estimations
[a-e] Values followed by the different superscripts are significantly different within the column
[*]Values are significantly different within the row, $p<0.05$

fruit and pineapple, *Borassus flabellifer* is one such fruit, locally available in Tamil Nadu. Hence, the aim of the present study was to evaluate the phytochemical profile, *in vitro* antioxidant and anti-inflammatory activity of the *Borassus flabellifer* seed in the freeze-dried form.

Phytochemical screening serves as a baseline for identification of pharmacological activities of plant extracts like antioxidant, anticancer, anti-inflammatory and antimutagenic.[28] The compounds present in the aqueous *Borassus flabellifer* seed powder extract were identified using Gas Chromatography-Mass Spectrometry and were found to possess various pharmacological activities that are listed in (Table 1). Results of the qualitative and quantitative phytochemical analysis indicate that the freeze-dried *Borassus flabellifer* seed powder is a rich reservoir of bioactive compounds which can positively influence its antioxidant and anti-inflammatory potential. In support to the findings of the present study, previously conducted studies identified the presence of phytochemicals such as such as tannins,

Table 5: Anti-inflammatory activity of freeze-dried *Borassus flabellifer* seed powder.

S. No	Concentration (µg/mL)	% inhibition of free radicals	
		***Borassus flabellifer* seed powder**	**Acetyl salicylic acid**
1	100	6.47 ± 0.39[a]	39.24 ± 0.07[a, *]
2	200	11.19 ± 0.81[b]	46.56 ± 0.02[b, *]
3	400	26.49 ± 0.81[c]	54.38 ± 0.01[c, *]
4	600	33.70 ± 0.32[d]	61.32 ± 0.01[d, *]
5	800	41.04 ± 1.04[e]	72.86 ± 0.02[e, *]

Mean ±Standard deviation of three independent estimations

[a-e]Values followed by the different superscripts are significantly different within the column

[*]Values are significantly different within the row, $p<0.05$

flavonoids, saponins, glycosides and terpenoids in the *Borassus flabellifer* seed coat[29] and alkaloids, flavonoids, glycosides, saponins, tannins, phytosterols, triterpenoids, phenols in the immature *Borassus flabellifer* fruits, stating that these bioactive components have contributed to their excellent antioxidant potential.[30] Results of the present study explain that, although the *Borassus flabellifer* seed and seed coat were subjected to freeze-drying technique, the phytochemicals have been retained.

Consumption of fruits is known to protect the human body from several diseases like cancer, diabetes, neurodegenerative diseases, heart and brain vascular diseases. This is because they possess protective properties due to the presence of antioxidants which protects the cells and their structures from oxidative damage.[31] Antioxidants are considered powerful since they can neutralize the toxic effects caused by free radicals through different mechanisms like binding of transition metal ion catalyst, preventing chain initiation reactions and reducing capacity/power.[32] This being said, it is important to incorporate natural antioxidant sources in the diet instead of synthetic antioxidants and drugs which can lead to health risks.

The antioxidant activity of the *Borassus flabellifer* seed powder was quantified using DPPH, H_2O_2 and NO free radical scavenging assays. Results indicate that the standards were more effective in scavenging the free radicals than the study sample. However, among the free radicals, NO was most effectively scavenged by the sample as indicated by the IC_{50} value since, smaller the IC_{50} value, greater the antioxidant activity. The IC_{50} value of Nitric oxide radical scavenging assay was 13.489 µg/mL while the IC_{50} value of DPPH and Hydrogen peroxide radical scavenging assay was 415.91 µg/mL and 1080.19 µg/mL respectively. Nitric oxide, an important chemical mediator is generated in the body by specific nitric oxide synthesis (NOSs). This metabolizes arginine to citralline and forms NO through a five-electron oxidative reaction. Excess NO formation in the body can be alarming as it leads to the onset of numerous diseases.[33] Fruits, being natural sources of antioxidants can serve as potent therapeutic agents in scavenging NO and regulating the pathological conditions caused by excess nitric oxide generated along with its oxidative product.[34] Phenolic compounds present in fruits are often associated with their antioxidant capacity. Among these phenolic compounds, flavonoids are known to have strong antioxidant potential[35] and the TFC of *Borassus flabellifer* seed powder is 5.1 mg QE/100g. Wijewardana *et al.*[2] stated that, the antioxidant activity of *Borassus flabellifer* fruit pulp powder obtained through different drying techniques was determined and it was found that, the freeze-dried sample exhibited strong radical scavenging activity among others. Similarly, the present study highlights that the freeze-dried *Borassus flabellifer* seed powder exhibits potent antioxidant activity and this can be due to the presence of flavonoids, tannins and other phenolic compounds in the sample.[34]

Table 5 shows that although the standard exhibits a stronger anti-inflammatory activity than the sample, it can be safely stated that the *Borassus flabellifer* seed powder exhibits fair anti-inflammatory activity. This activity can be attributed to the presence of flavonoids[36] and other phenolic compounds such as alkaloids, tannins, steroids, phenols in the sample.[37] During inflammation, free radicals produce reactive oxygen species (ROS) causing oxidative stress to the body, leading to cellular damage. Steroid drugs, nonsteroidal anti-inflammatory drugs (NSAIDs) and immunosuppressant's are generally used to treat inflammatory diseases and requires long-term treatment resulting in side effects such as bleeding gastrointestinal and peptic ulcers.[38] To overcome this, alternative therapy is important. Secondary metabolites from plants can be used to treat inflammation and pain.[39] It can be inferred from the study results that the seed and seed coat of the *Borassus flabellifer* fruit can be

a good alternative to synthetic drugs for treating pain, protein denaturation and vascular permeability which accompanies inflammation.[40]

CONCLUSION

The present study highlights that the aqueous extract of *Borassus flabellifer* seed and seed coat when freeze-dried exhibited good antioxidant and anti-inflammatory activity. This can be attributed to their rich phytochemical profile. Hence, freeze-dried fruit powders can be considered an important functional food. However, it is important to study other *in vitro* aspects of the freeze-dried *Borassus flabellifer* seed powder like antimicrobial, anti-diabetic, anti-cancer and hypocholesterolemic activity. Further, human interventional studies should be carried out to validate the *in vitro* therapeutic activities of the *Borassus flabellifer* seed.

Authors Contributions

Design of study protocol - Saira Mariam Banu[1], Nora Vigasini. K[1*]

Data analysis and interpretation - Saira Mariam Banu[1], Shanmugapriya Surenderan[2]

Writing of manuscript - Saira Mariam Banu[1], Nora Vigasini. K[1*]

Critical revision of the manuscript - Nora Vigasini. K[1*], Shanmugapriya Surenderan[2]

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

DPPH: 2, 2-diphenyl- 1 – picrylhydrazyl; **H_2O_2:** Hydrogen peroxide; **NO:** Nitric oxide; **GC-MS:** Gas Chromatography-Mass Spectrometry; **TTC:** total tannin content; **TPC:** total phenol content; **TFC:** total flavonoid content; **GAE:** gallic acid equivalents; **TAE:** tannic acid equivalents; **QE:** quercetin equivalents; **RT:** retention time; **MF:** molecular formula; **MW:** molecular weight.

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