

Evaluation of *in vitro* antioxidant and anti-inflammatory potentials of *Tapinanthus bangwensis* leaves

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Abstract: Excessive production of reactive oxygen and nitrogen species under pathological conditions contributes to oxidative stress and chronic inflammation, both of which are central to the development of various diseases. Plant-derived antioxidants and anti-inflammatory agents have gained attention for their potential in mitigating these processes. This study evaluated the antioxidants and anti-inflammatory activities of *Tapinanthus bangwensis* leaf extracts using aqueous and acetone solvents. Phytochemical analyses revealed significantly higher total phenolic content (27.21 ± 0.18 mg GAE/100 g) and total flavonoid content (18.69 ± 0.31 mg QE/100 g) in the aqueous solvent compared to the acetone solvents. In antioxidants assays, total antioxidant capacity, reducing power, ferric reducing antioxidant power, and free radical scavenging (DPPH and ABTS), consistently showed greater activity in the aqueous solvent. In anti-inflammatory assays, the aqueous solvent demonstrated significantly higher inhibition of protein denaturation in bovine serum albumin ($IC_{50} = 64.56 \pm 2.34$ μ g/mL) and egg albumin denaturation ($IC_{50} = 389.45 \pm 3.50$ μ g/mL). Conversely, the acetone solvent exhibited stronger activity in trypsin inhibition ($IC_{50} = 425.50 \pm 12.45$ μ g/mL) and hypotonic solution-induced hemolysis ($IC_{50} = 107.75 \pm 2.87$ μ g/mL) assays, suggesting the presence of distinct bioactive compounds with membrane-stabilizing and enzyme-inhibitory effects. The findings supported the traditional use of *Tapinanthus bangwensis* extracts in the management of diseases. *Tapinanthus bangwensis* leaf is a promising source of natural therapeutic agents that could be used to prevent oxidative stress and inflammation in the body.

Introduction

Plants have long been recognized as valuable sources of bioactive compounds with therapeutic potential [1-3]. Among these, antioxidant and anti-inflammatory agents derived from medicinal plants are particularly important due to their role in managing chronic diseases [2, 4-6]. Oxidative stress caused by an excess of reactive oxygen species (ROS) is a key contributor to cellular damage and inflammation [7]. These processes are closely linked and are involved in the development of various conditions such as cancer, cardiovascular disease, neuro-degenerative disorders, and metabolic syndromes [8]. The increasing concern over the safety and side effects of

synthetic drugs has led to a growing interest in identifying safer, plant-based alternatives that possess antioxidant and anti-inflammatory potentials. Natural antioxidants, in particular, are gaining attention not only for their ability to neutralize harmful free radicals but also for their broader pharmacological properties [9]. Many traditional medicinal plants are rich in flavonoids, tannins, phenolic compounds, and other phytochemicals known to exert antioxidant and anti-inflammatory effects [10-12].

Tapinanthus bangwensis (Taba), known as African mistletoe, is a semi-parasitic plant widely distributed across tropical Africa. It attaches to various host plants, drawing nutrients and water from them. The biological activity and chemical composition of Taba are often influenced by the species of the host plant [13]. In this study, Taba Leaf (Taba L) was harvested specifically from *Theobroma cacao* (Cocoa tree), a common host in West African agroecosystems. Traditional medicine practitioners have used Taba L for generations to treat ailments such as high blood pressure, epilepsy, and inflammatory conditions [14-16]. Solvent selection plays a crucial role in extracting bioactive compounds from plants [17]. Different solvents can extract different classes of compounds based on their polarity [18]. Water, a highly polar solvent, tends to extract hydrophilic substances like glycosides and some phenolics, while acetone, which is less polar, can dissolve a broader range of compounds, including hydrophilic and lipophilic constituents [19]. Using aqueous and acetone solvents provides an opportunity to compare the biological activity of the resulting extracts and to better understand which types of compounds may be responsible for the plant's therapeutic effects. Despite its widespread use, scientific validation of these medicinal claims remains limited. The study is intended to contribute to the scientific understanding of this medicinal plant and to explore its potential as a source of natural therapeutic agents. This study aims to evaluate the *in vitro* antioxidant and anti-inflammatory activities of aqueous and acetone extracts of Taba L.

Materials and methods

Collection of plant materials: Fresh Taba leaves were collected within the University site. The plant was taxonomically identified and authenticated in the Herbarium unit of the Department of Biological Sciences of the Olusegun Agagu University of Science and Technology, Ondo State, Nigeria (OAUSTECH/H/1064).

Preparation of extract: The leaves were air-dried, ground into powder, and stored for preparation of extracts. 50.0 g of powdered sample was separately soaked in 500 mL of acetone and distilled water, respectively. After 72 hrs., with intermittent shaking at regular intervals. The solutions recovered were evaporated with a rotary evaporator at 40°C and the concentrated sample was kept in freezer prior to use [20].

Total phenolic content, TPC: The TPC of the extracts was obtained using the Folin-Ciocalteu method [21, 22].

Total flavonoid content, TFC: The TFC was obtained spectrophotometrically using Park et al. [23].

Total antioxidant capacity, TAC: The TAC of the extracts was obtained using phosphomolybdate method [24].

Reducing power, RP: The RP of each extract was assessed following the method by Oyaizu [25].

Ferric reducing antioxidant power, FRAP assay: The FRAP assay was carried out by the method [26].

2, 2-diphenyl-1-picrylhydrazyl, DPPH, radical scavenging activity: This was determined by the method [27].

2, 2-azinobis (3-ethyl-benzothiazoline 6- sulphate), ABTS, free radical scavenging activity: The ABTS activity was based on the method outlined by Re et al. [28].

Inhibition of bovine serum albumin, BSA: This was carried out according to the method of Sakat et al. [29].

Inhibition of egg albumin denaturation, EAD: The test was conducted as described by Boutennoun et al. [30].

Inhibition of hypotonic solution-induced hemolysis, HIH: This was evaluated using the method [31].

Trypsin inhibition: The test was assessed using the modified method of Sakat et al. [29].

Statistical analysis: Data are expressed as mean±SD. One-way ANOVA was used for the analysis, with Dunnett's post hoc test applied to compare multiple groups. Analyses were performed with GraphPad Prism version 10 and a $p < 0.05$ was considered indicative for significance difference.

Results and discussion

Excessive production of ROS and reactive nitrogen species (RNS) during pathological conditions can lead to oxidative stress, which plays a central role in triggering and sustaining chronic inflammation [32]. These reactive species activate inflammatory signaling pathways, contribute to tissue damage, and are implicated in the development of various diseases, including cancer, cardiovascular disorders, and neurodegeneration [32, 33]. Plant-derived antioxidant (acetone) and anti-inflammatory (AI) agents have gained attention for their ability to neutralize free radicals and modulate inflammatory responses [34]. In this study, the antioxidant and anti-inflammatory activities of *Tapa* L demonstrated that the choice of solvent, aqueous or acetone, significantly affects the extraction of bioactive compounds and their resulting biological activities. The antioxidant evaluation of *Tapa* L extracts revealed higher levels of total phenolics, flavonoids, TAC, RP, and FRAP in the aqueous extract compared to the acetone extract (**Table 1**). Specifically, aqueous showed significantly higher phenolic content (27.21 ± 0.18 mg GAE/100 g) and flavonoid concentration (18.69 ± 0.31 mg QE/100 g) than the acetone extract (20.50 ± 0.43 mg GAE/100 g and 12.18 ± 0.32 mg QE/100 g, respectively). These elevated levels of phenolic and flavonoid compounds are of particular interest, given the well-documented role of these secondary metabolites in neutralizing ROS, chelating pro-oxidant metals, and stabilizing free radicals [35, 36]. Concisely, TAC, RP, and FRAP assays further supported this, as the aqueous extract consistently exhibited stronger antioxidant activity in all parameters assessed. These results suggest that the superior antioxidant activity of the aqueous extract is largely attributable to a higher concentration of hydrophilic polyphenolic constituents, a trend observed in previous studies where aqueous extracts of *Severinia buxifolia* showed greater antioxidant potential compared to acetone extracts [37].

Table 1: Polyphenol contents, reducing activities of aqueous and acetone extracts of *Tapinanthus bangwensis* leaf

	acetone extract	aqueous extract
TPC (mg GAE/100 g)	20.50 ± 0.43^a	27.21 ± 0.18^b
TFC (mg QE/100 g)	12.18 ± 0.32^a	18.69 ± 0.31^b
TAC (mg AAE/100 g)	08.07 ± 0.34^a	13.95 ± 0.14^b
RP (mg AAE/100 g)	08.20 ± 0.25^a	10.52 ± 0.34^b
FRAP (mg Fe ²⁺ /100 g)	34.47 ± 0.29^a	38.23 ± 0.56^b

Values are mean±SD. Values with different letters across the same row are significantly different

TPC=Total phenolic content; TFC=Total flavonoid content; TAC=Total antioxidant capacity
RP=Reducing power; FRAP=Ferric reducing antioxidant power

In addition, the radical scavenging activities of the aqueous extract, as demonstrated in the DPPH and ABTS assays, were significantly higher to that of the acetone extract in a concentration-dependent manner (**Figures 1 and 2**). This enhanced scavenging ability underscores the potential of the aqueous in mitigating oxidative stress by directly interacting with and stabilizing free radicals. These findings align with earlier reports which attribute strong acetone properties to phenolic-rich plant extracts [20, 38], suggesting that the bioactive compounds extracted could significantly contribute to the prevention of oxidative-stress-related diseases such as cancer, cardiovascular disorders, and neurodegenerative conditions. The anti-inflammatory potential of the extracts was evaluated using several *in vitro* models, including inhibition of protein denaturation, trypsin inhibition, and heat

induced hemolysis (HIH) in **Figures 3-6**. Interestingly, the aqueous demonstrated significantly higher inhibitory effects in BSA and EAD assays in a concentration-dependent manner, which are well-established models for assessing anti-inflammatory effects through the stabilization of protein structures under inflammatory conditions. The inhibition of protein denaturation observed may be attributed to the presence of polyphenols and flavonoids in the aqueous extract, as these compounds have been reported to prevent the unfolding and aggregation of proteins during stress [39]. This supports the notion that *Tapa L* particularly its aqueous, may exert anti-inflammatory effects by stabilizing lysosomal membranes and preventing the release of inflammatory mediators. Conversely, in the trypsin inhibition and HIH assays, the acetone showed significantly stronger inhibitory activity than the aqueous extract in a concentration-dependent manner. This suggests that non-polar or semi-polar compounds extracted more effectively in acetone might interact more directly with proteolytic enzymes and membrane components, thereby stabilizing cellular structures and reducing inflammation-induced damage. It is known that enzymes like trypsin and other proteinases play crucial roles in inflammatory processes by degrading tissue proteins and facilitating the recruitment of inflammatory cells [40]. The inhibition of these enzymes by the acetone of *Tapa L* may indicate the presence of specific lipophilic compounds that interfere with protease activity and membrane permeability [41].

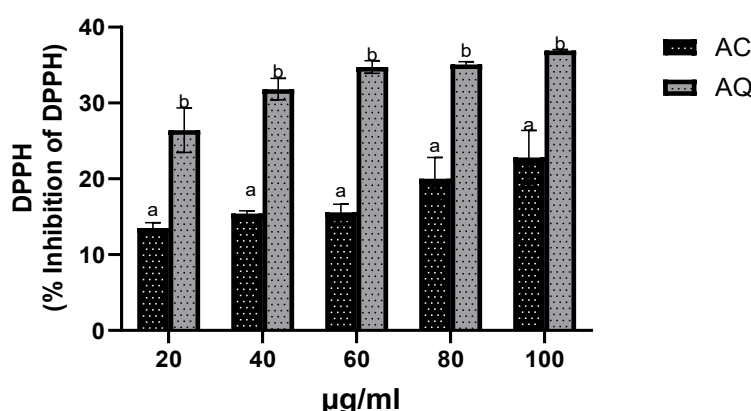


Figure 1: Inhibitory effect of the acetone and aqueous extracts of *Tapinanthus bangwensis* on DPPH

Bars are mean±SD. Different letters within the same bars indicate significant differences
AC=Acetone extract; AQ=Aqueous extract; DPPH= 2, 2-diphenyl-1-picrylhydrazyl scavenging power

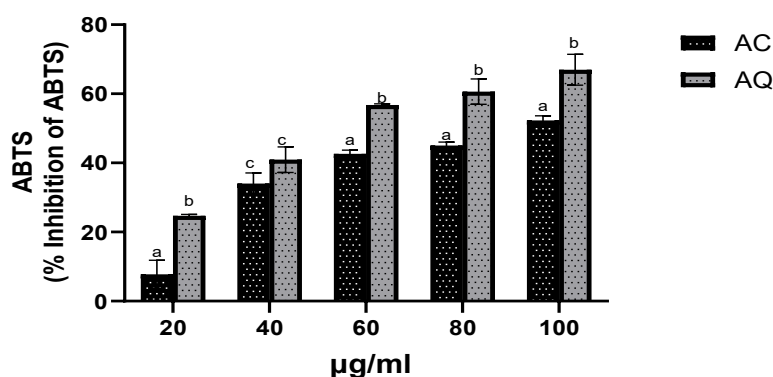


Figure 2: Inhibitory effect of the acetone and aqueous extract of *Tapinanthus bangwensis* on ABTS.

Bars are mean±SD. Different letters within the same bars indicate significant differences.
AC=Acetone extract; AQ=Aqueous extract; ABTS=2, 2-azino bis (3-ethyl-benzothiazoline 6- sulphate) scavenging power

The differences in the activities of the aqueous and acetone across the different assays emphasize the diverse mechanisms through which phytochemicals can exert their biological effects. While the aqueous appears to be more effective in directly scavenging free radicals and stabilizing denatured proteins, the acetone may act more effectively at the level of enzyme inhibition and membrane stabilization (**Table 2**). This solvent-dependent variation in activity also highlights the complexity of phytochemical interactions and the importance of extraction methods in determining therapeutic efficacy.

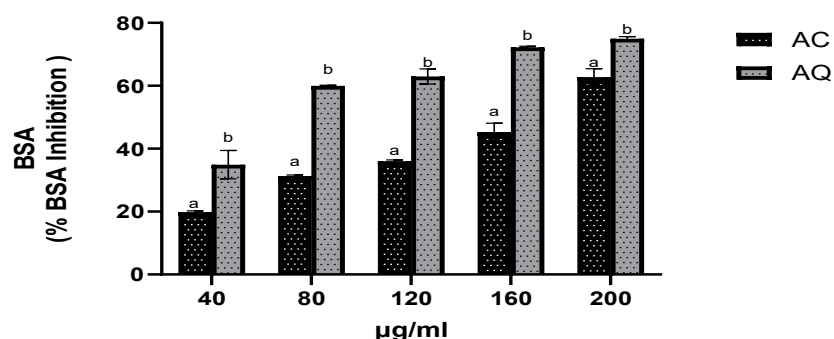


Figure 3: Inhibitory effect of the acetone and aqueous extracts of *Tapinanthus bangwensis* leaf on BSA

Bars are mean±SD. Different letters within the same bars indicate significant differences
AC=Acetone extract; AQ=Aqueous extract; BSA=Bovine serum albumin

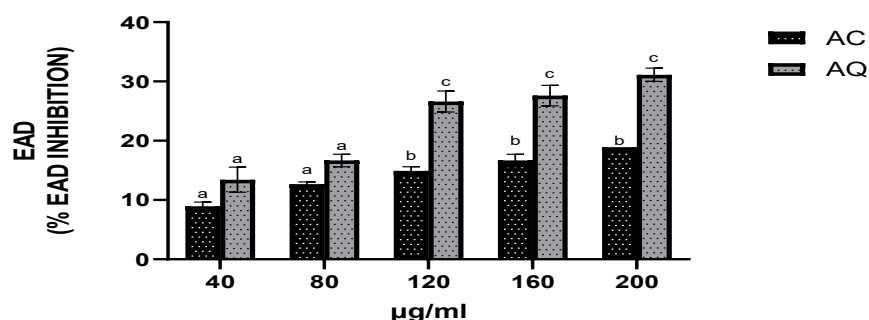


Figure 4: Inhibitory effect of the acetone and aqueous extracts of *Tapinanthus bangwensis* leaf on EAD

Bars are mean±SD. Different letters within the same bars indicate significant differences
AC=Acetone extract; AQ=Aqueous extract; EAD=Egg albumin denaturation

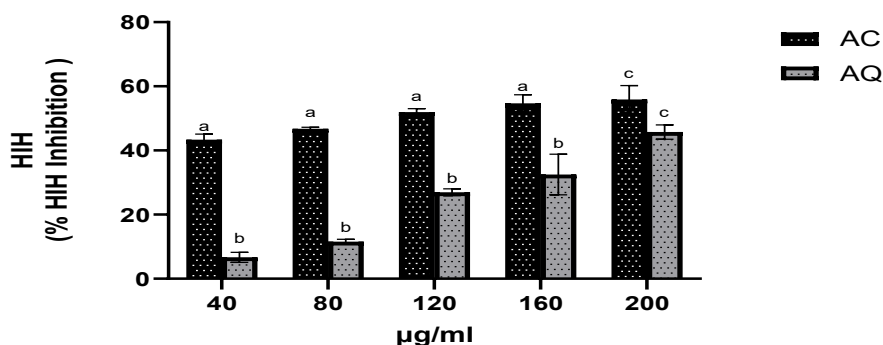


Figure 5: Inhibitory effect of the acetone and aqueous extracts of *Tapinanthus bangwensis* leaf on HIH

Bars are expressed in mean±SD. Different letters within the same bars indicate significant differences
AC=Acetone extract; AQ=Aqueous extract; HIH= Hypotonic solution- induced hemolysis

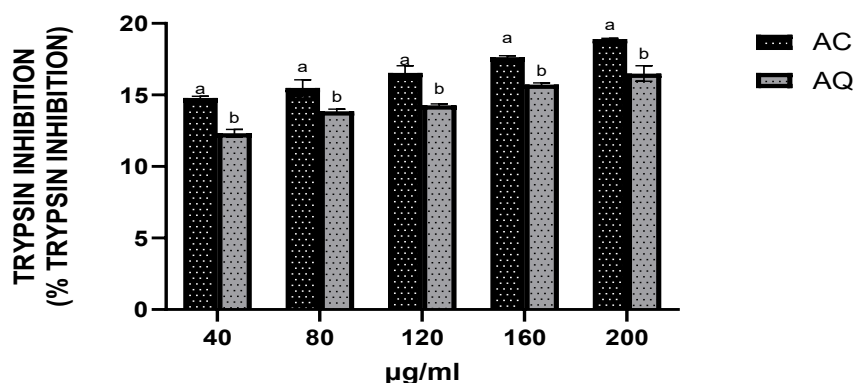


Figure 6: Inhibitory effect of the acetone and aqueous extracts of *Tapinanthus bangwensis* leaf on trypsin

Bars are mean±SD. Different letters within the same bars indicate significant differences
AC=Acetone extract; AQ=Aqueous extract

Table 2: IC₅₀ (µg/mL) of acetone and aqueous extracts of *Tapinanthus bangwensis* leaf

	Acetone extract	Aqueous extract
DPPH	189.05±04.48 ^a	108.19±02.56 ^b
ABTS	110.54±02.48 ^a	53.56±01.52 ^b
BSA	174.82±0 5.10 ^a	64.56±02.34 ^b
EAD	474.25±06.75 ^a	389.45±0 3.50 ^b
HIH	107.75±02.87 ^a	214.80±04.12 ^b
Trypsin inhibition	425.50±12.45 ^a	465.80±011.50 ^b

Values are mean±SD. Values with the different superscripts are significant differences

DPPH=2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

ABTS=2, 2-azinobis (3-ethyl-benzothiazoline 6- sulphate) (ABTS) free radical scavenging activity

BSA=Inhibition of bovine serum albumin, HIH=Inhibition of hypotonic solution-induced hemolysis (HIH)

EAD=Inhibition of egg albumin denaturation (EAD)

Conclusion: *Tapinanthus bangwensis* leaf contains a diverse range of bioactive compounds with significant antioxidant and anti-inflammatory potential. The aqueous, richer in phenolics and flavonoids, exhibited stronger free radical scavenging and protein stabilization activities, while the acetone demonstrated superior membrane stabilization and protease inhibition effects. These complementary activities highlight the therapeutic promise of *Tapinanthus bangwensis* leaf and validate its traditional medicinal use.

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