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IN VITRO EVALUATION OF THE INFLUENCE OF EXTRACTION SOLVENTS ON ANTIOXIDANT AND ANTI-INFLAMMATORY POTENTIALS OF DRIED LEAF OF *BRYOPHYLLUM PINNATUM* LAM.

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SUMMARY

This study investigated the effect of different extraction solvents on the phenolic contents (TPC), antioxidant activities, and anti-inflammatory potentials of dried-leaf extracts of *Bryophyllum pinnatum* Lam. The analyses were performed spectrophotometrically, using a Folin-Ciocalteu reagent for the estimation of the total phenolic contents. The ferric reducing power (FRP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assays were carried out to assess the antioxidant activities of aqueous, ethanolic, ethyl acetate, and n-hexane extracts of the plant, whereas the stabilization of heat-treated albumin and inhibition of protease action were performed to evaluate the anti-inflammatory potentials of the extracts. The results obtained indicate that the solvent nature significantly influenced the amount of TPC, antioxidant and anti-inflammatory potentials of the extracts. The aqueous extract had the highest TPC. The FRP was equally high in the aqueous and ethanol extracts. The ethanolic extract had the highest radical scavenging activity and anti-inflammatory potentials. The results show that water and ethanol could be suitable solvents for the extraction of bioactive compounds from the dried leaf of *B. pinnatum* vis-à-vis antioxidant and anti-inflammatory potentials.

Key words:

Bryophyllum pinnatum Lam., total phenolics, antioxidant activity, anti-inflammatory, solvent

Abbreviations:

DPPH-1,1-diphenyl-2-picrylhydrazyl; FRP-ferric reducing power; IAD-Inhibitions of albumin denaturation; IPA-Inhibition of protease activity; TPC-Total phenolic content

INTRODUCTION

Plants have been used extensively in folklore medicine due to their health-promoting properties. This is attributable to their high contents of bioactive compounds, which have been found to protect against the progression of degenerative diseases, dysregulations of inflammatory responses, and accumulation of oxidants in the body system (Zhang et al., 2011; Oso et al., 2018a). Plants containing these beneficial properties have been asserted as sources of natural antioxidants (Oso et al., 2019). *Bryophyllum pinnatum* Lam., a succulent perennial shrub belonging to the family Crassulaceae, has been used conventionally in the treatments of wounds and burns. The plant is also known as a 'life plant', whose leaf extracts have been found to protect against the initiation of renal calcifications in Lithiatric Rats (Yadav et al., 2016). However, there might be possible limitations to its effectiveness in human nutrition due to the fact that it could cause cardiac poisoning in grazing animals (Reppas, 1995). The phytochemical profiling of aqueous extract of the leaf of *B. pinnatum* revealed that it contains terpenoids, alkaloids, lipids, steroids, fatty acids, bufadienolides, phenolics, and flavonoids (Kamboj & Saluja, 2009; Oso, 2020). These phytochemicals are known to contribute immensely to the antioxidant properties of natural materials. It has also been stated that the antioxidant properties of the plant depend significantly on several factors such as the processing method, extraction technique,

and nature of extracting solvents (García-Márquez et al., 2012; Oso et al., 2018b; Oso & Olowookere, 2018). This study aimed to assess the influence of different solvents on the total phenolic content, antioxidant activity, and anti-inflammatory potential of dried leaf extracts of *B. pinnatum*.

MATERIAL AND METHODS

Chemicals

Ethyl acetate and n-hexane were products of JHD, GuanghuaSci-Tech, China, Folin-Ciocalteu reagent was a product of LobaChemie, Mumbai, India, and albumin was a product of Kem Light, Indian.

Preparation of extracts

Fresh leaves of *B. pinnatum* were harvested and air-dried at a temperature of $30 \pm 1^\circ\text{C}$. The sample (10.0 g) was powdered and immersed separately in 100 mL of water, ethanol, ethyl acetate, and n-hexane for 120 minutes at 29°C . Then, the mixtures were filtered. The filtrate of each solution was used for the subsequent determinations of total phenolics, antioxidant and anti-inflammatory potentials.

Experimental analysis

The total phenolic content in each filtrate was estimated as described by Singleton et al. (1999), using freshly prepared Folin-Ciocalteu reagent and sodium carbonate. The optical density of the reaction mixture was taken at 725 nm after 10 minutes. The amount of the estimated total phenolics was expressed in mg per 100 g of dried weight as Gallic acid equivalent (mg GAE/100 g). The antioxidant properties of the extracts were assessed through the reduction of potassium ferricyanide to potassium ferrocyanide and diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging capacity as described by Oyaizu (1986) and Choi et al. (2002), respectively. The reducing power was presented in mg per 100 g of dried weight as Ascorbic acid equivalent (mg AAE /100 g) The anti-inflammatory properties of the extracts were evaluated through the inhibitions of albumin denaturation and protease activities according to Williams et al. (2002) and Kunitz (1974), respectively. The results of the DPPH radical scavenging capacity, inhibitions of albumin denaturation, and protease activities were presented as the concentration ($\mu\text{g/mL}$) at which the extract decreased the absorbance concentration by 50% (IC_{50}).

Molecular docking

The putative studies on possible inhibitory mechanisms of the extracts were carried out through a molecular docking study which involved the interactions of caffeic acid and *p*-coumaric acid with structures of bovine serum albumin and trypsinogen (an inactive preform of trypsin). Caffeic acid and *p*-coumaric acid are phenolic compositions of *B. pinnatum* leaf extracts reported by Omojokun et al. (2021). The theoretical assessment was compared with ibufenac, a known anti-inflammatory compound. The 3D SDF structures of caffeic acid (PubChem CID: 689043), *p*-coumaric acid (PubChem CID: 637542), and ibufenac (PubChem CID: 15250) were obtained from the PubChem database (www.pubchem.ncbi.nlm.nih.gov) (Kim et al., 2023). The structures of bovine serum albumin and trypsinogen with PDB IDs 4OR0 and 2PTN were downloaded from www.rcsb.org (Berman et al., 2000). The blind docking of the selected compounds to the protein targets (bovine serum albumin and trypsinogen) was carried out using PyRx-Python Prescription 0.8 (The Scripps Research Institute) (Trott & Olson, 2010) with the grid centre set at x: 53.1650, y: 23.9873, z: 98.0448 and x: 2.4869, y: 7.5322, z: 23.7707, respectively with the dimensions (Armstrong) set at x: 25.0000, y: 25.0000, z: 25.0000. The interactions were visualized through the Protein-Ligand Interaction Profiler server (<https://projects.biotec.tu-dresden.de/plip-web/plip>) (Adasme et al., 2021).

Statistical analysis

Using IBM SPSS Statistics 20, the one-way analysis of variance (ANOVA) was employed for the result analysis. Differences between the means computed were analyzed using the Duncan post hoc test at $p < 0.05$. The data are presented as means $\pm$ standard deviation.

RESULTS AND DISCUSSION

The influence of extracting solvents on the antioxidant capacities of *B. pinnatum* was demonstrated by the ability of each of the extracts to reduce ferric ions and also break the free radical chain through hydrogen atom donation. The study revealed that the amount of phenolic content and the extent of antioxidant capacities of the botanical were determined by the nature of the solvent used (Fig. 1 and Tab. 1). The TPC mean values among the extracts were found to be significantly different at the $p < 0.05$ level. The aqueous extract had the highest TPC (mg GAE/100 g),

whereas the n-hexane extract showed the lowest TPC. The results also showed that the antioxidant capacity assessed by ferric reducing power was higher in aqueous and ethanol extracts than those of ethyl acetate and n-hexane. The ferric reducing power of the aqueous and ethanol extracts were not significantly different from each other ($p < 0.05$), with the values of 590.97 ± 35.82 and 585.04 ± 12.46 mg AAE/100 g respectively. However, the extracts of ethyl acetate and n-hexane had significantly ($p < 0.05$) lower ferric reducing power (FRP) than the aqueous and ethanol extracts. Similarly, the n-hexane extract showed the least activity towards DPPH radical neutralization. The values of the anti-radical index in the aqueous extract and the ethyl acetate extract were not significantly different ($p < 0.05$). The ethanolic extract had the highest anti-radical activity as established by the shown least inhibition concentration at 50% (Tab. 1).

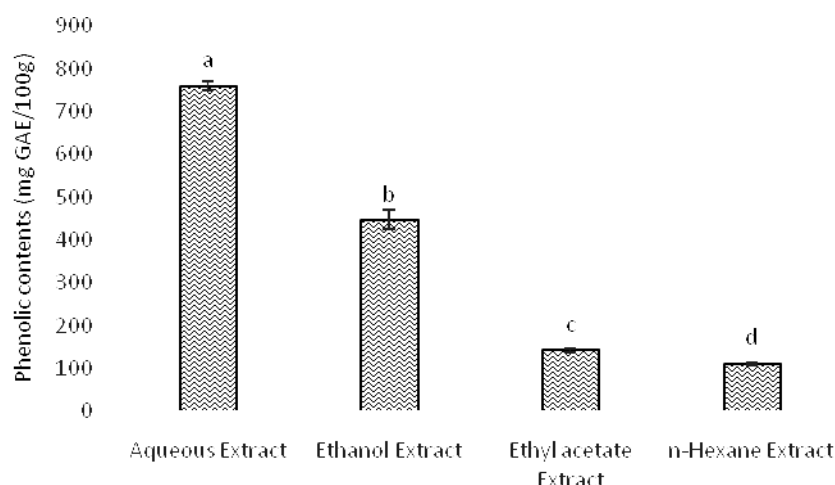


Figure 1. Phenolic contents (mg GAE/100 g) of dried *Bryophyllum pinnatum* leaf extracts using different extraction solvents (The bars with different letters are significantly different at $p < 0.05$)

Table 1. Ferric reducing power (FRP) (mg AAE /100 g) and IC_{50} values of the DPPH radical scavenging activity of dried *Bryophyllum pinnatum* leaf extracts

	FRP (mg AAE/100 g)	IC_{50} (μ g/ml) DPPH
Aqueous Extract	590.97 ± 35.82^a	795.30 ± 42.40^a
Ethanol Extract	585.04 ± 12.46^a	344.93 ± 23.49^b
Ethyl acetate Extract	43.52 ± 3.19^b	816.51 ± 30.75^a
n-Hexane Extract	214.59 ± 6.58^c	7098.41 ± 144.66^c

Legend: Data expressed as mean $\pm$ standard deviation of triplicate experiments. Values with different superscripts within a column are significantly different at $p < 0.05$.

These observations are consistent with previous reports that the extraction of antioxidant compounds from plant materials and the antioxidant potentials of botanicals could be influenced by the chemical and physical properties of extracting solvents (García-Márquez et al., 2012; Oso et al., 2018b; Oso & Olowookere, 2018). This could unvaryingly affect the biological effectiveness of the extract. The observed quantity of TPC in the aqueous extract might be due to the strong polarity and high dielectric constant of water. The ability of each extract to reduce ferric ions was similar in both the aqueous and ethanolic extracts. The apparent reducing power of the aqueous and ethanolic extracts could be defined by the nature and quantity of antioxidant compounds in the extracts. Previous findings have shown that ethyl acetate has stronger potential for the extraction of phenolics and antioxidant compounds than water and ethanol (Li et al., 2009; Khasawneh et al., 2014). However, in this study, the ethanolic extract had the highest radical scavenging ability of all the analyzed extracts. The observed variances might be due to differences in the structures and properties of compounds in the respective plant materials. Moreover, the degree of solubilization of each antioxidant compound might be determined by the nature of extracting solvents. The phenolic contents of the aqueous and ethanolic extracts may be responsible for the activities of these extracts. This is consistent with earlier reports that plant extracts from polar solvents usually have higher antioxidant potential than those from less polar solvents (Do et al., 2014; Rajendran et al., 2014; Oso & Olowookere 2018). Therefore, the extracting solvent with high polarity could be advantageous for the isolation of antioxidant compounds from dried

leaf *B. pinnatum* leaves. The interplay between the antioxidant potential of natural products and anti-inflammatory potential has been a probable target in drug discovery for the management of diseases that could originate from an imbalance in the maintenance of redox status (Oso et al., 2018c). The results of the anti-inflammatory potentials for the extracts are presented in Table 2. The extracts showed varying degrees of anti-inflammatory activity. The results revealed that the IC₅₀ values of inhibition of protein denaturation ranged from 252.00±9.07 µg/mL to 1434.67±366.73 µg/mL. The maximum anti-inflammatory potential was identified in the ethanolic extract, followed by the aqueous extract (332.00±27.78 µg/mL) and the ethyl acetate extract (567.67±22.99 µg/mL). The least potential *in vitro* anti-inflammatory activity against albumin denaturation was observed in the n-hexane extract. Similar trends with significant differences ($p < 0.05$) were observed in the actions of the extracts against protease activities.

Table 2. IC₅₀ values of inhibition of albumin denaturation (IAD) and inhibition of protease activity (IPA) of different dried *Bryophyllum pinnatum* leaf extracts

	IAD IC ₅₀ (µg/ml)	IPA IC ₅₀ (µg/ml)
Aqueous Extract	332.00±27.78 ^a	537.33±68.21 ^a
Ethanol Extract	252.00±9.07 ^b	312.33±16.89 ^b
Ethyl acetate Extract	567.67±22.99 ^c	1603.67±341.87 ^c
n-Hexane Extract	1434.67±366.73 ^d	-

Legend: Data expressed as mean± standard deviation of triplicate experiments. Values with different superscripts within a column are significantly different at $p < 0.05$.

The biological effects of the extracts against albumin denaturation and protease activity revealed that the nature of the extracting solvent could also affect the potency of the plant material vis-à-vis anti-inflammatory properties. Protein denaturation, which occurs when proteins lose their tertiary structures, is one of the major observations in patients with inflammatory disorders such as arthritis (Arya et al., 2014; Elisha et al., 2016). The interplay between inflammatory disorders and protein denaturation could be due to changes in the antigenicity of some endogenous proteins. The prevention of protein denaturation is considered one of the biological functions of many non-steroidal anti-inflammatory drugs such as ibufenac and diclofenac (Grant et al., 1970). This study revealed that the inhibition of albumin denaturation by the ethanolic extract showed promising activity when compared to other extracts considered. Moreover, the actions of the extract against protease activity were parallel to the observed actions of the extracts against protein denaturation. Proteases are involved in a wide variety of biological processes such as the metabolism of many biomolecules, signal transduction, and modulation of protein-protein interactions (López-Otín & Bond, 2008). However, the unregulated release of proteases could also exacerbate tissue injury and inflammation (Hyun et al., 2008; Ugajin et al., 2009; Pescosolido et al., 2014). The binding modes of phenolic compounds previously identified by Omojokun et al. (2021) in the binding site of albumin and trypsin were studied through intermolecular docking simulations comparable to ibufenac, a standard anti-inflammatory drug. This study was carried out to predict the putative inhibitory mechanisms of *B. pinnatum* phenolics. The energy values were computed from the docked conformations of complexes formed through the interactions between the compounds and proteins. Ibufenac has the highest affinity for albumin with an affinity value of -6.48±0.16 kcal/mol (Tab. 3, Fig. 2). The interactions of ibufenac and the selected phenolics with the albumin structure were found to be hydrophobic with no hydrogen bond interaction. It can be inferred that ibufenac and the phenolics have similar binding modes with albumin as they were found to interact at the same pocket containing Leu115 and Tyr160. As shown in Table 3, both caffeic acid and *p*-coumaric acid formed strong H-bond interactions with trypsinogen. Caffeic acid interacted through hydrogen bonds at Ser190, Ser195, and Ser214 and salt bridge at His57. The frequency of hydrogen bonds could be responsible for the strong binding energy. *p*-Coumaric acid was found to be involved in hydrophobic interactions at Val213, hydrogen bonding at Ser190, and Ser195, and salt bridge formation at His57 with trypsinogen, whereas only hydrophobic interactions occurred between trypsinogen and ibufenac, (Fig. 3). A better understanding of the molecular interactions between the compounds and selected targets may form the basis for the observed biological potentials of *B. pinnatum* extracts vis-à-vis albumin denaturation and protease hydrolysis.

Table 3. Computed binding scores of the selected phenolics with bovine serum albumin and trypsinogen

Protein	Ligands	Binding Energy (kcal/mol)	Interacting Residues		
			Hydrophobic interaction	Hydrogen Bonds	Salt bridge
Albumin	Caffeic acid	-5.53±0.04	Leu115, Tyr137, Tyr160		

	<i>p</i> -Coumaric acid	-5.55±0.05	Leu115, Pro117, Ile141, Tyr160		
	ibufenac	-6.48±0.16 [#]	Leu115, Phe133, Lys136, Tyr137, Tyr160		
Trypsinogen	Caffeic acid	-5.70±0.14 [*]		Ser190, Ser195, Ser214	His57
	<i>p</i> -Coumaric acid	-5.05±0.46	Val213	Ser190, Ser195	His57
	ibufenac	-5.13±0.13	Leu99		

Legend: [#]Significant differences among the compounds docked with albumin; ^{*} Significant differences among the compounds docked with trypsinogen

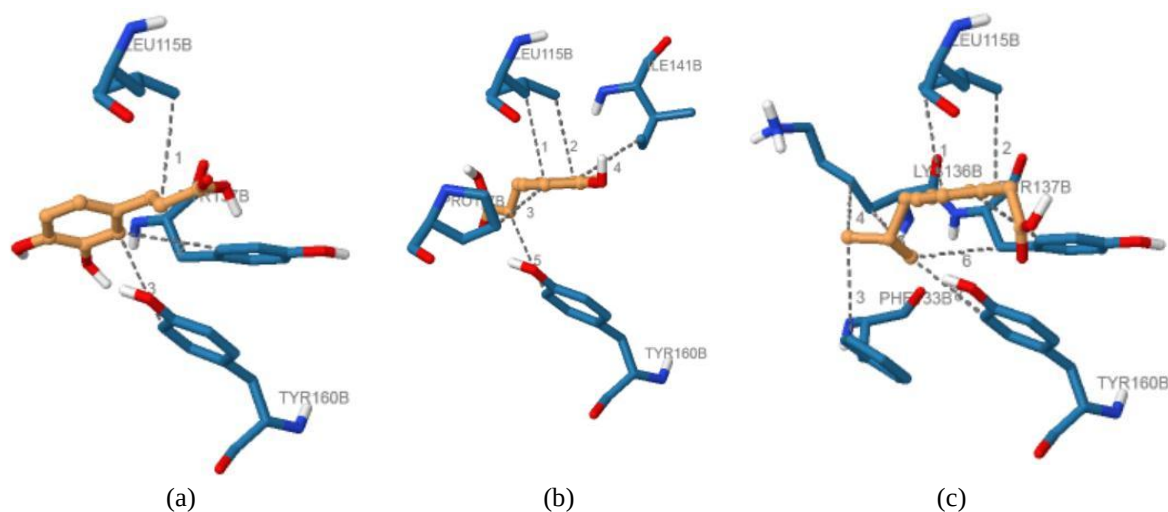


Figure 2. 3D interactions of albumin with (a) Caffeic acid (b) *p*-Coumaric acid (c) ibufenac

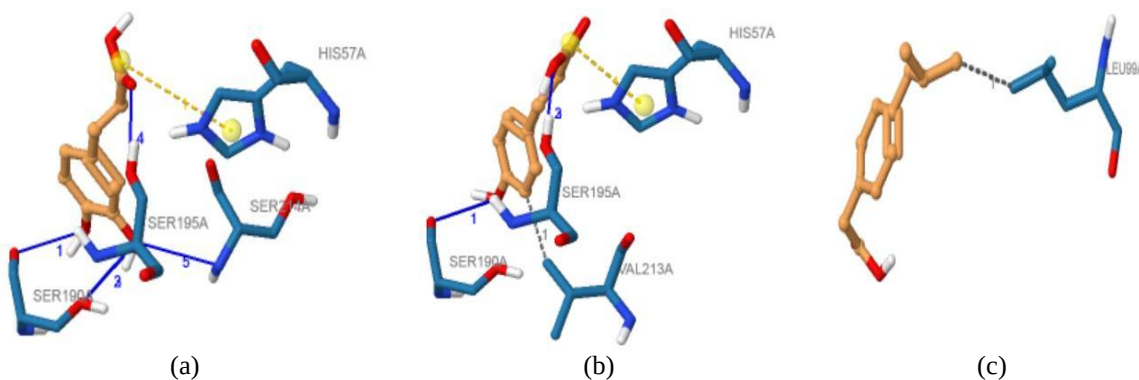


Figure 3. 3D interactions of trypsinogen with (a) Caffeic acid (b) *p*-Coumaric acid (c) ibufenac

CONCLUSION

On the basis of the results obtained and literature reviewed, this study revealed that the phenolic content, antioxidant potential, and anti-inflammatory properties of dried *B. pinnatum* leaves could differ depending on the nature of extracting solvents. In addition to high antioxidant and anti-inflammatory properties, the aqueous and ethanolic extracts were found to have higher phenolic contents than those of the ethyl acetate and n-hexane extracts. It could also be argued that the ethanolic extract of dried *B. pinnatum* leaves could possess an anti-inflammatory effect

against protein denaturation and protease activities. Therefore, this study suggests that extracts from polar solvents might be effective in the extraction of antioxidant compounds from dried *B. pinnatum* leaves. Further research is suggested on the clinical and pharmacological importance of this plant relative to its therapeutic potential against inflammatory disorders.

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Conflict of interest: The authors declare that they have no conflict of interest.

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