

# Impact of drying processes on *Bryophyllum pinnatum* phenolic constituents and its anti-inflammatory and antioxidative activities in human erythrocytes

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## Abstract

The effect of drying on the phytoconstituents, antioxidative, and anti-inflammatory properties of *Bryophyllum pinnatum* leaves was investigated. The phenolic constituents were characterized using HPLC-DAD. The aqueous extraction was done and various assays (Inhibition of membrane stabilization, albumin Denaturation and heat-induced hemolysis, malondialdehyde (MDA), and reduced glutathione (GSH) contents, as well as superoxide dismutase (SOD) activity), were carried out on human erythrocytes. The fresh portion (89.12 µg/ml) exhibited the highest potential to inhibit heat-induced hemolysis compared to the standard drug—Diclofenac (91.51 µg/ml). Freeze-dried sample showed the highest inhibitory potential on albumin denaturation ([Freeze-dried-330.72 µg/ml], [Diclofenac-318.63 µg/ml]) and membrane destabilization ([Freeze-dried-331.93 µg/ml], [Diclofenac-289.57 µg/ml]) when compared with Diclofenac. Similarly, the freeze-dried sample showed the highest GSH and SOD level and lowest MDA level when human erythrocytes challenged with tertiary butyl hydroperoxide (tBHP) were treated with the extract. This study confirms the retention of a considerable quantity of bioactive constituents of plants when freeze-dried.

## Practical applications

The ideal method of drying *Bryophyllum pinnatum* and possible anti-inflammatory potential was investigated. This work may apply to the development of anti-inflammatory agents from a natural source with little or no side effect in managing inflammation.

## KEYWORDS

anti-inflammatory, antioxidative, *Bryophyllum pinnatum*, drying, phenolics

## 1 | INTRODUCTION

Drying is the most common way of preserving medicinal herbs through the removal of moisture content (Poos & Varju, 2017). Drying reduces the quality of active ingredient, color, flavor, and aroma, inhibits microbial growth (Oztekin & Martinov, 2007), reduces antioxidant properties (Oboh, Ademiluyi, & Omojokun, 2017), and alter the phenolic constituents of the medicinal plants (Omojokun, Oboh,

& Ademiluyi, 2018). To maintain the quality of most plant herbs during storage the use of various methods of drying (e.g., vacuum drying, cabinet/air drying, freeze-drying, oven drying, and so forth) is needed, the difference in the different drying methods is the energy demand required to remove the moisture content (Mahapatra & Nguyen, 2007) in a bid to improve the plant foods' shelf life. In most developing countries, various plants of indigenous origin are used to cure many diseases in herbal medicine (Okwu & Josiah, 2006).

*Bryophyllum pinnatum*, a perennial herb with common names of cathedral bells, miracle leaf, leaf of life, Good luck leaf, and so on (Jain, Patel, Shah, Patel, & Joshi, 2010; Nagaratna & Prakash, 2015) belongs to the family Crassulaceae. Studies have confirmed the antimicrobial property, antihypertensive potential, antidiabetic activity, wound healing property, hepatoprotective activity, and anti-inflammatory properties (Ojewole, 2002) of *B. pinnatum*. The various health-promoting potential of the plant is attributed to the numerous bioactive constituents present in it. Bufadienolides, a vital component identified with *B. pinnatum*, were reported to possess antibacterial, antitumor, cancer preventive, and insecticidal action (Muhammad et al., 2012). Other phytochemicals present in the plant include alkaloids, saponins, flavonoids, and tannins (Nwali, Okaka, Ibiam, & Aja, 2012). The phytoconstituents (e.g., flavonoids and phenolic acids) have been reported to potentiate anti-inflammatory and antioxidative properties. (Okwu & Josiah, 2006).

Inflammation is defined as the arranged cascade of changes in the cells and fluids of living tissue responding to injury (Stankov, 2012). About 2000 years ago, Celsius proposed four principal effects of the inflammatory response which are: Tissue tumor-swelling, elevated tissue temperature, redness of vascular tissue at the site of inflammation, and intensive noxious stimulus sensation (Stankov, 2012). A brief response which results in healing is termed acute inflammation. In contrast, chronic inflammation is an extended, dysregulated, and inadequate response that involves active inflammation, tissue destruction, and tissue repair attempts (Zhou, Hong, & Huang, 2016). Conventional treatments of inflammation include the use of corticosteroids, Nonsteroidal Anti-Inflammatory Drugs [NSAIDS], and herbs (Christian, 2007). Recent reports presented various complications (e.g., renal failure) associated with the frequent use/consumption of NSAIDS (Zhang, Donnan, Bell, & Guthrie, 2017). Given this, the need for alternative therapy is sacrosanct, hence our reason to explore this potential in *B. pinnatum*, having confirmed that regulated consumption of medicinal plants is presented with minimal side effects. *B. pinnatum*, like other medicinal herbs and plant materials, are usually eaten raw or processed into dried form when it's offseason for all year round availability (Omojokun et al., 2018). However, information is scanty on the effects of drying on its biological activities, especially the anti-inflammatory and antioxidative potential of this plant. Hence, this study is aimed at investigating the influence of various drying methods (freeze-drying, sun-drying, oven-drying, and air-drying) on the phytoconstituents as well as the anti-inflammatory and antioxidative potential of *B. pinnatum* leaves using some in vitro analysis.

## 2 | MATERIAL AND METHODS

### 2.1 | Materials

#### 2.1.1 | Sample collection and preparation

*B. pinnatum* leaves were plucked from a farm orchard in Akure, Ondo State, Nigeria. Authentication was done at the Department of Crop,

Soil and Pest Management, Federal University of Technology, Akure, Nigeria. The leaves were rinsed under a running tap, weighed, and then, divided into five equal portions viz; the first portion was sun-dried to constant weight using direct sunlight at an average temperature of 35°C, the second portion was oven-dried to constant weight using a laboratory heat drying oven at a temperature of 40°C, the third portion was freeze-dried at -4°C, the fourth portion was air-dried to constant weight at room temperature (approximately 25°C) kept away from the intensity of the sun while the fresh portion (fifth portion) was pulverized, filtered, and kept in the laboratory refrigerator for further analysis. The dried samples were milled into a fine powder and kept in an airtight container for further analysis.

#### 2.1.2 | Chemicals and reagents

All chemical used were of analytical grade. Acetonitrile, formic acid, acetic acid, caffeic acid, and *p*-coumaric acid were purchased from Merck (Darmstadt, Germany). Quercetin, Catechin, Rutin, Luteolin-7-O- $\beta$ -D-glycoside, Luteolin, and other chemicals were acquired from Sigma Chemical Co. (St. Louis, MO, USA). The water used was glass distilled.

## 2.2 | Methods

### 2.2.1 | Aqueous extract preparation

The aqueous extracts of the dried samples were subsequently prepared by soaking 5 g of the ground samples in 100 ml of distilled water for 24 hr at 37°C. The mixture was later filtered through Whatman No. 2 filter paper, and the filtrate was then stored in the refrigerator for subsequent analysis.

### 2.2.2 | High-performance liquid chromatography—diode array detector (HPLC-DAD) characterization

The slightly modified method described by Carvalho et al. (2016) was followed to identify and quantify the phenolic acids and flavonoids in *B. pinnatum*. *B. pinnatum* extracts at 12 mg/ml was injected onto reversed phase Phenomenex C<sub>18</sub> column (4.6 mm × 250 mm) packed with 5  $\mu$ m diameter particles. The mobile phases were 0.5% (v/v) aqueous formic acid (solvent A) and 1% (v/v) acetic acid in acetonitrile (solvent B). The binary elution system was as follows: 2% B at initial 5 min to wash the column, a linear gradient was 8% B (25 min), 12% B (45 min), and 24% B (60 min). After 80 min, the organic phase concentration was brought back to 2% (B) and lasted 10 min for column equilibration. Flow rate of 0.6 ml/min and injection volume 40  $\mu$ l. Quantifications were carried out by integration of the peaks using the external standard method, at 280 nm for catechin; 327 for caffeic acid and *p*-coumaric acid; and 366 for quercetin, rutin, luteolin-7-O- $\beta$ -D-glycoside, and luteolin.

The wavelengths used were 254 nm for gallic acid and ellagic acid; 280 nm for catechin; 327 for chlorogenic acid and caffeic acid; and 356 nm rutin, quercetin, and luteolin. The extract and mobile phase were filtered through 0.45  $\mu\text{m}$  membrane filter (Millipore), and then, degassed by ultrasonic bath prior to use. Stock solutions of standards references were prepared in the HPLC mobile phase at a concentration range of 0.025–0.500 mg/ml. Chromatography peaks were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200 to 700 nm). All chromatography operations were carried out at ambient temperature and in triplicate.

### 2.2.3 | Inhibition of albumin denaturation

The anti-inflammatory activity via the inhibition of albumin denaturation was carried out by modifying the method of (Odeyemi, Anthony, & Graeme, 2015). The absorbance was read at 660 nm, and the percentage of protein inhibition was calculated by the equation:

$$\% \text{ Inhibition} = (1 - A/A^{\circ}) \times 100.$$

where A = absorbance of the test sample and  $A^{\circ}$  = absorbance of control.

### 2.2.4 | Preparation of human erythrocytes

Blood of twenty (20) healthy human subjects (both genders, 18–35 years old) with no history of previous chronic diseases and not under treatment for any current chronic or acute diseases (as self-declared by the volunteers), was collected in heparinized tubes. The study was approved by the Animal and Research Ethical Committee of the Faculty of Basic and Applied Sciences, Elizade University, Ilara-mokin. Erythrocytes were isolated by centrifugation at 4,000 rpm for 10 min at room temperature and washed three times with 6.6 mM of phosphate buffer, pH 7.4, containing 150 mM of NaCl.

### 2.2.5 | Human red blood cells (HRBC) membrane stabilization

The principle concerned in this method was the stabilization of the HRBC membrane by hypotonicity-induced membrane lysis. Blood was collected (2ml) from healthy volunteers who had not taken any NSAID for 2 weeks before the experiment and mixed with an equal volume of sterilized Alsevers solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% NaCl in distilled water) and centrifuged at 3,000 rpm. The hemoglobin contents of the supernatant solution were estimated spectrophotometrically at 560 nm (Gupta, Chauhan, Prakash, & Mathur, 2013).

The percentage of HRBC membrane stabilization was after that calculated as:

$$\% \text{ Protection} = 100 - \frac{\text{Optical density of drug treated sample}}{\text{Optical density of control}} \times 100.$$

### 2.2.6 | Heat-induced hemolysis

The reaction mixture (2 ml) consisting of 1 ml of the test sample solution and 1 ml of 10% red blood cells suspension; instead of the test sample only saline was added to the control test tube. Diclofenac sodium was taken as a standard drug. The method described by Gupta et al., 2013 was followed, and the absorbance of the supernatants taken at 560 nm (Gupta et al., 2013). Thereafter, the percent hemolysis was calculated.

$$\% \text{ Hemolysis} = \frac{\text{OD of test}}{\text{OD of control}} \times 100.$$

$$\% \text{ Protection} = 100 - \frac{\text{OD of test}}{\text{OD of control}} \times 100.$$

### 2.2.7 | Treatment of human erythrocytes

Human erythrocytes (400  $\mu\text{l}$ ) and 350  $\mu\text{l}$  of phosphate-buffered saline (PBS) were mixed in the presence or absence of 250  $\mu\text{l}$  of the oxidative stress inducer; tBHP (4 mM; tBHP will be prepared in 6.6 mM phosphate buffer, pH 7.4, containing 150 mM NaCl). To the mixture, 333  $\mu\text{l}$  of different concentrations of aqueous *B. pinnatum*. (10, 100, and 1,000  $\mu\text{g}/\text{ml}$ ) was added, and the mixture was incubated for 2 hr at 37°C. Untreated erythrocytes incubated in PBS was used as the control group, while those incubated in PBS in the presence of 4 mM tBHP served as the positive control. Erythrocytes suspension was used for the determination of malondialdehyde (MDA) and reduced glutathione (GSH) content. After incubation, the biochemical analysis was performed.

### 2.2.8 | Determination of lipid peroxidation

Lipid peroxidation was determined by measuring the MDA content. After incubation, treated and untreated erythrocytes were added to 300  $\mu\text{l}$  of distilled water, 300  $\mu\text{l}$  of 40% TCA, and 500  $\mu\text{l}$  of 0.8% TBA. The mixture was heated in a water bath for 1 hr. After cooling, the mixture was centrifuged at 3,000g for 10 min, and the MDA content was measured in the supernatant at 540 nm using a spectrophotometer (Ohkawa, Ohishi, & Yagi, 1979).

### 2.2.9 | Determination of nonprotein thiols (NPSH)

The level of NPSH (i.e., GSH) in the erythrocytes was determined by the modified method of Ellman (Ellman, 1959). After incubation,

erythrocytes were deproteinated by adding 1 ml of TCA (40%), and the mixture was centrifuged at 2,000 rpm for 5 min. About 20  $\mu$ l of 5,5-dithiobis (2-nitrobenzoic acid) (DTNB, 10 mM final concentration) was added to 100  $\mu$ l of potassium phosphate buffer and the formation of 5-thio-2-nitrobenzoic acid, which was proportional to reduced GSH concentration, was monitored at 412 nm using a spectrophotometer. The result was expressed as a percentage of control.

### 2.2.10 | Determination of superoxide dismutase activity

Briefly, 0.1 ml of the erythrocytes was diluted in 0.9 ml of distilled water to make 1 in 10 dilutions. An aliquot of 0.2 ml of the diluted microsome was added to 2.5 ml of 0.05 M carbonate buffer pH 10.2 to equilibrate in a cuvette, and the reaction started by the addition 0.3 ml of 0.3 M of adrenaline. The reference cuvette contained 2.5 ml of carbonate buffer, 0.3 ml of substrate (adrenaline) and 0.2 ml of distilled water. The increase in absorbance at 480 nm was monitored (Fridovich, 1995).

## 2.3 | Data analysis

Analysis of variance model with the level of significance set to  $p < .05$  was used for HPLC characterization statistical analysis. These analyses were performed using the free software R version 3.1.1. (R Core Team, 2014). Also, the results of replicate experiments were pooled and expressed as mean  $\pm$  standard deviation (SD). A one-way analysis of variance (ANOVA) was used to analyze the mean, and the post hoc treatment was performed using Duncan multiple range test. Significance was accepted at  $p < .05$  (Zar, 1984). The E.C.<sub>50</sub> (extract concentration causing 50% inhibition/antioxidant activity) was performed using nonlinear regression analysis.

## 3 | RESULT

The fresh and differently dried *B. pinnatum* extract characterized using HPLC-DAD showed that catechin (peak a), caffeic acid (peak b), *p*-coumaric acid (peak c), rutin (peak d), quercetin (peak e), luteolin-7-O- $\beta$ -D-glycoside (peak f), and luteolin (peak g) in addition to other minor compounds were identified and quantified as shown in Figure 1. However, the most predominant flavonoid is found to be Rutin with the highest content in the fresh sample (8.65 mg/g) followed by the freeze-dried sample (7.84) while sun-dried (4.86 mg/g) and air-dried (4.87 mg/g) samples had the least rutin content with no significant difference in the values as shown in Table 1. Also, caffeic acid was found to be absent in the sun-dried and air-dried sample, while only the oven-dried sample had Luteolin-7-O- $\beta$ -D-glycoside.

Figure 2 represents the effect of drying on *B. pinnatum* inhibition of albumin denaturation using Diclofenac as a standard drug. The result revealed that the extracts inhibited albumin denaturation in a

dose-dependent manner (0–428.78  $\mu$ g/ml). Interestingly, there was no significant difference ( $p > .05$ ) at the maximum inhibitory concentration (500  $\mu$ g/ml) between freeze-dried *B. pinnatum* (76.27%) and standard drug Diclofenac sodium (76.87%). Furthermore, as revealed by the EC<sub>50</sub> values (Table 2), the air-dried sample exhibited the least inhibitory activity. At the same time, freeze-dried (330.72  $\mu$ g/ml) had the highest inhibitory effect of all the drying methods compared with Diclofenac used as the standard drug (318.63  $\mu$ g/ml).

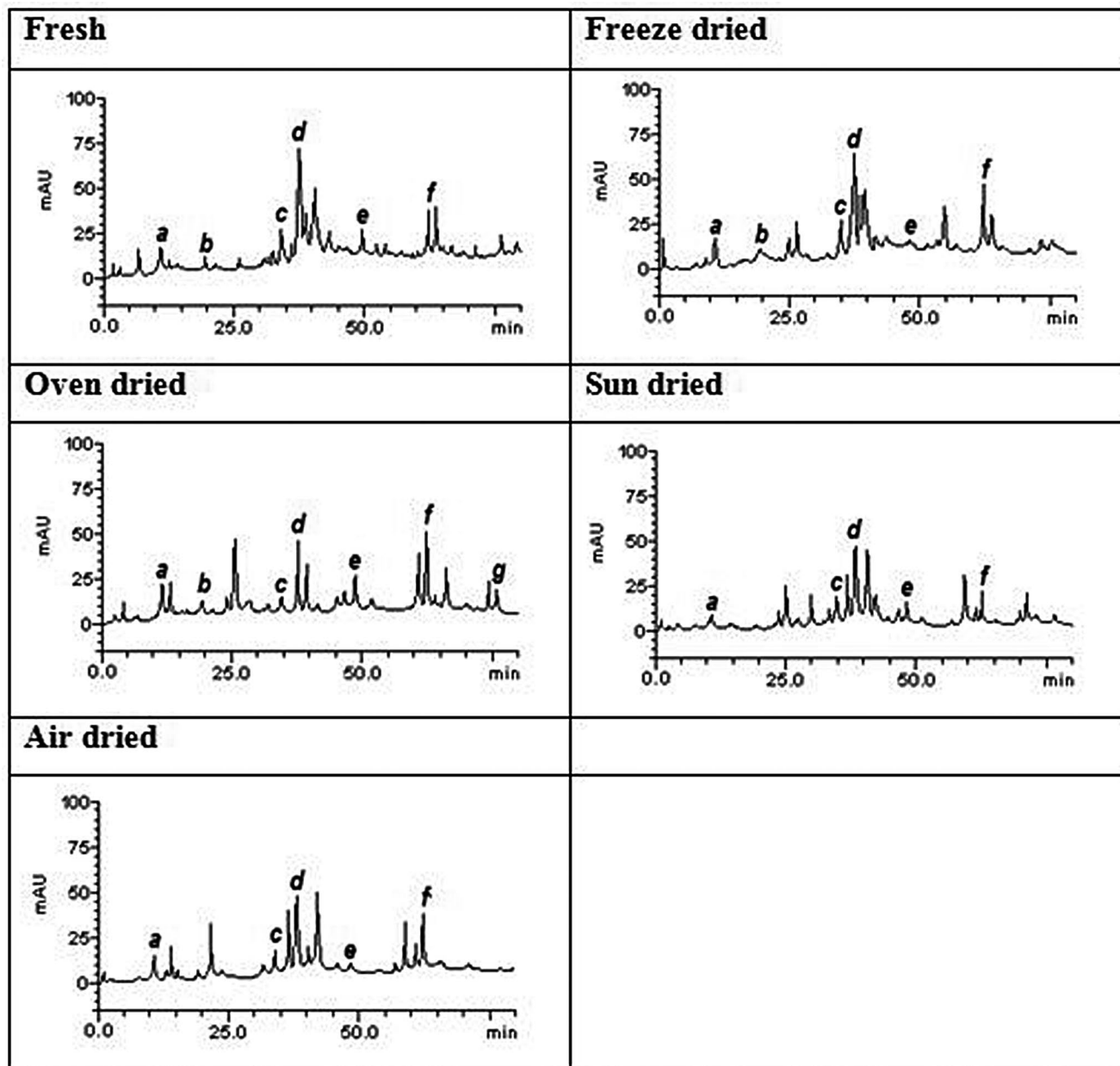
Figure 3 represents the human red blood cells (HRBC) membrane stabilization potential of aqueous extract of differently *B. pinnatum*. The result showed that the extracts stabilized the membrane in a dose-dependent manner (0–397.40  $\mu$ g/ml). As revealed by the EC<sub>50</sub> values (Table 2), air-dried sample (397.40  $\mu$ g/ml) exhibited the least stabilization potential while freeze-dried (331.93  $\mu$ g/ml) had the highest of all the drying methods as compared with Diclofenac used as the standard drug (289.57  $\mu$ g/ml).

Presented in Figure 4 is the result of an assay depicting the potential of the aqueous extract of differently dried *B. pinnatum* leaves to reverse heat-induced hemolysis in HRBC. Interestingly, the fresh extract (89.12  $\mu$ g/ml) showed the highest potential even when compared with the standard drug Diclofenac (91.51  $\mu$ g/ml). However, there was no significant ( $p > .05$ ) difference in their hemolysis inhibitory potential. As observed in previous results, air-dried (128.23  $\mu$ g/ml) sample still followed the same trend of having the lowest potential, as shown in Table 2.

As depicted in Figure 5, tBHP-induced oxidative stress caused a significant increase in the MDA level in comparison to the control ( $p < .05$ ). Interestingly, the rise in the MDA level was markedly attenuated in the presence of differently dried *B. pinnatum* extracts. However, freeze-dried *B. pinnatum* extract exhibited the highest reduction potential, while the air-dried had the least. The ability of *B. pinnatum* extract to reverse cellular degradation in the human erythrocytes when challenged with tertiary butyl hydroperoxide (tBHP) was assessed. The cellular level of nonprotein thiol (GSH) and superoxide dismutase (SOD) reduced significantly ( $p < .05$ ) when challenged with tBHP as compared with the control. The co-incubation of erythrocytes with the stressor (tBHP) increased the GSH and SOD levels significantly ( $p < .05$ ). However, the level of increase is significantly ( $p < .05$ ) lower than that of the control, as shown in Figures 6 and 7, respectively. The freeze-dried closely followed by the fresh sample had the highest increase in GSH and SOD levels after the human erythrocytes challenged with tBHP was treated with *B. pinnatum* extracts while there was no significant difference ( $p > .05$ ) between the GSH and SOD levels of oven-dried, sun-dried, and air-dried. This finding indicates that differently dried *B. pinnatum* extracts inhibited the exogenous oxidative stress-mediated by tBHP to increase GSH and SOD levels.

## 4 | DISCUSSION

The use of *B. pinnatum* leaves in wound treatment, hypertension, and various diseases are explored and established in folklore. Supporting



**FIGURE 1** High performance liquid chromatography characterization of phenolics and flavonoids profile of *B. pinnatum* extracts. Catechin (peak a), caffeic acid (peak b), *p*-coumaric acid (peak c), rutin (peak d), quercetin (peak e), luteolin (peak f) and luteolin-7-O- $\beta$ -D-glycoside (peak g)

**TABLE 1** Phenolics and flavonoids composition of *Bryophyllum pinnatum* leaf extracts

	Fresh	Freeze dried	Oven dried	Sun dried	Air dried
Compounds	mg/g	mg/g	mg/g	mg/g	mg/g
Catechin	1.32 $\pm$ 0.05 <sup>b</sup>	2.65 $\pm$ 0.01 <sup>a</sup>	2.59 $\pm$ 0.01 <sup>a</sup>	1.13 $\pm$ 0.04 <sup>c</sup>	1.49 $\pm$ 0.03 <sup>b</sup>
Caffeic acid	0.49 $\pm$ 0.01 <sup>c</sup>	1.37 $\pm$ 0.03 <sup>a</sup>	1.18 $\pm$ 0.01 <sup>b</sup>	-	-
<i>p</i> -Coumaric acid	2.78 $\pm$ 0.02 <sup>a</sup>	2.71 $\pm$ 0.02 <sup>a</sup>	1.13 $\pm$ 0.04 <sup>c</sup>	1.42 $\pm$ 0.01 <sup>b</sup>	1.08 $\pm$ 0.05 <sup>c</sup>
Rutin	8.65 $\pm$ 0.03 <sup>a</sup>	7.84 $\pm$ 0.01 <sup>a</sup>	4.95 $\pm$ 0.02 <sup>b</sup>	4.86 $\pm$ 0.03 <sup>b</sup>	4.87 $\pm$ 0.01 <sup>b</sup>
Quercetin	1.29 $\pm$ 0.01 <sup>b</sup>	1.03 $\pm$ 0.04 <sup>c</sup>	2.53 $\pm$ 0.01 <sup>a</sup>	1.39 $\pm$ 0.02 <sup>b</sup>	0.61 $\pm$ 0.02 <sup>d</sup>
Luteolin	3.26 $\pm$ 0.01 <sup>c</sup>	5.02 $\pm$ 0.02 <sup>a</sup>	5.12 $\pm$ 0.05 <sup>a</sup>	2.17 $\pm$ 0.01 <sup>d</sup>	4.37 $\pm$ 0.04 <sup>b</sup>
Luteolin-7-O- $\beta$ -D-glycoside	-	-	1.39 $\pm$ 0.03 <sup>a</sup>	-	-

Note: Results are expressed as mean  $\pm$  SE of three determinations. Averages followed by different letters differ by Tukey test at  $p < .05$ .

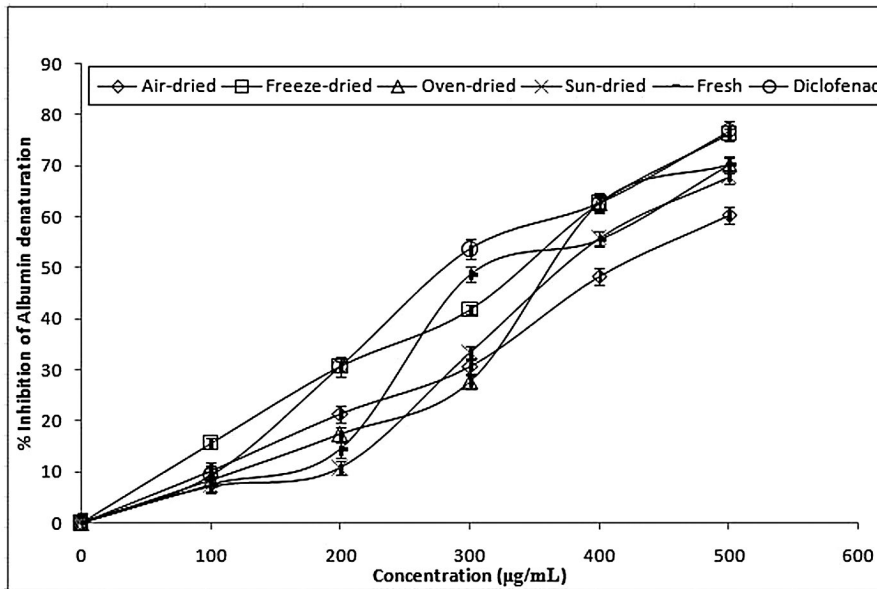


FIGURE 2 Effect of drying on *B. pinnatum* leaf inhibition of albumin denaturation

Sample	EC <sub>50</sub> values (µg/ml)		
	Inhibition of albumin denaturation	Membrane stabilization	Inhibition of heat-induced hemolysis
Diclofenac	318.63 ± 7.7 <sup>a</sup>	289.57 ± 8.3 <sup>a</sup>	91.51 ± 3.7 <sup>a</sup>
Freeze-dried	330.72 ± 6.3 <sup>b</sup>	331.93 ± 6.9 <sup>b</sup>	105.53 ± 3.7 <sup>b</sup>
Oven-dried	377.93 ± 5.7 <sup>d</sup>	405.85 ± 5.8 <sup>d</sup>	121.46 ± 4.7 <sup>c</sup>
Sun-dried	393.81 ± 7.1 <sup>d</sup>	369.24 ± 3.7 <sup>c</sup>	116.39 ± 3.7 <sup>c</sup>
Air-dried	428.78 ± 8.7 <sup>e</sup>	397.40 ± 8.7 <sup>d</sup>	128.23 ± 5.2 <sup>d</sup>
Fresh	364.35 ± 6.7 <sup>c</sup>	370.54 ± 3.6 <sup>c</sup>	89.12 ± 3.2 <sup>a</sup>

TABLE 2 EC<sub>50</sub> values of the effects of drying methods on *Bryophyllum pinnatum* membrane stabilization and inhibition of albumin denaturation and heat-induced hemolysis

Note: Values represent mean ± standard deviation (n = 3). Values with the same superscript number on the same column are not significantly ( $p < .05$ ) different.

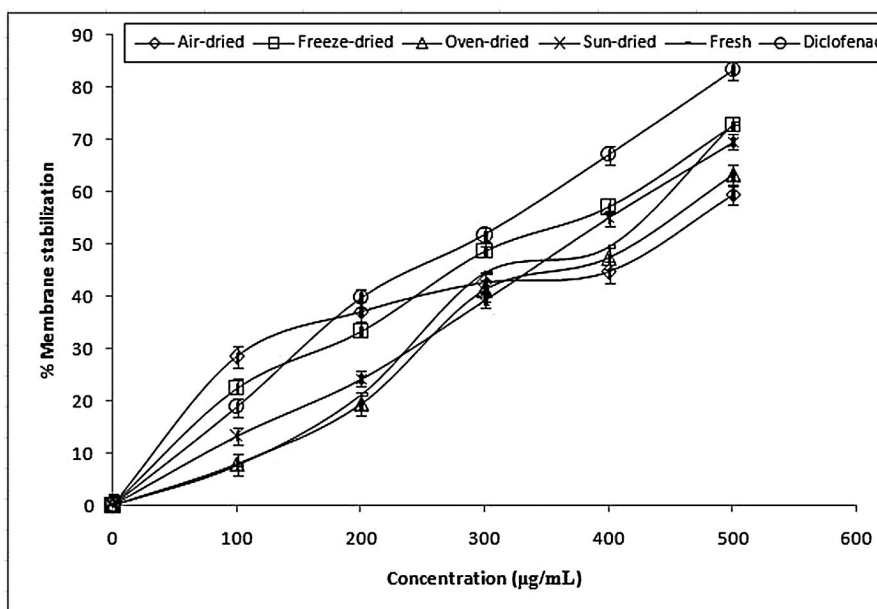


FIGURE 3 Effect of drying on *B. pinnatum* leaf membrane stabilizing potential in human erythrocytes

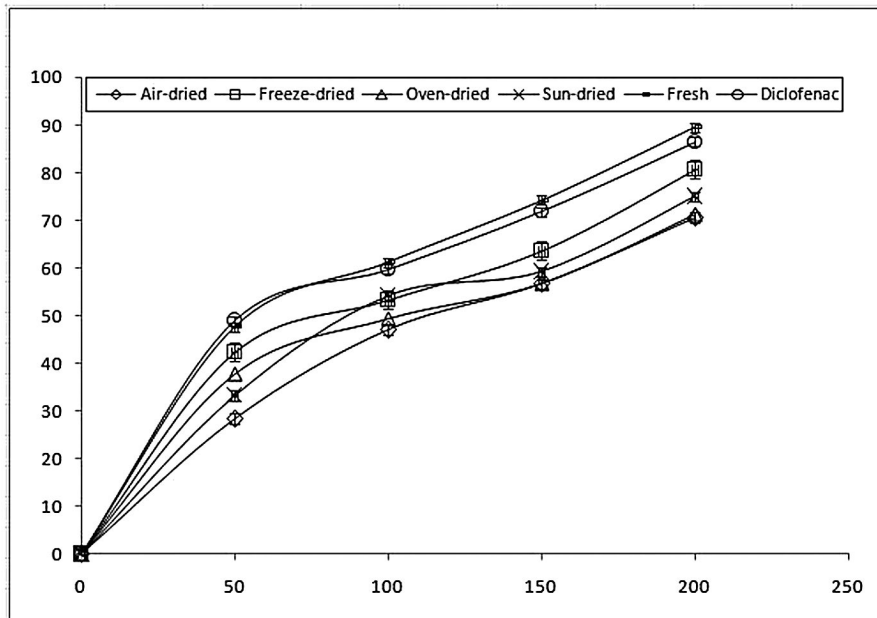


FIGURE 4 Effect of drying of *B. pinnatum* leaf on the protection of hemolysis in human erythrocytes

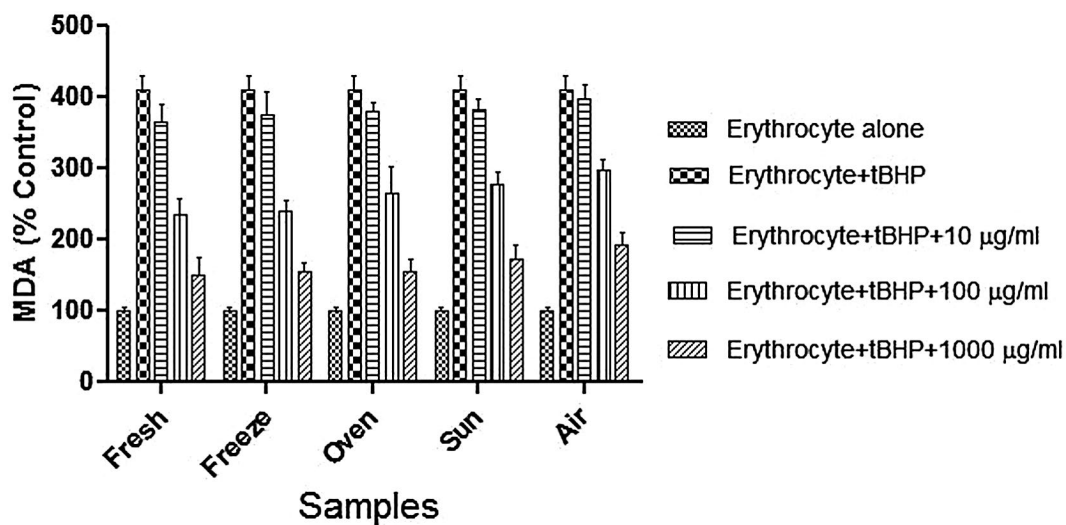
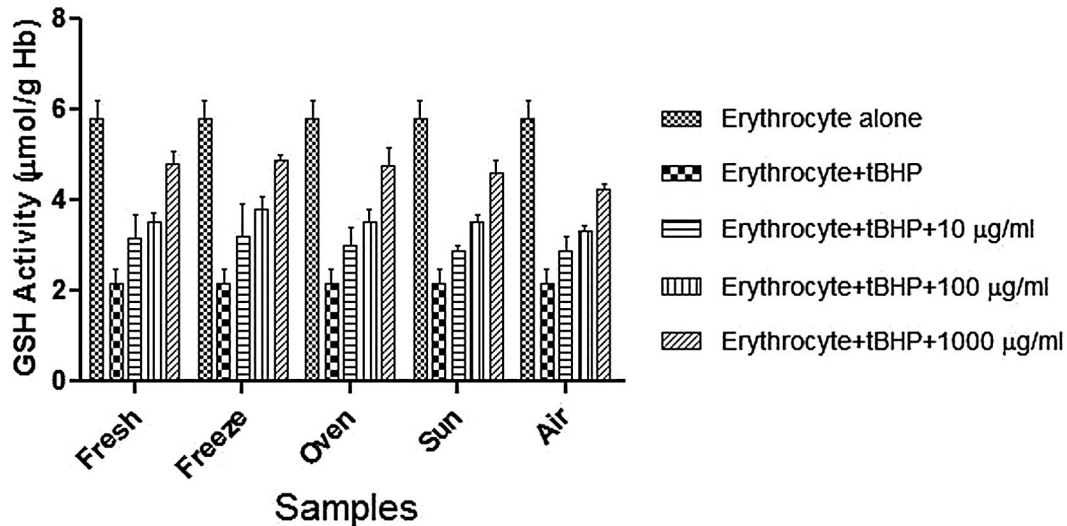


FIGURE 5 Effect of drying of *B. pinnatum* on malondialdehyde (MDA) content in human erythrocytes challenged with tertiary butyl hydroperoxide (tBHP)

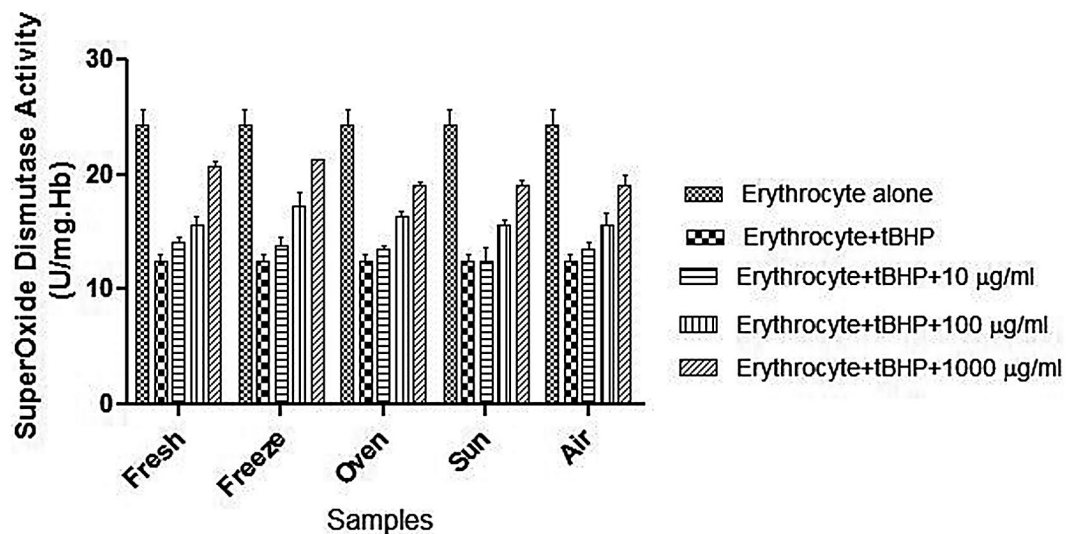
shreds of evidence have been released from some research findings affirming different potentials of *B. pinnatum* leaves (Akinpelu, 2000; Ojewole, 2002).

Protein tissue denaturation is adjudged to be a significant cause of inflammatory diseases. It is believed that tissue protein denaturation account for autoantigens production in certain inflammatory diseases (Umapathy et al., 2010). Functional foods or medicinal plants with the potential of preventing the denaturation of proteins would, therefore, be a worthwhile therapeutic anti-inflammatory agent. This is evident in the potential of freeze-dried *B. pinnatum*, which showed the highest inhibition of 76.27% at a dose concentration of 500 µg/ml even when compared with Diclofenac sodium (76.87%) used as the standard drug.

Erythrocyte membrane is believed to be analogous to the lysosomal membrane, hence human red blood cells (HRBC) membrane stabilization is a widely accepted method used for the in vitro anti-inflammatory study (Shenoy et al., 2010). This stabilization suggests that lysosomal membranes may also be stabilized by some natural products. An inflammatory response is limited by stabilization of the lysosomal membrane through the prevention of the liberation of lysosomal contents of activated neutrophil, such as bacterial, proteases, and enzymes, which gives rise to further tissue injury and inflammation after extracellular discharge. Extracellular activities produced by lysosomal enzymes is adjudged an indicator of acute and chronic inflammation (Leelaprakash & Dass, 2011). NSAIDs can also function by preventing the synthesis of prostaglandin, thereby



**FIGURE 6** Effect of drying of *B. pinnatum* on reduced glutathione (GSH) level in human erythrocytes challenged with tertiary butyl hydroperoxide (tBHP)



**FIGURE 7** Effect of drying of *B. pinnatum* on superoxide dismutase activity in human erythrocytes challenged with tertiary butyl hydroperoxide (tBHP)

blocking the autocooid synthesis that inhibits the COX-1 enzyme or prevents the lysosomal membrane from the breakdown (Rajendran & Lakshmi, 2008). Findings from this study correlate with Leelaprakash and Dass (2011) previous report, which states that *Enicostemma axillare* stabilized the HRBC membrane. Thus, we affirm the scientific suggestion and hypothesis, which says that the inhibition of lysosomal enzymes or stabilization of the lysosomal membrane is the mechanism of action of NSAIDs.

Stabilization of HRBC membrane by hypotonicity-induced membrane lysis as reported by Leelaprakash and Dass (2011) can be taken as an in vitro measure of anti-inflammatory activity of plant extracts. The protective ability of *B. pinnatum* extract against heat-induced erythrocyte membrane lysis in this study could be adjudged as a significant fact buttressing its anti-inflammatory activity. The result obtained showed that the fresh portion (89.124 µg/ml) of

the sample showed there was no significant difference ( $p > .05$ ) in the inhibition percentage of heat-induced hemolysis when compared with the standard drug—Diclofenac (91.508 µg/ml). Unpublished reports have it that fresh extract of *B. pinnatum* is majorly made use of in African traditional medicine to treat inflammation accompanied with a high temperature in infants, findings from this has shed more light on the scientific rationale behind this practice.

Tertiary Butyl Hydroperoxide (tBHP) is reported to cause oxidative stress (Anandita & Parames, 2012). Exposure of the cell or tissue to external toxins like tBHP causes membrane permeability and formation of water pores (Anandita & Parames, 2012). tBHP is reduced in the red cell cytosol by GSH or hemoglobin. Once hemoglobin binds to tBHP, it forms t-butoxy radicals that react with lipids in the tissue membrane to initiate peroxidation (Krukoski et al., 2009). Cascade of reactions such as hydroperoxide decomposition is undertaken to



form products like aldehydes, MDA, ketones, hydrocarbon gases, and so forth (Chen, Chiang, Wang, & Lii, 2000).

It is becoming evident that unchecked consumption of plant extract without any consideration of the dosage may pose a severe health problem. Reppas (1995) reported cattle poisoning upon consumption of a large amount of *B. pinnatum*, which led to hypersalivation, ataxia, severe cardiac arrhythmia, labored respiration, and eventually death. Plant extracts contain a variety of chemical compounds that can interact with biomolecules in erythrocytes, leading to the perturbation of the pro-oxidant/antioxidant balance of the cell, as well as membrane disruption. In this study, we investigated for the first time the effect of drying and potential protective effect of *B. pinnatum*, against tBHP-induced oxidative damage in human erythrocytes. tBHP is widely used as an inducer of oxidative stress in cells and tissues. tBHP is known to act mainly by assembling arachidonic acid (AA) from the membrane phospholipids under cytotoxic conditions. This leads to increased intracellular AA and MDA formation, a by-product of lipid peroxidation (Bhattacharya, Gachhui, & Sil, 2011). Exposure of human erythrocytes to tBHP in this current study effectively caused a significant increase in MDA formation, which was attenuated by co-treatment with the aqueous extracts of differently dried *B. pinnatum* leaves.

The harmful effect of tBHP results in the alterations of the cell membrane fluidity and permeability, which is associated with GSH depletion (Krukoski et al., 2009). Glutathione, a very vital cellular non-antioxidant defense, protects the organism from oxidative stress by scavenging free radicals and detoxifying xenobiotics (Chaves, Leonart, & Nascimento, 2008). However, excess of free radicals generation caused by oxidative stress inducers like tBHP, the GSH level decreases below the normal level. In the current study, we found that the GSH level in tBHP-treated erythrocytes was drastically reduced when compared with the control value. Still, it was significantly increased when co-treated with the aqueous extract of differently dried *B. pinnatum*. Accumulating evidence indicates that oxidative stress in erythrocytes can result in increased membrane permeability and loss of essential fatty acids (Petiboisab & Délérís, 2005; Çimen-Burak, 2008).

In a bid to reduce its degradation and nutrient loss, freeze-drying is an effective drying method employed for drying bioactive substances that are thermosensitive. Encapsulation by freeze-drying has commonly been used for polyphenol-rich foods, for example, red wine. This process helps minimize the loss of nutrients and biological activity as well as increase product shelf life (Fang & Bhandari, 2010). The observed lowest phenolic constituents of the air-dried *B. pinnatum* leaves may be due to deterioration as a result of the slow dehydration process, which took place under room temperature. Reducing the moisture content of the air-dried sample took a long time, which may be as a result of the leaf thickness/texture, this may lead to degradation of bioactive substances, and thus, encourage some microbial growth. Recently, we affirmed that because of similarities in the secondary metabolites of a particular plant family, each plant family needs a unique drying method for an appreciable retention of its bioactive substances (Obboh, Omojokun, &

Ademiluyi, 2016). Findings from this research work and similar work where air-dried *Moringa oleifera* potentiated better biological activity than other conventional drying methods (Ademiluyi, Aladeselu, Obboh, & Boligon, 2018) affirms our previous suggestion that each plant family needs a unique drying method. Taking into consideration that *Moringa oleifera* leaf is a thin-leafed plant compared to our thick test sample. Thus, we confirm that the best conventional drying method that soothes a particular polyphenol-rich plant with appreciable retention of its bioactive substances and overall biological activity may be dependent on the plant family as well as its leaf size or orientation, morphology, and texture which are strong determinants that affects the drying time. However, freeze-drying remains the best method owing to its apparent retention of bioactive substances and overall biological activity observed in this study.

## 5 | CONCLUSION

Findings from this study indicate that the differently dried extracts of *B. pinnatum* possess anti-inflammatory properties via the stabilization of HRBC membrane, inhibition of heat-induced hemolysis, and albumin denaturation. The extracts also protected human erythrocytes against tBHP-induced lipid peroxidation and increased GSH level of erythrocytes previously challenged with tBHP. This study gives an idea that this medicinal plant is a potent anti-inflammatory agent, which could as well, point a lead as a future treatment for various diseases such as cancer as anti-inflammatory agents are hypothesized as cancer therapeutic agents of the future. In the light of this study, air drying remained a poor drying method that will impact negatively on the anti-inflammatory and antioxidant properties as well as phenolic constituents of the *B. pinnatum* leaves. However, for optimum retention of bioactive substances and biological activity (anti-inflammatory and antioxidative potential), we suggest that the plant be freeze-dried for off-season availability and use.


## ACKNOWLEDGMENTS

The authors will like to appreciate all healthy volunteers who gave the blood samples used for this study. The efforts of Ms. Alajo Olanike and Omojokun Dolapo in blood sample collection is highly appreciated.

## CONFLICT OF INTEREST

The authors confirm that there is no conflict of interest in the work.

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**How to cite this article:** Omojokun OS, Oboh G, Ademiluyi AO, Oladele JO, Boligon AA. Impact of drying processes on *Bryophyllum pinnatum* phenolic constituents and its anti-inflammatory and antioxidative activities in human erythrocytes. *J Food Biochem*. 2021;45:e13298. <https://doi.org/10.1111/jfbc.13298>