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A comparative assessment of the *in vitro* antioxidant potential of *Tapinanthus preussii* (African mistletoe) leaf aqueous and ethanolic extracts

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Abstract
The significance of antioxidant properties in medicinal plants lies in their ability to combat detrimental free radicals, which are linked to various conditions like diabetes mellitus, cancer, atherosclerosis, and visual impairment. *Tapinanthus preussii* is a hemiparasitic epiphyte species of African mistletoe in the Loranthaceae family. It thrives mainly in the wet tropical biome across Southeast Nigeria to West Central Tropical Africa, showcasing diverse medicinal potentials. This study aimed to compare the total phenolic contents, flavonoid contents and various antioxidant capacities of *Tapinanthus preussii* crude leaf aqueous and ethanolic extracts. The choice of solvents was guided by the common practices of herbal remedy preparation in the region, ensuring the study's relevance and practical implications. The *in-vitro* evaluation included several electron transfer reactions (e.g. TAC, TEAC, FRAP, DPPH, hydroxyl radical scavenging, and nitric oxide radical scavenging assays). The analysis revealed significantly ($p<0.05$) higher total phenolic contents (1968.89 µg/ml GAE) and total flavonoid contents (13120.99 µg/ml QE) in the ethanolic extract compared to the aqueous extract (360.34 µg/ml GAE and 280.99 µg/ml QE respectively). The ethanolic extract exhibited significantly ($p<0.05$) higher TAC (1870.54 µg/ml AAE), FRAP (3372.99 µg/ml AAE), trolox equivalent antioxidant capacity (38363.64 ug/ml trolox) and reductive potential (09.59 µg/ml AAE) compared to the aqueous extract (497.10 µg/ml AAE, 2275.95 and 6415.5 ug/ml trolox µg/ml AAE, 04.07 µg/ml AAE respectively). These results highlights the superior antioxidant potential of the ethanolic extract, indicating ethanol as a more efficient solvent for extraction contributing to the plant's overall antioxidant capacity. In conclusion, the findings suggest that the ethanolic extract of *Tapinanthus preussii* leaves showed promising potential as a relatively effective solvent for extracting natural antioxidants. These antioxidants extracted from the plant may offer valuable natural compounds with applications in healthcare and medicine with therapeutic benefits against diseases associated with free radical damage.

Keywords: Medicinal plants, *Tapinanthus preussii*, African mistletoe, free radicals, antioxidants

1. Introduction
Medicinal plants are a rich source of natural antioxidants, including phenolic acids, flavonoids, and tannins, which have stronger antioxidant activity than typical dietary plants [1]. A vast array of natural antioxidants have been identified and isolated from various plant materials, such as oilseeds, cereal crops, vegetables, fruits, leaves, roots, spices, and herbs. Numerous epidemiological studies have consistently demonstrated a strong correlation between a high dietary intake of fruits and vegetables and a reduced risk of developing chronic diseases, such as cancer and cardiovascular disease [2]. The involvement of oxidative and nitrosative stress in the development and progression of various acute and chronic clinical disorders has prompted the idea that antioxidants can serve as prophylactic agents for better health [3]. This implies that altering dietary habits by increasing the consumption of plant-based foods, which are abundant in bioactive phytochemicals, could offer additional health benefits beyond basic nutrition, potentially reducing the risk of chronic diseases [4].
Tapinanthus preussii also commonly known as *Tapinanthus preussii* Engl. Tiegh.,is a species of the mistletoe plant in the Loranthaceae family, native to Africa [5]. The genus Tapinanthus is derived from the Greek words *tapeinos*meaning "low" or "humble" and*anthos*meaning flower.

It may contain some 40 species, with 30 being accepted [6]. The species *Tapinanthus preussii* is a hemiparasitic epiphyte that grows primarily in the wet tropical biome, and its native range is South East Nigeria to West Central Tropical Africa, Angola, Cameroon, and Gabon [7, 8]. The plant is threatened by habitat loss due to the impact of diamond mining in its natural habitat, which is subtropical or tropical moist lowland forests [8]. It is estimated that over 5,000 individual phytochemicals exist in fruits and vegetables [9]. However, a significant percentage remains unidentified, necessitating this research to fully comprehend the comparative availability of antioxidants in *Tapinanthus preussii* leaves using indegenous solvents like water and ethanol

Antioxidant properties in plants are crucial due to their ability to neutralize harmful free radicals, which are linked to conditions like diabetes, cancer, atherosclerosis, and vision loss [10]. Plants synthesize and accumulate various antioxidants which play a vital role in protecting cells from oxidative stress caused by reactive oxygen species (ROS) [11]. These antioxidants help maintain a balance between energy-linked functions and the control of ROS within plant cells, thus safeguarding against cellular damage and disease development [11] Additionally, phenolic antioxidants, a significant group of plant compounds, exhibit promising antioxidant activity both *in vitro* and *in vivo*, highlighting the importance of these natural molecules in promoting health and preventing oxidative damage [12]. The different types of antioxidants found in plants include ascorbic acid, glutathione, phenolic acids, carotenoids, ellagic acid, resveratrol, flavonoids, phytoestrogens, and glucosinolates [11]. Carotenoids are pigments responsible for the vibrant colors of fruits and vegetables known for their antioxidant properties. Ellagic acid is a phenolic compound found in various fruits (Particularly strawberries, raspberries and blackberries), nuts (Walnuts and pecans) as well as vegetables, known for reducing cancer risk and lowering cholesterol. Resveratrol, found in grapes and wine, supports cardiovascular and cognitive health. Flavonoids, a large group of phytonutrients, possess antioxidant properties and anticancer activity. Phytoestrogens mimic estrogen in the body and may reduce the risk of cancer, heart disease, and osteoporosis. Glucosinolates, mainly found in cruciferous vegetables, help regulate inflammation, metabolic function, and stress responses, and are associated with cancer prevention [11]. The concentrations of different antioxidants in body fluids and tissues can vary significantly, with some like glutathione or ubiquinone primarily present within cells, while others such as uric acid are more uniformly distributed. Some antioxidants are unique to specific organisms and can play crucial roles in pathogens, acting as virulence factors [13].

Antioxidants are substances that, when present in low concentrations relative to an oxidizable substrate, significantly delay or prevent the oxidation of other molecules. This definition applies to all oxidation processes, both radical and non-radical, but becomes experimentally useful when associated with the concept of the oxidant that needs to be neutralized [14]. It is important to note that antiradical and antioxidant activities are distinct. Antiradical activity refers to a compound's ability to react with free radicals, while antioxidant activity involves inhibiting oxidation processes [15]. There is a growing body of evidence suggesting that oxidative stress plays a role in the development of various disorders and diseases, which has garnered significant attention from scientists and the general public regarding the role of antioxidants and antiradicals in maintaining human health, preventing, and possibly treating diseases [16]. Oxidation is a chemical process that involves the transfer of electrons or hydrogen from a substance to an oxidizing agent. This process can generate free radicals, which have the potential to initiate chain reactions. When these chain reactions take place within a cell, they can lead to cellular damage or even cell death. Antioxidants play a vital role in halting these chain reactions by eliminating free radical intermediates and inhibiting other oxidation processes. Antioxidants achieve this by undergoing oxidation themselves, acting as reducing agents like thiols, ascorbic acid, or polyphenols [17]. While oxidation reactions are essential for life, they can also be harmful. To counteract this, plants and animals have evolved intricate systems comprising various types of antioxidants, including glutathione, vitamin C, vitamin A, and vitamin E, along with enzymes such as catalase, superoxide dismutase, and different peroxidases.

Considering their mode of action, the antioxidants can be classified as primary, secondary or co-antioxidants. Primary antioxidants are able to donate a hydrogen atom rapidly to a lipid radical, forming a new radical, which is more stable. Secondary antioxidants react with the initiating radicals (Or inhibit the initiating enzymes), or reduce the oxygen level without generating reactive radical species [18]. Therefore, these secondary antioxidants can retard the rate of radical initiation reaction by elimination of initiators. This can be performed by deactivating high energy species (Singlet oxygen), absorbing UV light, scavenging of oxygen, chelating metal that catalyses free radical reaction, or inhibiting enzymes, such as peroxidases, NADPH oxidase, xanthine oxidase, among other oxidative enzymes [19, 20] The most effective antioxidants are those that interrupt the free radical chain reaction. Usually containing aromatic or phenolic rings, these antioxidants donate H^+ ion to the free radicals formed during oxidation becoming a radical themselves. These radical intermediates are stabilized by the resonance delocalization of the electron within the aromatic ring and formation of quinone structures [21]. In addition, many of the phenolics lack positions suitable for molecular oxygen attack. Both synthetic antioxidants (butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate) and natural botanicals that contain phenolics (Flavonoids) function in this manner. Botanical extracts with antioxidant activity generally quench free radical oxygen with phenolic compounds [22]. The bivalent transition metal ions, Fe^{2+} in particular, can catalyze oxidative processes, leading to the formation of hydroxyl radicals, and can also decompose hydroperoxides via Fenton reactions. Therefore, chelating these metals molecules (Ferritin and ceruloplasmin) can effectively reduce oxidation [22, 23]. Antioxidants can also be categorized into two major groups based on their solubility in water (Hydrophilic) or lipids (Lipophilic). Generally, water-soluble antioxidants interact with oxidants in the cell cytosol and blood plasma, while lipid-soluble antioxidants safeguard cell membranes from lipid peroxidation [17]. These antioxidants can either be produced in the body or obtained through dietary sources [24].

The catalytic enzyme systems of antioxidants act by different mechanisms as they neutralize or divert the reactive oxygen species. The superoxide dismutase converts

0_2* into H_2O_2, which is then detoxified to water either by catalase in the peroxysomes or by glutathione peroxidase in the mitochondria, cytosol and nucleus. Glutathione peroxidase belongs to a group of selenoenzymes that require selenium for their biosynthesis. Sequel to that, the intake of food or dietary supplements containing selenium is crucial for antioxidant enzyme defences. Glutathione reductase (G_{red}) is also important as it regenerates glutathione (GSH) that is used as a hydrogen donor by glutathione peroxidase with the help of NADPH [25]. Glutathione peroxidase can also transform hydroperoxide lipids into alcohols (LOH). It is a low molecular weight tripeptide composed of glutamate, cysteine, and glycine being the main intracellular redox buffer. The capacity of GSH to regenerate the most important antioxidant molecules is linked with the redox state of the glutathione disulphide/glutathione (GSSG/GSH) couple [26]. GSH effectively scavenges ROS (HO*, H_2O_2, LOO* and $ONOO^-$) either directly or indirectly as a cofactor of several detoxifying enzymes, such as glutathione peroxidase (GPx), glutathione-S-transferases (GST), among others. In the neutralization process of ROS, GSH is oxidized to glutathione disulphide (GS-SG), which can be further reduced to two molecules of GSH by the enzyme glutathione reductase (G_{red}). It is also able to regenerate other antioxidant molecules such as Vitamins C and E. GSH can react with a variety of electrophilic xenobiotics in reactions catalysed by glutathione-S-transferases (GST) generating products with higher solubility and thus easier to eliminate. It can neutralize NO*, resuming in the formation of S-nitrosoglutathione (GSNO) [27]. Vitamin E is a liposoluble vitamin present in the membranes thus playing an important role in the prevention of lipid peroxidation. Among the eight forms of vitamin E, α-tocopherol is the most active form in humans. ROS (hydroxyl and peroxyl radicals, etc.) react with vitamin E, generating a poorly reactive phenolic radical (Vitamin E'). Vitamin C then reacts with vitamin E' producing vitamin C radical (Vitamin C) and regenerating vitamin E [28] reported synergistic effects between ascorbic acid and α-tocopherol in protecting an *in vitro* biological model system. Both radicals (Vitamin E and vitamin C) are poorly reactive species because of its unpaired electron [29].

2. Materials and Methods

2.1 Equipment/Apparatus

Measuring cylinder, beakers, micropipettes, spatula, Gallenkamp water bath, Spectrophotometer (Spectrum Lab 21 A), Gallenkamp Muffle furnace, Gallenkamp drying oven, MSE minor/Bench centrifuge, desiccator, Mettler H-80 weighing balance, Flame Emission photometer (Corning 421), laboratory mill (Corona, Landers Y CIA, SA), Atomic absorption spectrophotometer (Perkin-Elmer Model 403), and refrigerator (Jouan VX 380E).

2.2 Reagents/chemicals

Absolute ethanol, distilled water, concentrated and dilute sulphuric acid, Fehlings solution, sodium phosphate, lead acetate, hydrochloric acid, potassium bismuth, phenazone, acetone, lead acetate, iodine solution, Wagner's reagent, Dragendorff's reagent, ferric chloride, petroleum ether, copper acetate, acetic anhydride, ammonia, methylated spirit (Sigma Aldrich Laboratory, Germany,). All other reagents/chemicals used were of analytical grade and products of British Drug House (BDH) England, E. Merck, Darmstadt, Germany and Aldrich Chemical Company, USA.

2.3 Collection, identification and authentication of *Tapinanthus preussii* leaves

The collection of *Tapinanthus preussii* leaves, an African mistletoe that parasitizes cocoa (Theobroma cacao), was carried out in a cocoa plantation in Kumba, Southwest Region of Cameroon, around midday in January 2023. A botanist at the Limbe Botanic Gardens in the Southwest Region of Cameroon identified and authenticated the leaves, and a voucher specimen was deposited in the herbarium for future reference.

2.4 Preliminary treatment of *Tapinanthus preussii* leaves for extraction

The freshly harvested leaves underwent a thorough cleansing with clean water to eliminate any impurities. Subsequently, they were air-dried in the shade for four days at room temperature and then subjected to oven drying at 40 °C until a consistent weight was reached. Following this, the dried leaves were finely powdered using an electric grinder. The resulting powdered leaves were preserved in hygienic, airtight, high-density polyethylene containers at -4 °C until they were prepared for extraction [30, 31].

2.5 Extraction procedure of *Tapinanthus preussii* leaves

The pulverized leaves were subjected to two distinct extraction solvents, namely ethanol and water, following standard procedures [32].

2.5.1 Ethanolic extraction

To extract the bioactive compounds from the ground leaves, a total of 250 grams were soaked in 1000 ml of 95% ethanol, maintaining a ratio of 1:4 for 72 hours with intermittent stirring. The resulting mixture was then filtered using a sieve and cotton wool. The filtrate was evaporated to dryness at 40 °C in a water bath and further freeze-dried to achieve a consistent weight. The concentrated crude extract was stored in airtight, polyethylene containers at -4 °C in a refrigerator until required for *in-vitro* antioxidant assays.

2.5.2 Aqueous extraction

The powdered leaves, weighing 250 grams, underwent cold maceration in 1300 ml of distilled water. The mixture was shaken intermittently for 72 hours, and the resulting supernatant was filtered using a sieve and cotton wool. Subsequently, the extract was freeze-dried until a consistent weight was achieved. The concentrated crude extract was then collected and stored in airtight, polyethylene containers at -4 °C in a refrigerator until required for *in-vitro* antioxidant assays.

2.6 Estimation of *in-vitro* polyphenolic contents and antioxidants of *Tapinanthus preussii* leaf crude aqueous and ethanolic extracts

2.6.1 Estimation of Total Phenolic Content

The total phenolic content of the crude extracts was determined using the Folin-Ciocalteau assay method as described by McDonald *et al*. [33]. The assay was based on the reduction of Folin-ciocalteau reagent (Phosphomolybdate and phosphotungstate) by the phenolic compounds. The reduced Folin-ciocalteau reagent was blue

and thus detectable with a spectrophotometer in the range of 500-750nm.

2.6.2 Estimation of Total Flavonoid Content
Determination of the total flavonoid content of the plant extracts was based on the aluminium chloride colorimetric method as described by Miliauskas *et al*., [34]. The assay was based on the reaction between flavonoids and aluminium chloride to generate a characteristic mixture that has maximum absorption at 510nm.

2.6.3 Determination of Total Antioxidant Capacity
The determination of the total antioxidant capacity was carried out according to the method of Prieto *et al*., [35]. The method was based on the reduction of molybdenum (IV) to molybdenum (V) by the extract and the subsequent formation of a green phosphate-molybdenum (V) complex at acid P^H.

2.6.4 Determination of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity
The hydrogen donating or radical scavenging properties of extracts were determined using the stable radical DPPH according to the method of Blois [36] as described by Brace *et al*., [37]. The assay was based on the ability of the antioxidant compounds to reduce DPPH by donation of hydrogen. The change in colour from deep violet to light yellow was measured spectrophotometrically at 517nm.

2.6.5 Lipid Peroxidation Assay
Lipid peroxidation was determined using a modified thiobarbituric acid reactive species (TBARS) assay of Ohkowa *et al*., [38] as described by Nabasree and Bratati [39]. The assay was based on the generation of malodialdehyde by lipid peroxidation of egg yolk homogenate which serve as lipid-rich media [40]. The malondialdehyde (MDA) reacts with the thiobarbituric acid under acidic condition to form an MDA-TBA adduct. The pink coloured product was measured spectrophotometrically at 532nm

2.6.6 Estimation of Hydroxyl Radical Scavenging Activity (Deoxyribose Assay)
The hydroxyl radical scavenging potential of the extracts was determined using the deoxyribose assay as described by Neergheen *et al*., [41]. This method was based on the competition between deoxyribose and the test compound for hydroxyl radicals generated from the iron (III)-ascorbic acid-EDTA-H_2O_2 system.

2.6.7 Estimation of Nitric Oxide Radical Scavenging Activity
Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH, interact with oxygen to produce nitrite ions which were measured by Griess reaction [42].

2.6.8 Estimation of Reductive Potential (RP)
The reductive potential of the extract was determined according to the method of Oyaizu, [43] and as described by Gulcin *et al.*, [44]. This method was based on the transformation of iron III to iron II in the presence of the test sample in a buffered solution.

2.6.9 Ferric reducing antioxidant power (FRAP) assay
FRAP assay was determined according to the method of Benzie and Strain, [45], based on the ability of antioxidant to reduce Fe^{3+} to Fe^{2+} in the presence of 2, 4, 6-tri (2-pyridyl)-s-triazine (TPTZ), forming an intense blue Fe^{2+}- TPTZ complex with an absorption maximum at 539 nm. A 300 mol/1 acetate buffer of pH 3.6 (3.1g of sodium acetate + 16 ml of glacial acetic acid made up to 1 L with distilled water), 10 mmol/L 2, 4, 6- tri (2-pyridyl)-s-triazine, 3.1 mg/ml in 40 mmol/ 1 HCL and 20 mmol/1 of ferric chloride were mixed together in the ratio of 10:1:1, respectively to give the FRAP working reagent.

2.6.10 Trolox Equivalent Antioxidant Capacity (TEAC) Assay
The radical scavenging activity of the extracts were measured by 2, 2^1 azinobis (3-ethyl-benzothiazoline-6-sulphonate (ABTS) radical cation assay by Re *et al*., [46] as described by Chanda and Dave [47]. The ABTS radical formed quickly reacts with hydrogen donors to form colourless 2, 2^1-azinobis (3-ethyl-benzothiazoline 6-sulphate) (ABTS).

2.6.11 Determination Metal Chelating Activity
Ferrozine can quantitatively chelate with Fe^{2+} and form a complex with a red colour. This reaction was limited in the presence of other chelating agents and resulted in a decrease of the red colour of the ferrozine-Fe^{2+} complexes [48].

2.7 Statistical Analysis
Data collected were subjected to statistical analysis using SPSS version 20. The results were expressed as mean±SEM. One-way Analysis of variance (ANOVA) was also used to compare the means of some of the parameters measured and where significant differences were observed, at 95% confidence level, $p<0.05$ for all treatments carried out compared to the control Duncan's New Multiple Range test [49] was used to separate the means.

3. Results
3.1 Total Antioxidant capacity (TAC), Ferric reducing Antioxidant Potential (FRAP), Trolox Equivalent Antioxidant capacity and Reduction potential of *Tapinanthus preussii* crude Aqueous and Ethanolic leaf extracts
The antioxidant properties of *Tapinanthus preussii* crude aqueous and ethanolic leaf extracts were assessed and presented in Figures 3.1a, b and c. The evaluations included measuring the total antioxidant capacity (TAC), ferric reducing antioxidant potential (FRAP), trolox equivalent antioxidant capacity and Reduction potential. The results showed that the ethanolic extract had a significantly ($p<0.05$) higher TAC (1870.54 µg/ml AAE), FRAP (3372.99 µg/ml AAE) and trolox equivalent antioxidant capacity (38363.64 ug/ml trolox) compared to the aqueous extract (497.10 µg/ml AAE, 2275.95 and 6415.5 ug/ml trolox µg/ml AAE, respectively). Additionally, the ethanolic extract exhibited a better reductive potential (09.59 µg/ml AAE) than the aqueous extract (04.07 µg/ml AAE), all expressed as ascorbic acid equivalent (AAE).

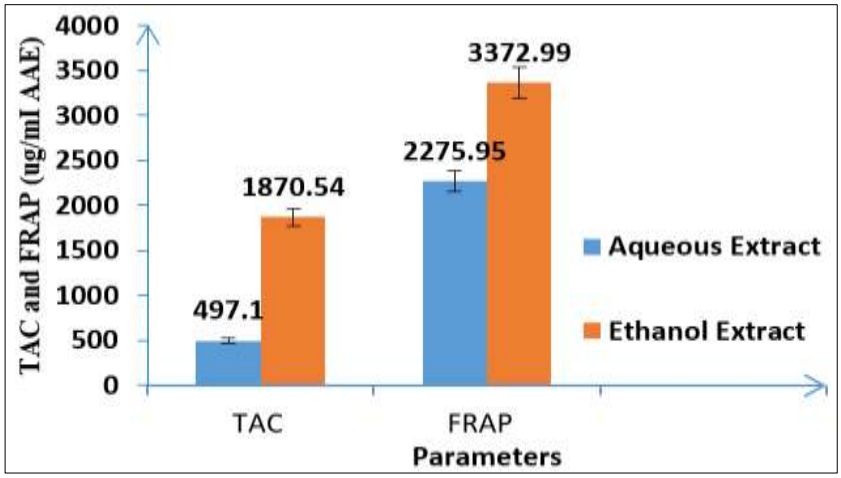

Fig 3.1a: Total antioxidant capacity (TAC) and ferric-reducing antioxidant potential (FRAP) of *T. preussii* leaf crude aqueous and ethanolic extracts. Values represent mean±standard error of mean, AAE= Ascorbic acid equivalent

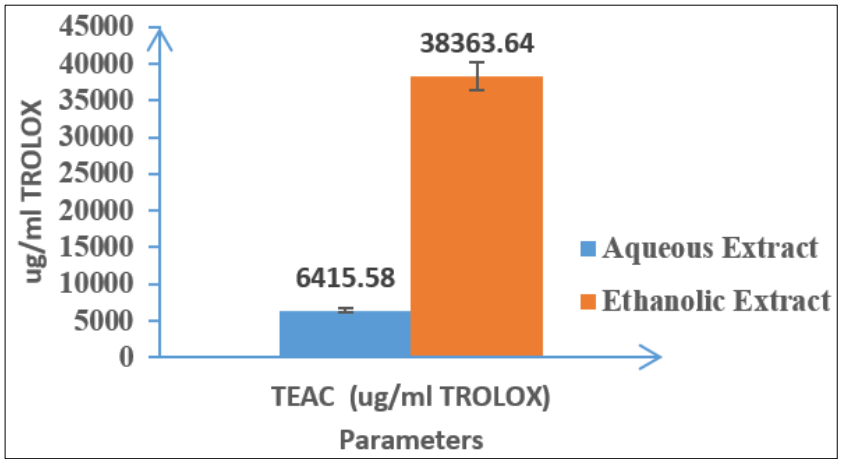

Fig 3.1b: Trolox equivalent antioxidant capacity (TEAC) of *T. preussii* leaf crude aqueous and ethanolic extracts. Values represent mean±standard error of mean

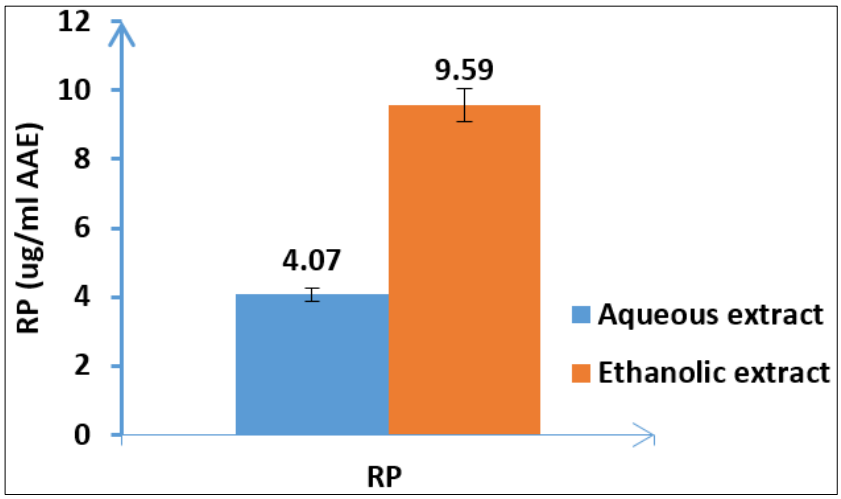

Fig 3.1c: Reduction potential (RP) of *T. preussii* crude aqueous and ethanolic leaf extracts. Values represent mean±standard error of mean (SEM)

3.2 Estimation of total phenolic (TPC) and total flavonoid contents (TFC) in *Tapinanthus preussii* leaf crude aqueous and ethanolic extracts

The total phenolic and total flavonoid contents of *Tapinanthus preussii* leaf crude aqueous and ethanolic extracts were evaluated and summarized in Figure 3.2a and b, respectively. The analysis revealed a significantly (p<0.05) higher level of total phenolic content (TPC) expressed in gallic acid equivalent (GAE) in the ethanolic extract (1968.89 µg/ml GAE) compared to the aqueous extract (360.34 µg/ml GAE). Similarly, the total flavonoid content, expressed as quercetin equivalent (QE), was notably higher in the ethanolic extract (13120.99 µg/ml QE) than in the aqueous extract (280.99 µg/ml QE), with statistical significance (p<0.05).

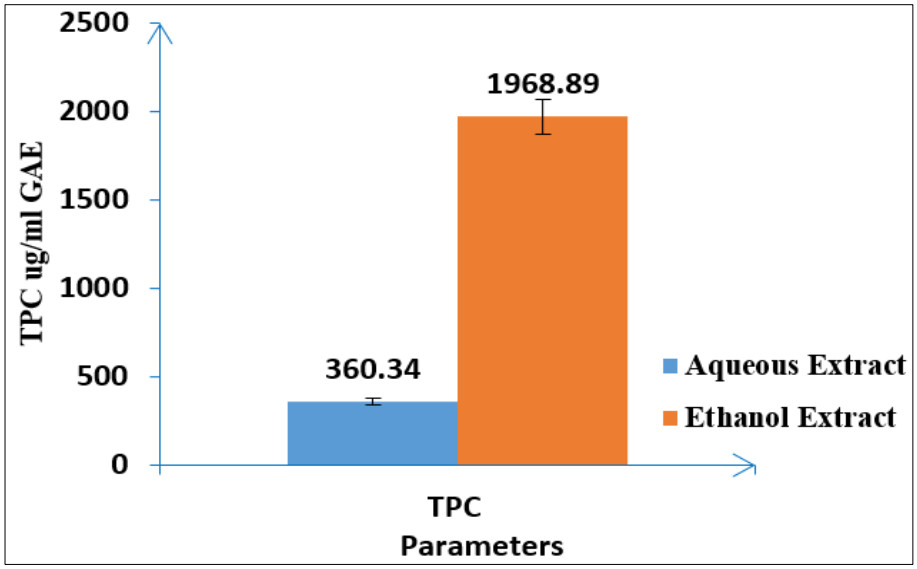

Fig 3.2a: Estimation of Total phenolic in crude Aqueous and Ethanolic extracts of *T. preussii* leaves. Values represent mean±standard error of mean (SEM)

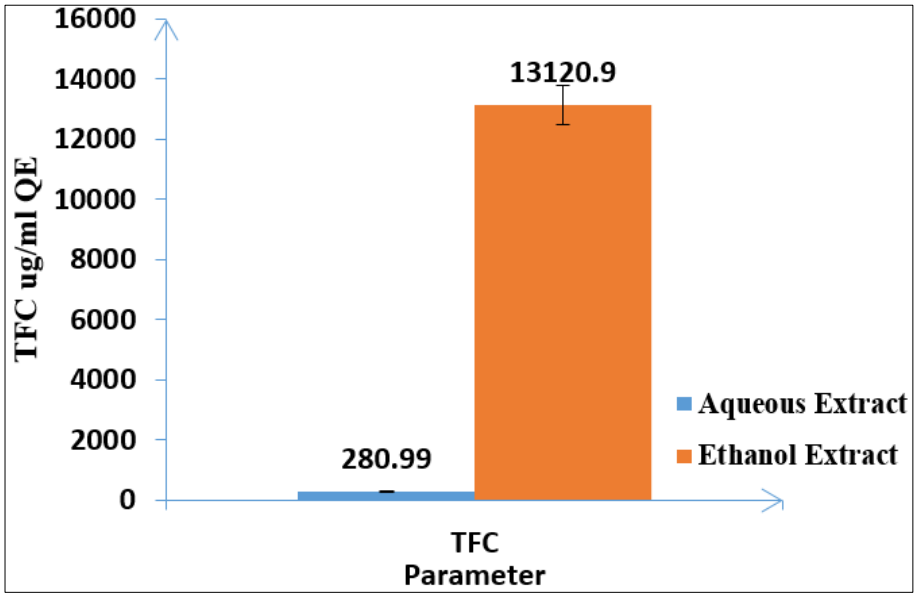

Fig 3.2b: Estimation of Total flavonoid contents in crude Aqueous and Ethanolic extracts of *T. preussii* leaves. Values represent mean±standard error of mean (SEM)

3.3. Nitric Oxide radical scavenging activity (NO) of *Tapinanthus preussii* crude aqueous and ethanolic leaf extracts

The nitric oxide (NO) radical scavenging ability, expressed as percentage inhibition and IC_{50}, of *Tapinanthus preussii* leaf crude aqueous and ethanolic extracts is presented in Figure 3.3a and b respectively. The aqueous and ethanolic extracts exhibited nitric oxide scavenging activity ranging from 0.65-74.93% and 22.85-90.25%, respectively, at a concentration range of 200-1000 µg/ml. The IC_{50} value, which represents the concentration required for 50% inhibition, was significantly higher for the ethanolic extract (789.32 µg/ml) than for the aqueous extract (471 µg/ml). However, both extracts had significantly higher IC_{50} values compared to the ascorbic acid standard (76.30 µg/ml). These findings suggest that the ethanolic extract has a stronger nitric oxide scavenging potential compared to the aqueous extract.

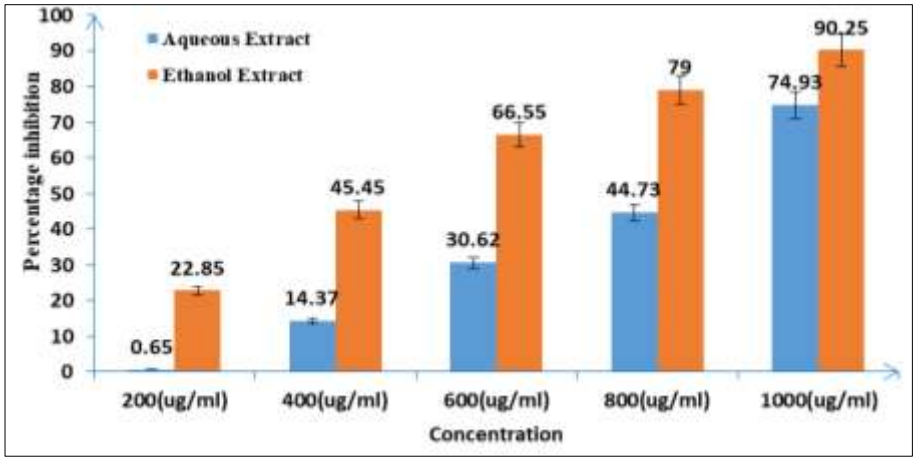

Fig 3.3a: Percentage inhibition of Nitric oxide scavenging activity (NO) of *Tapinanthus preussii* crude leaf extracts. Values represent mean±standard error of mean (SEM)

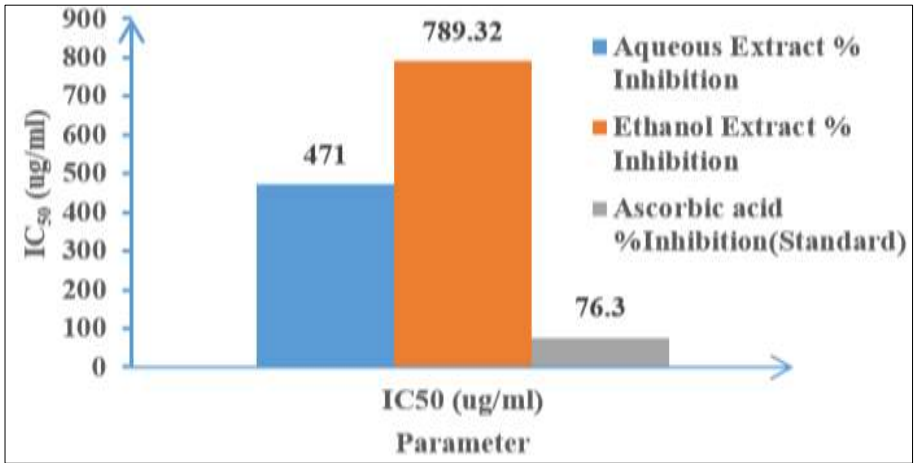

Fig 3.3b: IC_{50} of Tapinanthus preussii Aqueous and Ethanolic leaf extracts scavenging activity of Nitric oxide (NO)

3.4 Hydroxyl radical scavenging activity of *Tapinanthus preussii* leaf crude aqueous and ethanolic extracts

The hydroxyl radical scavenging activity of *Tapinanthus preussii* aqueous and ethanolic crude leaf extracts is presented in Figure 3.4a and b, with the percentage inhibition and IC_{50} values shown respectively. At a concentration range of 20-100 µg/ml, the aqueous extract exhibited a percentage inhibition ranging from 1.15±0.39 to 16.34±2.81%, while the ethanolic extract ranged from 41.21+0.79 to 51.95±0.84%. The mannitol standard, on the other hand, ranged from 4.44±0.67 to 42.36±0.39 at a concentration range of 0.02 - 0.1. The IC_{50} values obtained for the aqueous, ethanolic extracts, and the mannitol standard were 92.78, 267.06, and 0.12 µg/ml, respectively. These results suggest that the ethanolic extract of *Tapinanthus preussii* has a better hydroxyl radical scavenging activity compared to the aqueous extract.

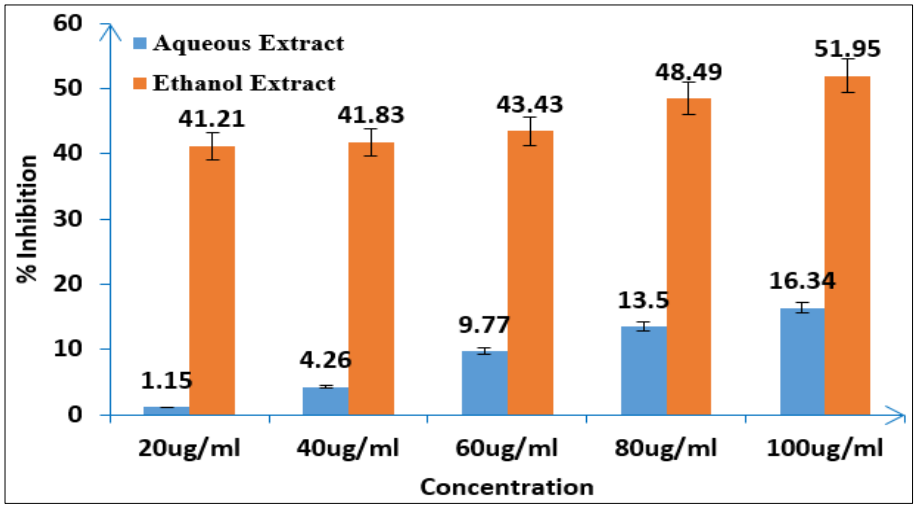

Fig 3.4a: Hydroxyl Radical Scavenging Activity of *Tapinanthus preussii* aqueous and ethanolic Crude leaf extracts. Values represent mean±standard error of mean (SEM).

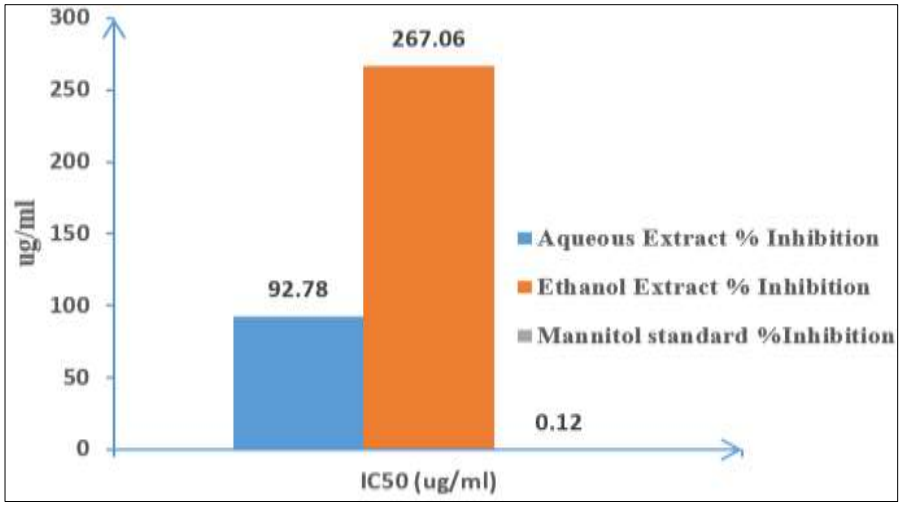

Fig 3.4b: The IC_{50} of hydroxy radical scavenging activity of Tapinanthus preussii aqueous and ethanolic leaf extracts

3.5. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of *Tapinanthus preussii* crude aqueous and ethanolic leaf extracts: The DPPH radical scavenging activity of *Tapinanthus preussii* aqueous and ethanolic crude leaf extracts, presented as percentage inhibition and IC_{50} values, can be seen in Figure 3.5a and b, respectively. In the concentration range of 31.25 to 500 µg/ml, the DPPH radical scavenging activity of the aqueous and ethanolic crude leaf extracts ranged from 20.79±4.06 to 86.43±0.97% and 26.95±4.35 to 87.24±0.24%, respectively. The IC_{50} values obtained for the aqueous extract, ethanolic extract and standard, were 118.46, 65.32, and 8.85 µg/ml, respectively. A lower IC_{50} value indicates better scavenging ability and antioxidant power. Therefore, the ethanolic extract demonstrated superior inhibition of the DPPH radical compared to the aqueous extract. Ascorbic acid, being a pure substance, exhibited the highest antioxidant efficacy among the tested samples.

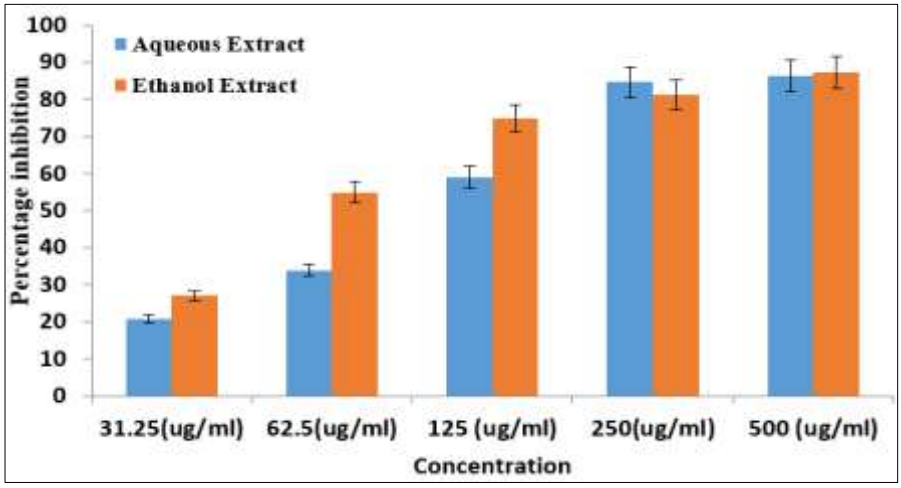

Fig 3.5a: Percentage inhibition of 2, 2^1-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of *Tapinanthus preussii* leaf extracts. Values represent mean±standard error of mean (SEM)

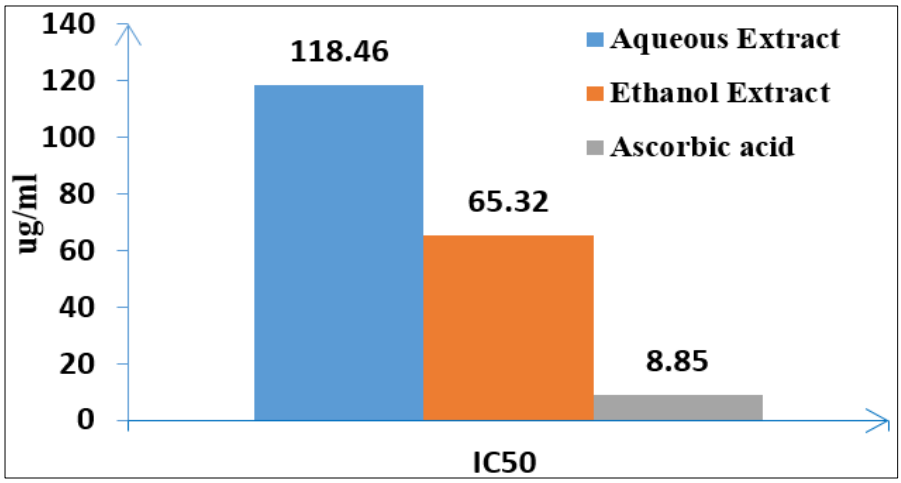

Fig 3.5b: The IC_{50} of DPPH radical scavenging activity of *Tapinanthus preussii* aqueous and ethanolic leaf extracts

3.6. The effect of *Tapinanthus preussii* aqueous and ethanolic crude leaf extracts on lipid peroxidation.

The percentage lipid peroxidation inhibition activity (LPIA) and IC_{50} values of *Tapinanthus preussii* crude leaf extracts are presented in Figure 3.6a and b, respectively. The ethanolic extract exhibited a higher percentage lipid peroxidation inhibition (LPI) at a concentration range of 62.5 to 1000 µg/ml (05.85±0.55 to 58.70±1.00%) compared to the aqueous extract (07.30±1.59 to 50.00±3.20%). The IC_{50} value of the ethanolic extract was also lower (695.44 µg/ml) than that of the aqueous extract (879.69 µg/ml) implying that the ethanolic extract had a better antioxidant capacity than the aqueous extract. However, the IC_{50} value of the standard Botulene hydroxyl toluene (BHT) was lower (287.42 µg/ml) than that of both extracts, indicating that the standard has a better antioxidant capacity than the two extracts as expected since it is a pure substance.

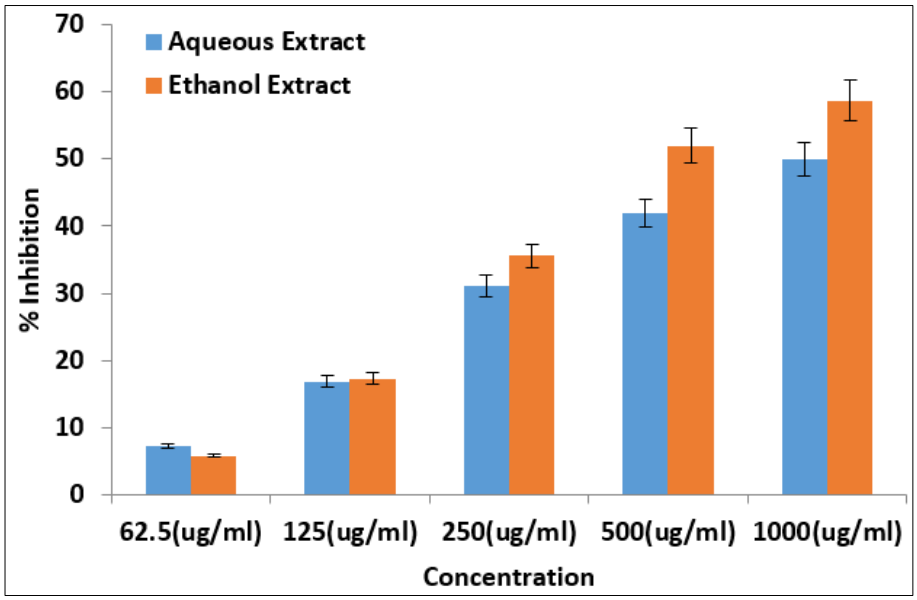

Fig 3.6a: Percentage Lipid peroxidation inhibition activity (LPIA) of *Tapinanthus preussii* crude aqueous and ethanolic leaf extracts. Values represent mean±standard error of mean (SEM)

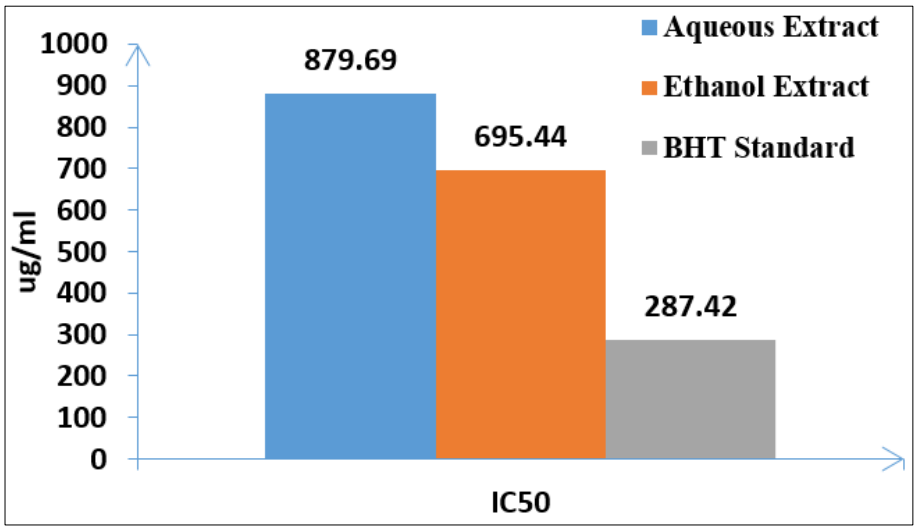

Fig 3.6b: The IC50 of Lipid peroxidation inhibition activity (LPIA) of *Tapinanthus preussii* crude aqueous and ethanolic leaf extracts

3.7. Metal (ferrous ion) chelating activity of *Tapinanthus preussii* crude leaf extracts.

Figures 3.7a and b display the percentage inhibition of metal (Iron II) chelating ability and the IC_{50} values of *Tapinanthus preussii* crude aqueous and ethanolic leaf extracts respectively. In the concentration range of 20-100 µg/ml, the aqueous extract exhibited a slightly higher inhibition of chelating ability (6.17±1.33 to 29.14±0.35%) compared to the ethanol extract (0.31±0.20 to 17.81±1.38%). The standard EDTA demonstrated significantly ($p<0.05$) higher inhibition (8.36±0.74 to 39.45±0.74%) at a lower concentration range (0.02 to 0.1 µg/ml) than both extracts. The IC_{50} value for the aqueous extract was lower (197.58 µg/ml) than that of the ethanolic extract (230.09 µg/ml), while the standard EDTA had a significantly lower IC_{50} (0.12 µg/ml) compared to both extracts. Consequently, the aqueous extract displayed superior ferrous ion chelating activity compared to the ethanolic extract.

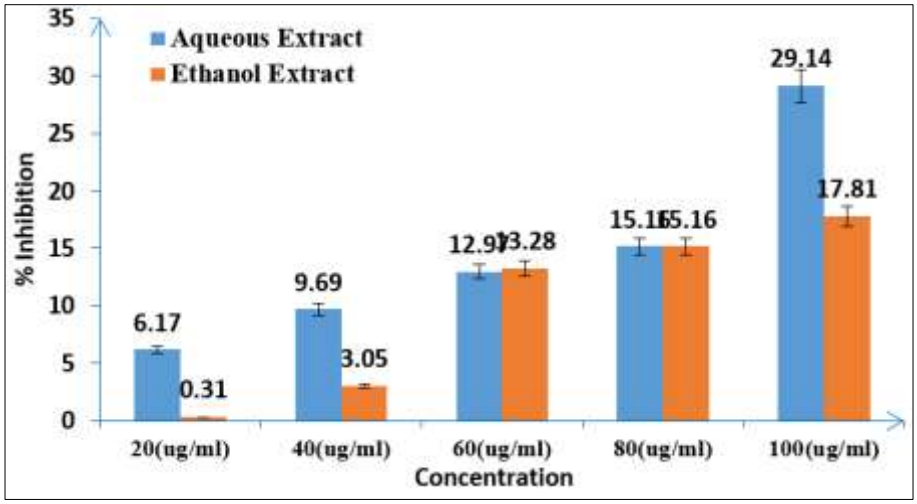

Fig 3.6a: Percentage inhibition of metal chelating activity of *Tapinanthus preussii* leaf extracts. Values represent mean±standard error of mean (SEM)

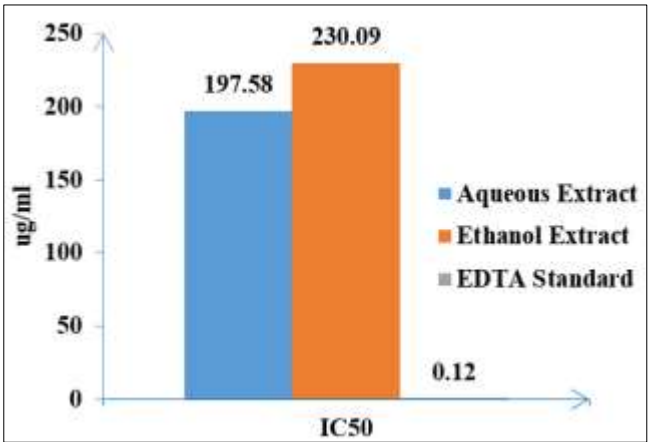

Fig 3.6b: IC_{50} metal chelating activity of *Tapinanthus preussii* leaf extracts. Values represent mean±standard error of mean (SEM)

4. Discussion

Under physiological conditions, the body has a widespread antioxidant defense system that protects against the adverse effects of free radical production. This system works by either scavenging the reactive oxygen species (ROS) or protecting the antioxidant defense mechanisms [50]. Free radicals are often generated as by-products of normal biological and aberrant metabolic reactions. They are molecules, ions, or atoms with one or more unpaired electrons, making them generally reactive, and are referred to as reactive oxygen species (ROS). Examples of ROS include superoxide radicals, hydroxyl radicals, singlet oxygen, and hydrogen peroxide [51]. The efficiency of the antioxidant defense mechanism is altered in a wide variety of pathological conditions, such as diabetes, cardiovascular disorders, atherosclerosis, and neoplastic diseases [52]. The ineffective scavenging or detoxification of free radicals may play a crucial role in determining tissue damage [53]. Plants often contain substantial amounts of antioxidants, including alpha-tocopherol (Vitamin E), carotenoids, ascorbic acid (Vitamin C), flavonoids, and tannins. These plant-derived antioxidants offer resistance against oxidative stress, helping to maintain the body's delicate balance and protect against the harmful effects of free radicals. Therefore the body's antioxidant defense system plays a vital role in protecting against the adverse effects of free radical production. Understanding the importance of this system and the role of plant-derived antioxidants is crucial for maintaining overall health and preventing various pathological conditions.

Several techniques have been employed to determine the antioxidant activity of plant materials *in vitro*. This is essential because of the complex nature of phytochemicals [47, 54]. These methods involve scavenging free radicals, inhibiting lipid peroxidation, and utilizing various other mechanisms [55]. The purpose of these *in vitro* assessments is to allow for rapid screening of plant materials, as those with low antioxidant activity *in vitro* are likely to exhibit little activity *in vivo* and, consequently, have limited potential for disease prevention. The mode of preparation and administration of herbal remedies is fundamental to determining their efficacy for pharmacological evaluations [56]. The selection of water and alcohol as the solvents for this study was based on the prevalent local practices of herbal remedy preparation. By aligning the experimental conditions with the traditional methods used in the region, the researchers aimed to ensure the relevance and applicability of the findings to the local context.

Phenolic compounds are secondary metabolites derived from phenylalanine and tyrosine, which are found ubiquitously in plants and exhibit a wide range of variations [57]. These phytochemical compounds are categorized into several groups, with flavonoids being a prominent class known for their potent antioxidant activities [55]. Flavonoids are recognized as powerful chain-breaking antioxidants and are crucial constituents due to their scavenging ability, which is attributed to the presence of hydroxyl groups [58]. They possess high redox potentials, making them effective reducing agents, hydrogen donors, and singlet oxygen quenchers [59]. Plant materials rich in phenolics are increasingly being utilized in the food industry because they retard oxidative degradation of lipids and enhance the quality and nutritional value of food items [60]. The results of this study revealed that the ethanolic extracts of *Tapinanthus preussii* had a higher total phenolic content compared to the aqueous extract. This suggests that the ethanolic extraction method may be more effective in extracting phenolic compounds from this plant material, which could have significant implications for its potential applications in the food industry. The findings revealed a consistent trend in the flavonoid content of the extracts, mirroring the results seen with the phenolic content. This observation indicates that ethanol serves as a superior solvent for extracting phenols

from *Tapinanthus preussii* leaves. Phenols and polyphenols, including flavonoids, are secondary metabolites in plants that are believed to be positively correlated with the antioxidant properties of plants [61]. Flavonoids, naturally present in plants, are associated with various health benefits. Research on flavonoid derivatives has demonstrated a wide array of antibacterial, antiviral, anti-inflammatory, anticancer, and anti-allergic activities [62]. Flavonoids exhibit potent scavenging abilities against oxidizing molecules, including singlet oxygen and various free radicals implicated in numerous diseases [63]. The molecular antioxidant response of phenolic compounds varies significantly based on their chemical structure. It is important to note that the presence of other chemical components in the extract, such as sugars or ascorbic acid, may introduce some interference in the antioxidant response of phenolic compounds [64]. Hence, the use of ethanol as a solvent for extracting phenols from *Tapinanthus preussii* leaves showed promising results, highlighting the potential benefits of phenols and flavonoids in plant extracts for their antioxidant activities and health-promoting effects.

The total antioxidant capacity of the plant extracts was evaluated spectrophotometrically. This method was based on the reduction of molybdenum (IV) to molybdenum (V) by the extracts, followed by the formation of a green phosphate/molybdenum (V) complex at an acidic pH, which exhibits maximum absorption at 765nm. The present study demonstrated that the *Tapinanthus preussii* ethanolic extract exhibited a higher antioxidant capacity for phosphomolybdate reduction compared to the aqueous extract. The trend observed in the results suggests a correlation between the total phenolic content (TPC) and total flavonoid content (TFC) extracted by the ethanolic solvent, and their corresponding total antioxidant capacity [65]. Recent studies have shown that many flavonoids and related polyphenols contribute significantly to the phosphomolybdate scavenging activity of medicinal plants [63]. This further supports the observed relationship between the phenolic and flavonoid content, as well as the overall antioxidant capacity of the *Tapinanthus preussii* extracts. Thus, the spectrophotometric analysis revealed that the *Tapinanthus preussii* ethanolic extract demonstrated a higher antioxidant capacity, which appears to be correlated with its higher phenolic and flavonoid content. This highlights the potential of this plant extract as a source of natural antioxidants with potential health benefits.

Natural compounds exhibiting reducing power activity have the ability to donate electrons, thereby reducing oxidized intermediates formed during lipid peroxidation. This dual role allows them to function as both primary and secondary antioxidants [47]. The level of reducing power is closely linked to antioxidant activity and can serve as a significant indicator of the overall antioxidant potential [66]. In this study, the ethanolic extracts of *Tapinanthus preussii* demonstrated superior antioxidant potential compared to the aqueous extracts of the plant. The enhanced antioxidant capacity of the ethanolic extract may be attributed to the presence of reductones, which act by breaking the free radical chain through hydrogen atom donation and can also interact with peroxide precursors to inhibit their formation [61].

When the same extracts were assessed for Ferric Reducing Antioxidant Power (FRAP), it was observed that the ethanolic extract of *Tapinanthus preussii* exhibited a greater ability to reduce Fe^{3+} to Fe^{2+} under acidic conditions. This suggests the presence of compounds in the extract that may not be solely dependent on the extraction solvent for their extraction. The superior antioxidant potential of the ethanolic extracts of *Tapinanthus preussii*, as evidenced by their reducing power activity and FRAP results, highlights the presence of compounds with significant antioxidant properties. These findings underscore the importance of considering the extraction method and the specific compounds present in plant extracts when evaluating their antioxidant capabilities [67].

Egg yolk homogenate, being a lipid-rich medium, is susceptible to rapid non-enzymatic peroxidation in the presence of ferrous sulfate, leading to the generation of malondialdehyde (MDA) and other aldehydes. These compounds are responsible for the formation of pink pigments when reacting with thiobarbituric acid (TBA). Oxygen radicals play a significant role in the peroxidation of biological membrane lipids, disrupting their normal functions. The ethanolic extract of *Tapinanthus preussii* leaves exhibited superior activity in inhibiting lipid peroxidation. This indicates that the extract contains components capable of effectively preventing lipid peroxidation. The observed effect may be attributed to the extract's ability to reduce the lag phase of lipid peroxidation and slow down the propagation rate. These actions are characteristic of a chain-breaking antioxidant, similar to the mechanism of action of the standard antioxidant butylated hydroxytoluene (BHT) [68]. The effectiveness of the *Tapinanthus preussii* ethanolic extract in inhibiting lipid peroxidation was further confirmed by the IC_{50} results obtained 695.44µg/ml, surpassing the aqueous extract though falling short of the 287.42µg/ml IC_{50} of the reference standard. This parameter provides valuable insights into the concentration of the extract required to inhibit lipid peroxidation by 50%. The ethanolic extract of *Tapinanthus preussii* demonstrated potent inhibition of lipid peroxidation, suggesting the presence of compounds capable of effectively combating oxidative damage. The extract's ability to mimic the actions of a chain-breaking antioxidant, as evidenced by its impact on the lag phase and propagation rate, underscores its potential as a natural source of lipid peroxidation inhibitors.

Nitric oxide is a highly reactive free radical that can induce adverse alterations in the structure of proteins, carbohydrates, nucleotides, and lipids. It also plays a significant role in cell and tissue destruction, sterile inflammation, and the formation of adhesions [69]. Nitric oxide is a potent vasodilator, relaxing arterial and venous smooth muscles, and it also inhibits platelet aggregation and adhesion formation, albeit to a lesser extent [70]. In this study, the ethanolic extract of *Tapinanthus preussii* displayed a higher percentage of nitric oxide scavenging activity compared to the aqueous extract. However, the nitric oxide scavenging activity of the ethanolic extract was not as potent as the standard, ascorbic acid, as confirmed by the better IC_{50} value of the ascorbic acid standard. The superior nitric oxide scavenging activity of the ascorbic acid standard can be attributed to its high phenolic content, which has been linked to antioxidant potentials. The phenolic compounds present in the standard likely contribute to its enhanced ability to scavenge nitric oxide radicals. Thus the ethanolic extract of *Tapinanthus preussii* exhibited higher nitric oxide scavenging activity compared

to the aqueous extract, but it was still less effective than the ascorbic acid standard. This difference can be explained by the higher phenolic content of the standard, which is known to be associated with potent antioxidant properties, including the ability to scavenge nitric oxide radicals.

DPPH, a stable radical, is commonly utilized to assess the potential of compounds as free radical scavengers or hydrogen donors and to evaluate the antioxidant activity of plant extracts [71]. In this study, both extracts exhibited significant levels of inhibition against the DPPH free radical. Notably, the ethanolic extract demonstrated the highest percentage inhibition of the DPPH radical, with an IC_{50} value that closely resembled that of the ascorbic acid standard. The superior DPPH radical scavenging activity of the ethanolic extract can be attributed to its high phenolic content, which surpassed that of the aqueous extract. This finding aligns with previous reports indicating a positive correlation between phenolic content and antioxidant activity [72]. Therefore, the ethanolic extract of *Tapinanthus preussii* exhibited remarkable DPPH radical scavenging activity, likely due to its elevated phenolic content. This study reinforces the association between phenolic compounds and antioxidant potential, highlighting the extract's efficacy as a free radical scavenger and supporting its potential application as a natural antioxidant source.

The hydroxyl radical, known for its extreme reactivity among reactive oxygen species, inflicts significant damage to biomolecules by abstracting hydrogen atoms from membrane lipids, leading to lipid peroxidation. In this *in vitro* study, the hydroxyl radical scavenging results indicated that the ethanolic extract of *Tapinanthus preussii* leaves exhibited notable scavenging activity against the radical, although it did not match the efficacy of the mannitol standard. The scavenging properties observed were influenced by the extraction solvent, despite the potential presence of water and ethanol-soluble components in the plant material. The IC_{50} values of both extracts from *Tapinanthus preussii* suggest that they possess reasonably effective antiradical scavenging abilities, albeit not reaching the level of the Mannitol standard. Hence, the ethanolic extract of *Tapinanthus preussii* demonstrated significant hydroxyl radical scavenging activity, highlighting its potential as a radical scavenger. While not as potent as the Mannitol standard, the extract's scavenging properties were influenced by the extraction solvent, underscoring the importance of extraction methods in determining the antioxidant capabilities of plant extracts [67].

Ferrous iron can trigger lipid peroxidation through the Fenton reaction and also expedite the peroxidation process by breaking down hydroperoxides into peroxyl and alkoxyl radicals, which can be harmful to biological systems [73]. The findings of this research indicated that the aqueous extracts of *Tapinanthus preussii* exhibited superior iron chelating capabilities compared to the ethanolic extract. However, the EDTA standard demonstrated the most effective iron chelation with the lowest IC_{50} value.

5. Conclusion

The efficacy of plant materials as antioxidants is best elucidated by their impact on various indicators of antioxidant activity. In this investigation, the ethanolic extract of *Tapinanthus preussii* demonstrated superiority in terms of total phenolic content, total antioxidant capacity, FRAP, nitric oxide scavenging activity, and DPPH radical scavenging activity. It also exhibited modest scavenging activity against hydroxyl radicals. Consequently, *Tapinanthus preussii* ethanolic extract emerges as a promising antioxidant and a potential natural chemotherapeutic agent against diseases linked to free radicals.

6. Acknowledgement

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