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Tapinanthus globiferus (A. Rich.) Tiegh. leaf extract and the antioxidant vitamins in heat-driven oxidation of beef meat in rats

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Abstract

Boiling and frying make meat suitable for consumption. This study evaluated oxidative changes in boiled and fried meat (180-200°C) and the antioxidant effect of *Tapinanthus* leaf extract in rats. Animals were assigned to groups A to E (n-4). Group A (control) took distilled water; the experimental groups B to E either received fried beef floss feed supplement, an oxidized lipid diet (OLD) and different treatment per kg body weight for two weeks as follows: Groups B (400 mg extract), C (33.33% OLD), D (OLD + 400 mg extract) and E (OLD + 1000 mg vitamin E). The extract showed detectable levels of polyphenols, cardiac glycosides and phytosterols, and demonstrated superoxide dismutase, catalase and glutathione antioxidant activities in a concentration-dependent manner. The beef floss contains high levels of free fatty acids, peroxides and carbonyl compounds. However, serum levels of vitamins E, C and A and malondialdehyde were not significantly ($p>0.05$) different between the groups, $F = 1.45, 1.39, 1.93$ and 2.44, respectively. The findings suggest that frying boiled beef meat promotes loss of fat and protein constituents and the formation of potential pro-oxidants, which did not influence the supposed required antioxidant potentials in *Tapinanthus globiferus*. Hence, recommending further studies on higher and chronic supplementation.

Keywords: Beef floss, Carbonyls, Malondialdehyde, Polyphenol, Pro-oxidant, *Tapinanthus globiferus*, Vitamins.

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Contents

Contribution of this paper to the literature
The study has provided data on how frying boiled beef meat changes some physicochemical characteristics of the meat, indicating oxidation, potential oxidative damage risk and recommend further research focus on the pathophysiological effects of excessive or chronic consumption.

1. Introduction

Oxidation occurs by several molecular mechanisms leading to generation of reactive oxygen precursors and free radicals. Free radical formation, if a molecule loses or gains an electron, can occur through exogenous and endogenous pathways. Input of energy from different sources, such as heating, has been shown to liberate free radicals exogenously. A temperature range of 100°C to 200°C has been employed in meat processing, involving boiling and frying, the common cooking methods. The high-temperature heating is shown to transform the meat to value-added products that are safer, producing good palatability and organoleptic values. However, thermal treatment of red meat, that are rich in triglycerides, phospholipids and cholesterol, has been shown to induce oxidation of the unsaturated fatty acid components, leading to the generation of various lipid oxidation products [1-3].

High temperature heating has been shown to initiate oxidation of lipid products by radical-mediated H-atom abstraction, mostly from the weak carbon-carbon double bond (-C_2H_4-) of the unsaturated fatty acid moiety to produce conjugated diene, which in presence of oxygen molecules, is propagated to peroxides (LOO^*) and alkoxyl (LO^*) radicals. These primary peroxyl (lipid) oxidation products are unstable, highly reactive and are further being oxidized to secondary lipid oxidation products such as aldehydes, ketones and epoxides, which can only be terminated by formation of stable non-radical products [1, 4]. Additionally, Redox reactions in metabolic pathways are a well-known source of endogenous radicals. Oxidation of oxygen, an aspect of cellular respiration in aerobic organisms, which is crucial to energizing many biological processes, releases reactive oxygen radicals and non-radical species, which at optimal concentrations are of certain biochemical significance [5, 6].

Continued high concentrations of exogenous or endogenous free radical release and the non-radical species, which can subsequently be converted to free radicals are well documented to generate oxidative stress. The oxidative stress resulting from a disproportion antioxidant defense mechanisms and free radicals is implicated in cellular damage and diseases [7-10]. It can initiate the disease, thus acting as a primary cause, results secondary to the disease or exacerbate disease progression. Thus, antioxidants have been employed as a useful index in the determination of oxidative stress in biological systems. It has been well reported to played significant positive clinical prognosis as an adjunct to management options in clinical studies [6, 7, 11].

Exogenous dietary antioxidant compounds have shown essential enhancement of antioxidant defenses in biochemical models, prompting continuous research focus on different natural antioxidant-based compounds and medicinal herbs [6, 8, 12]. *Tapinanthu species*, commonly called mistletoe, which belongs to the family Loranthaceae, WFO, World Flora Online [13], have shown antioxidant activities in a previous study [14]. The plant was reported to contain polyphenols and antioxidant vitamins, which can donate a proton to scavenge free radicals. The reducing potential has been assessed on several free radical models [15, 16].

While high temperature heating of red meat is well established to promote oxidation of its potential oxidative prone lipid components, the antioxidant activities of *Tapinanthus* has several times been reported. The aim of this study is to investigate the potential peroxide (s) products in beef meat boiled and then fried, a commonly globally adopted cooking method and the antioxidant capability of the methanol extract of *Tapinanthus globiferus* leaves *in rats.*

This study is one aspect of the research topic, evaluation of antioxidant potential and toxicity of *Tapinanthus globiferus* leaf extract on heat-induced lipid oxidation of beef meat in rats

2. Materials and Methods

2.1. Ethical Clearance

The research was conducted in Department of Biochemistry, Northwest University, Kano. Ethical approval was obtained from the Research and Ethics Committee (YUMSU/REC/02/V. II/7- 15.12.2025), Northwest University, Muhammad Buhari way, along Kofar Kabuga - Kofar Ruwa Road, $12^0$0'17^0N $8^0$28'47^0E. Kano. The animal handling and procedures were carried out in a humane and scientific manner as mandated.

2.2. Plant Identification, Collection and Extract Preparation

The *Tapinanthus globiferus* (A.Rich.) Tiegh. [13] leaves (BUKHAN624), as previously identified [16], were collected from *Azadirachta indica* host tree in Bayero University, Kano premises on 17th September, 2025. It was handpicked, washed under running tap water and air-dried under shade (30°C). The dried leaf samples were pulverized using a mechanical grinder, 100g of the powder leaf was extracted in 1 liter of methanol, ratio of 1:10 (w/v) of sample-to-Methanol. The mixture, shaken at intervals on an orbital shaker was macerated for 72 hours and filtered. The filtrate was lyophilized. The 400 mg/kg body weight dose (0.05g/ml/rat) was determined from the concentrate and administered [17, 18].

2.3. Preparation of Oxidized Lipid Diet

The oxidized lipid diet (OLD) was constituted with 33.33% fried shredded beef floss. The fresh beef meat was obtained from Kano metropolis abattoir, cut into smaller pieces (1cm) diagonally and boiled at high temperature (180–200 °C) for 60 minutes. The boiled meat was shredded using a homogenizer and then fried with vegetable oil for 45 minutes also at same temperature [2]. The fried shredded beef floss produced was supplemented in the animals' feed at ratio 1:2 of their diet daily for two weeks.

2.4. Animals and Experimental Design

Twenty female Wistar rats (100–120 g) weight range were used for the study. Animals were obtained from Department of Human Physiology, Bayero University, Kano. Acclimatized for 1 week and then randomly assigned

to five groups (groups A–E), with four animals per group and treated for 2 weeks/kg body weight of the animal as follows:

Group A – Control distilled water.
Group B – 400 mg extract.
Group C – 33.33% fried shredded beef floss supplemented feed (OLD).
Group D – OLD + 400 mg extract.
Group E – OLD + 1000 mg vitamin E.

2.5. Collection of Blood Sample and Preparation of Serum

At the end of the treatment day, animals were sacrificed using diethyl ether anesthesia, and 5 ml of blood samples were collected by cardiac puncture in non-vacuum plain AGARAY (CR-AG240722) blood collection tubes. After full clot retraction, the blood was centrifuged (1000 g for 5 min). Serum, the separated supernatant, was pipetted into another clean plain bottle and was used for biochemical analysis.

2.6. Biochemical Analysis

2.6.1. Quantitative Phytochemical Analysis of Tapinanthus globiferus Leaf Extract

Selected secondary metabolites were determined in the extract using procedures and methods described in Ezeonu and Ejikeme [19].

2.6.2. Screening of Tapinanthus Globiferus Leaf Extract for Antioxidant Enzyme Activity

Total activity of some enzymatic antioxidants was screened for at graded concentrations. Superoxide dismutase (SOD) was determined by epinephrine method, it is based on the ability of SOD to inhibit autooxidation of adrenaline [20]. Catalase was assayed calorimetrically at 620 nm and expressed as µmoles of H_2O_2 substrate consumed min/mL of the extract [19]. Glutathione activity was measured by the 5,5′-dithiobis-(2-nitrobenzoic acid (DTNB) method of Tietze [21] as modified by Adams, et al. [22]. Glutathione was oxidized by 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) to oxidized glutathione (GSSG) and 5-thio-2-nitrobenzoic acid (TNB). The GSSG is then reduced to GSH by glutathione reductase (GR) with NADPH. The rate of TNB formation measured at 412 nm is proportional to the sum of GSH and GSSG in the extract.

2.6.3. Physicochemical Analysis

The total Free fatty acid, peroxide value and the iodine value of the fried beef floss were evaluated using [23].

2.6.4. Determination of Antioxidant Vitamins

Modified method and the procedure of Rutkowski and Grzegorczyk [24] were adopted to measure the serum concentrations of vitamins A, C, E, nutrients as previously described in Oseni, et al. [16].

2.6.5. Determination of Malondialdehyde

Malondialdehyde (MDA) was measured calorimetrically [25, 26]. In principle, MDA reacts with thiobarbituric acid reactive substances (TBARS), in acidic medium at 100°C, to generate a pink/red-coloured chromogen, which was extracted with butanol and the absorbance was measured at 520-535nm.

3. Results

In this study, beef meat was boiled and fried at high temperatures. The fried beef floss was used as a supplement in the experimental animals' feed along with *Tapinanthus globiferus* leaf extract and a mega dose (maximum supplemental tolerable upper intake level) of vitamin E. The extract and fried beef floss were analyzed in relation to the antioxidant vitamins level in the animals. The results obtained were as shown in Tables 1-4 and Figure 1.

Table 1. Some secondary metabolite composition in methanol extract of *T. globiferus* leaf.

Total composition (mg/g)			
Flavonoids	**Phenolics**	**Cardiac glycoside**	**Phytosterol**
90.80 ± 0.54	149.06 ± 27.24	5.38 ± 0.02	17.47 ± 0.18

Note: Values are the mean ± SD of three replicate determinations.

Table 2. Selected total enzymatic antioxidant activity in *T. globiferus* methanol leaf extract.

	Substrate concentrations (µg/mL)		
Total enzyme activity	**1000**	**500**	**250**
Superoxide dismutase (ng/mL)	8.96 ± 0.00	5.16 ± 0.13	2.36 ± 0.13
Catalase (U/mL)	4.02 ± 0.01	2.41 ± 0.11	1.90 ± 0.06
Glutathione peroxidase (µmol/L)	110.50 ±0.02	123.83 ± 1.44	88.00 ± 0.00

Note: Values are the mean ± SD of three replicate determinations.

Table 3. Selected total pro-oxidant indices analysis of fried beef meat.

Oxidative indices	Total concentration
Free fatty acids (mg KOH/g)	1.63 ± 0.02
Peroxide value (meq O_2 /Kg)	1.73 ± 0.03
Iodine value (wij's)	88.45 ±1.60
Carbonyl content (µmol/g)	44.12 ± 0.73
Protein carbonyl content (nmol/mg)	31.21 ± 0.21

Note: Values are the mean ± SD of three replicate determinations.

Table 4. Serum antioxidant vitamins level and *T. globiferus* leaf extract effect in fried beef floss- fed rats.

Vitamins (mg/dL)	Normal feed		OLD-feed supplement		
	Distilled water (1ml)	Extract (400 mg)	OLD (33.33%)	OLD + 400 mg extract	OLD + 1000 mg vit E
E	0.75 ± 0.11^{d}	0.66 ± 0.05^{d}	0.63 ± 0.04^{d}	0.34 ± 0.05^{d}	0.80 ± 0.30^{d}
C	0.27 ± 0.05^{b}	0.18 ± 0.01^{b}	0.17 ± 0.03^{b}	1.12 ± 0.02^{b}	0.38 ± 0.20^{b}
A	2.30 ± 0.22^{f}	1.23 ± 0.01^{f}	1.21 ± 0.07^{f}	0.80 ± 0.09^{f}	0.78 ± 0.20^{f}

Note: Values are the mean ± SD of four samples (n=4). The OLD = Oxidized lipid diet.
Superscript letters along the same rows are not statistically different (P>0.05).

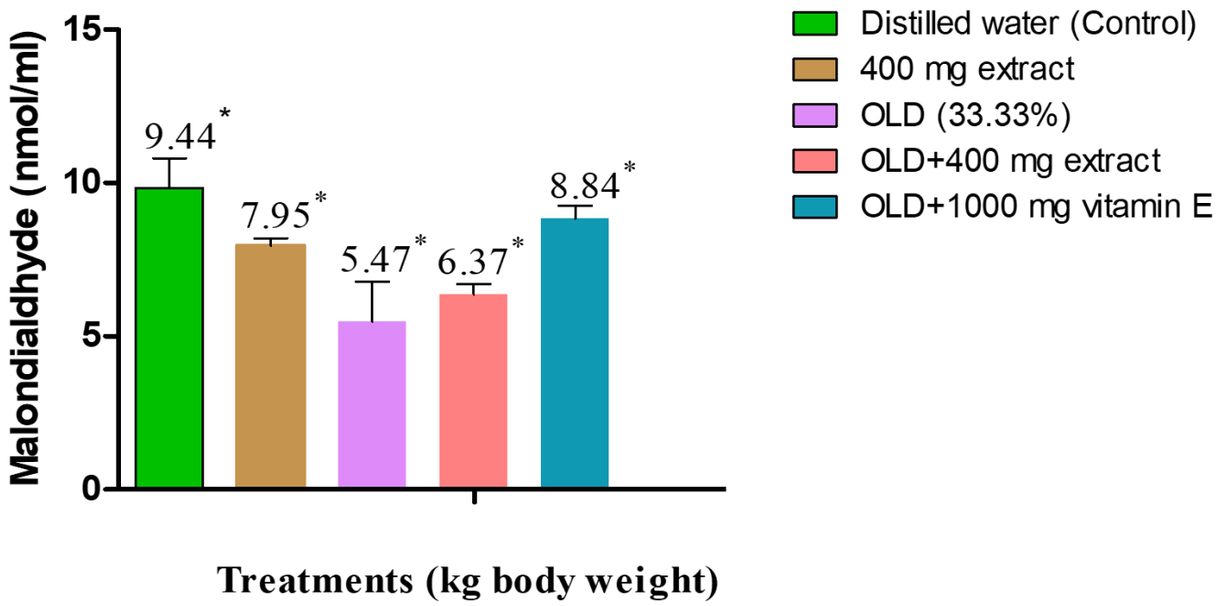

Figure 1. Serum malondialdehyde concentration and *T. globiferus* leaf extract effect in fried beef floss (OLD) fed rats.
Note: Bars are mean ± SD, (n=4). Mean* differences are not significantly different (p>0.05).

4. Discussion

Thermal processing and antioxidant capacity measurements are used to determine oxidation in food items and biological tissues [2, 27-29]. Organic peroxides (ROOH) can be released from the oxidation of lipids and proteins, which can subsequently be converted to reactive oxygen species (ROS). An increase in free radicals, non-radical intermediates of ROS and antioxidant disproportion can affect cellular components, necessitating continuous research focus on pro-oxidants and antioxidants.

Flavonoids and phenols were detected in the plant extract. These polyphenols are potent antioxidants. Polyphenols from different plant species in previous studies have demonstrated reducing activity in several radical molecules [30]. These secondary metabolites in the extract, which are believed to have antioxidant effects can enhance the antioxidant activity of *T. globiferus* during oxidative events. The concentrations obtained in this study is higher than what was reported earlier [16] and lower than the level reported elsewhere [31], suggesting seasonal and geographical influence on these bioactive compounds. The extract also demonstrated substantial superoxide dismutase, catalase and glutathione antioxidant activities on superoxide and peroxide radicals in a concentration-dependent manner, a further confirmation of potential antioxidant activity.

Free fatty acids (FFA), peroxide and iodine values, carbonyl and the protein carbonyl contents are indices used to evaluate oxidation in fats and oils and proteins [27-29]. The degree of unsaturated fatty acids in oil determines the oxidative stability and indicate the hydrolytic release of free fatty acids from the triacylglycerol of the oil. The permissible thresholds are 0.1- 0.3 % (0.20 - 0.60 mgKOH/g) for refined oils, depending on nature of the oil Codex, [32]. In this study, however, a slightly high value of FFA (0.82%) was seen in the fried beef, this may be an indication for heat transfer from the heated hot oil to remove moisture from the meat, this can hydrolyze triglyceride to produce the free fatty acid, which may subsequently promote further oxidation. The increase FFA in our study is similar to previous study, reported increase in FFA with number of frying cycles and times, being indicative of cumulative excessive heating or high temperature effects.

Iodine value is used to measure the total unsaturation in oil. It is the grams of iodine absorbed by 100 g of oil. According to the Codex Alimentarius Commission [32], permissible limits, if the value is higher than 40-45 (wijs) in tallow (beef meat), it can indicate oxidation effect. Therefore, the iodine value of the beef floss analyzed, which produced a higher value, suggests possible oxidation of its fat's constituent. On the same basis, peroxide value (PV), the number in milliequivalents of peroxide oxygen in a kilogram of oil (Meq O_2/Kg), which can liberate iodine from potassium iodide, is used to evaluate peroxides and hydroperoxides, the primary oxidation products which are formed during the initial stage of lipid oxidation. A low PV, if the concentration was determined over time during the initial stage of oxidation, indicates that the products are stable to oxidation. Decreased value could also imply a later oxidation stage in which the intermediate products are converted to final products. In this study, however, the PV was measured after the meat processing (boiling and frying) was completed, not intervally. A similitude of the widely adopted mode of cooking meats for human consumption. Thus, the lipid being tested is not likely to be in the initial stages of oxidation. Therefore, the low PV obtained in our result may indicate a later stage of the oxidative events; the peroxides might have been converted to secondary oxidized products as evidenced by the detected total carbonyl and protein carbonyl contents in the floss. These are secondary products of fats and protein oxidation, leading to final products of advanced oxidation state, suggesting that triglyceride, an oxidation-prone lipid in the meat, and some of the essential amino acids might have been oxidized. Frying boiled meat, a non-enzymatic oxidative

degradation, could induce oxidation of unsaturated fatty acids and might produce methyl (•CH_3), carbon (II) oxide (•CO), and aldehyde (•CHO) radical metabolites, and subsequently carbon dialdehyde lipid peroxidation end products. Cumulative increase in the generation of primary and secondary products of oxidation and the potential health risk implications is well documented [27, 29]. For instance, malondialdehyde (MDA) may react with DNA, proteins, and phospholipids by electrophilic addition, forming Schiff base adducts which are cytotoxic, neurotoxic, or mutagenic. Massad and Marıod [28] in their study also reported lower PV in oil used in frying chicken over time, documenting that the PV can increase or decline due to primary oxidative product. Yang, et al. [3] reported elevated carbonyl content in deep-fried beef and an elevated MDA was reported in fried beef compared to the raw, as reported by Hejazy, et al. [33].

Physical and chemical changes are produced in food cooked at high temperatures [28] During frying, which involved drying in heated hot oil, the moisture in the meat can turn to gas and the oil is absorbed by the meat, additionally, oxidation of triglyceride component of the meat can lead to formation of free fatty acid, hydroperoxides, producing a transient elevated peroxides which is further hydrolyzed to carbonyl and epoxide radicals, justifying the varying alterations in the physicochemical characteristics of the oil extracted from the beef floss observed in our study.

The diet-derived antioxidant nutrients such as tocopherol (vitamin E), ascorbate (vitamin C) and carotenoid (vitamin A), Zn, Mn and Se, as well as plant-derived polyphenols are commonly employed as antioxidant supplements [8, 34]. Vitamins E, C and A, the essential micro nutrients, acting as exogenous non-enzymatic antioxidants as well as the MDA were assessed in serum of the animals, the results showed that statistically, all the treatment groups were not significantly ($p>0.05$) different, F value produced 1.45,1.39, 1.93 and 2.44, respectively in the levels of vitamins E, C and A as well as the MDA. indicating that the concentration of the oxidized feed in the animals' diet and the duration of consumption might not be enough to induce adverse metabolic and oxidative events in the animals, therefore, conserving the supposed required non-enzymatic or enzymatic antioxidants action.

In conclusion, results from our study demonstrated that the frying causes high free fatty acid, iodine value, total and the protein carbonyl contents in the beef floss and a decrease in the peroxide value. These physicochemical changes are atribute of oxidation and may indicate that the meat might have been oxidized during the frying. The liberated products are potential pro-oxidants which could compromise enzymatic and non-enzymatic activity and introducing oxidative effects. However, the oxidative products did not produce a significant pro-oxidant effect on the antioxidant vitamins in this study. It is recommended that fried beef meat should be consumed with caution and, optimally, this may enable to prevent consequent excessive or overtime cumulative consumption potential oxidative effects. Further investigations are required to substantiate oxidative free effects and/or the pathophysiological implications of excessive and chronic consumption of fried beef meat.

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