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COMPARATIVE TOTAL PHENOLIC CONTENT, ANTI-LIPASE AND ANTIOXIDANT ACTIVITIES OF TWO NIGERIAN *AFRAMOMUM* SPECIES

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ABSTRACT

The anti-obesity drug development is presently not a bright story. So far, drugs reported to be effective have stimulated controversies due to side effects they elicit. Obesity and its co-morbidities continue however to constitute major problems in both developed and developing countries. This has resulted in a continuous search for novel, cost-effective, safe and potent alternatives. This study investigated the ethanolic extracts of two Nigerian *Aframomum* species for their anti-lipase and anti-oxidant activities as well as estimates of their polyphenol contents. Lipase activity was determined using glyceryltrioleate emulsion as a substrate and measuring the release rate of oleic acid from it. Percentage inhibition of lipase by the methanolic extracts of plants was determined spectrophotometrically at T€ and T ƒ € (30 minutes after incubation at 37ÚC). DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity of the extracts and that of gallic acid as control was measured using the stable radical DPPH method and absorbance at 515 nm using a spectrophotometer. The IC_{50} (half-maximal inhibitory concentration) value was calculated by linear regression analysis and the total phenolic content was determined by the Folin-Ciocalteau method at 765 nm. The standard curve was prepared by solutions of Gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of Gallic acid equivalent (w/w of dry mass). *Aframomum melegueta* exhibited the highest phenolic content of 60.4 ± 2.36 mgGAE/g**,** a percentage antioxidant activity of 86.6 % at 200µg/ml and percentage lipase inhibition of 89% at 1mg/ml while *Aframomum danielli* revealed a total phenolic content of33.3 ± 2.71mgGAE/g, a percentage antioxidant activity of 77.3% at 200µg/ml and percentage lipase inhibition of 73% at 1 mg/ml. The result provides some justifications for the use of these plants in ethno-medicine for the management of obesity. The species exhibited properties that are beneficial to health and therefore could find use as an alternative and/or complementary strategy in managing associated co-morbidities of obesity, and also as possible template for future anti-obesity drug development.

INTRODUCTION

Obesity is a leading but preventable cause of death worldwide, with increasing prevalence in adults. It has been viewed as one of the most serious public health problems of the 21st century [21,3]. it is a worldwide epidemic [16] that is characterized by excess body fat that has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/ or increased health problems [11,12]. In children, a healthy weight varies with age and sex and the term overweight rather than obese is often used in children as it is less stigmatizing [4]. This epidemic has received both national and international attention because of its detrimental impact on health, the enormous economic burden it imposes [29], and its increasing prevalence. Obesity is stigmatized in much of the modern world, though it was widely perceived as a symbol of wealth and fertility at other times in history, and still is in some parts of the world [12, 31].

People are considered obese when their body mass index (BMI), a measurement obtained by dividing a person's weight in kilograms divided by the square of the person's height in meters, exceeds 30kg/m^2 [32]. The adverse health consequences associated with obesity include cardiovascular disease[14,24]; stroke; type 2 diabetes mellitus[13]; hypertension; dyslipidemia; cancers of the breast, endometrium, prostate, and colon[27,30] gallbladder disease; osteoarthritis[7,8] respiratory problems, including asthma[6] and sleep apnea[35]; and perhaps depression[9,25].

Obesity is most commonly caused by a combination of excessive food energy intake, lack of physical activity, and genetic susceptibility, although a few cases are caused primarily by endocrine disorders, medications or psychiatric illness. Evidence to support the view that some obese people eat little yet gain weight due to a slow metabolism is limited; on average, obese people have a greater energy expenditure than their non-obese counterparts due to the energy required to maintain an increased body mass [1,23].

Management of obesity with synthetic drugs without any side effects is still a challenge to the medical system [33]. However, plant products have been researched into for safe, less toxic, natural and cost effectiveness alternatives [22,33]. Dieting and physical exercise are also a major main stay in the treatment of obesity. Anti-obesity drugs act through different mechanisms of actions which may be taken to reduce appetite or inhibit fat absorption together with a suitable diet. If diet, exercise and medication are not effective, a gastric balloon may assist with weight loss, or surgery may be performed to reduce stomach volume and/or bowel length, leading to earlier satiation and reduced ability to absorb nutrients from food [15]. Natural products no doubt provide a vast pool of pancreatic lipase inhibitors with potential for being developed into clinical products [5]. The lipase inhibitors work by inhibiting gastric and pancreatic lipases, the enzymes that break down triglycerides in the intestine. When lipase activity is blocked, triglycerides from the diet are not hydrolyzed into absorbable free fatty acids, and are excreted undigested instead.

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation

reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols [36]. Although oxidation reactions are crucial for life, they can also be damaging; plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, vitamin A, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Insufficient levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells. Oxidative stress which can be considered to be both the cause and the consequence of some diseases seem to play a significant role in many human diseases and since obesity does not just present as a disease but a harbinger of several other diseases, the use of antioxidants that are also able to control obesity by inhibiting fat absorption is particularly a good treatment for such diseases.

Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness [46]. Although initial studies suggested that antioxidant supplements might promote health, recent clinical trials of antioxidant supplements including beta-carotene, vitamin A, and vitamin E singly or in different combinations suggest that supplementation has no effect on all-cause mortality or possibly increases it [45].

Screening of plants is done by measuring the antioxidant activity through various *in-vitro* methods like the DPPH method, nitric oxide method among others. Several plants have been found to possess antioxidant activities including *Gingko biloba, Foeniculum vulgare, Emblica officinalis, Picrorrhiza kurroa, Cuscuta reflexa, Psoralen corylifolia, Santalum album.*

The IC_{50} is commonly used to determine the antioxidant activity of a substance in relation to a linear regression plot. It is a measure of how effective the plant is and it indicates how much of it is needed to inhibit a given biological process by half. In other words, it is the half minimal (50%) inhibitory concentration of a substance and the lower the IC_{50}, the higher the activity of the plant.

Although similar to alcohols, phenols have unique properties and are not classified as alcohols (since the hydroxyl group is not bonded to a saturated carbon atom). They have higher acidities due to the aromatic ring's tight coupling with the oxygen and a relatively loose bond between the oxygen and hydrogen. The acidity of the hydroxyl group in phenols is commonly intermediate between that of aliphatic alcohols and carboxylic acids (their pKa is usually between 10 and 12). Organisms that synthesize phenolic compounds do so in response to ecological pressures such as pathogen and insect attack, UV radiation and wounding [41]. As they are present in food consumed in human diets and in plants used in traditional medicine of several cultures, their role in human health and disease is an important subject of research [37-41]. Some phenols are germicidal and are used in formulating disinfectants. Others possess estrogenic or endocrine disrupting activity. Natural phenols are reactive species toward oxidation. The complex mixture of phenolics, found in food for example, can undergo autoxidation during the ageing process. Browning associated with oxidation of phenolic compounds has also been given as the cause of cells death in calli formed in *in-vitro* cultures. Those phenolics originate both from explant tissues and from explant secretions [42].

In *Vitis vinifera* grape, trans-resveratrol is a phytoalexin produced against the growth of fungal pathogens such as *Botrytis cinerea* [43]. Sakuranetin is a flavanone, a type of flavonoid. It can be found in *Polymnia fruticosa* and rice, where it acts as a phytoalexin against spore germination of *Pyricularia oryzae* [44]. Stilbenes are produced in *Eucalyptus sideroxylon* in case of pathogens attacks. Such compounds can be implied in the hypersensitive response of plants. High levels of phenolics in some woods can explain their natural preservation against rot.

The possession of phenolic content in many plants therefore explains the use of these plants in treatment of certain ailments. Previous studies reported it that the inclusion of the extract of *A. danielli*in biscuit recipe at a concentration of 400 *ppm* was found to be effective as an antioxidant [47]. Other studies have established the antiulcer, antimicrobial, anti-inflammatory, and sexual performance enhancing effects of the *A. melegueta* seed extract [48-51] which is very rich in the nonvolatile pungent compounds gingerol, shogaols, paradol, and related compounds [50]. Some others reported the *in vitro* and *in vivo* antioxidant potentials of the methanolic seed extract of *Aframomum melegueta* [52], the acetylcholinesterase inhibitory activity and antioxidant properties of phenolic-rich extracts from the two species [53].

However,][18this study investigated the ethanolic extracts of *Aframomumm elegueta* and *Aframomum danielli* mainly for their potential lipase inhibitory activities supported with the total phenolic content and antioxidant activities which confers possibly, a wide range of therapeutic benefits on them and also seek to justify the ethno-medicinal claim that these food/medicinal plants possess these potentials for an all-round health status.

RESULTS AND DISCUSSION

Lipase inhibitory activities of *A. melegueta, A. danielli* and the reference standard Orlistat:

TABLE 1: **P**ercentage lipase inhibition of Orlistat® at different concentrations

Reference standard (Orlistat®)	T_0	T_{30}	$T_0 - T_{30}$	% inhibition
1 mg/mL	2.363 ± 0.0011	2.163 ± 0.0055	0.200	74
0.5 mg/mL	2.322 ± 0.0180	1.920 ± 0.0130	0.402	49
0.25 mg/mL	2.223 ± 0.0130	1.817 ± 0.0136	0.406	48

Inhibition of Pancreatic lipase is one of the most widely studied mechanisms for determining natural products potential efficacy as anti-obesity agents [5] and a key enzyme in dietary triacylglycerol absorption, hydrolyzing triacylglycerols to monoacylglycerols and fatty acids [18]. However, only a few substances interact directly with lipases themselves. One example is tetrahydrolipstatin (Orlistat®); a derivative of the naturally occurring lipase inhibitor produced from *Streptomyces toxytricini*[2] hence, Orlistat® was used as a positive control in this study.

TABLE 2: **P**ercentage lipase inhibition of *Aframomum melegueta* at different concentrations

Aframomum melegueta	**T_0**	**T_{30}**	**$T_0 - T_{30}$**	**% inhibition**
1 mg/mL	1.64 ± 0.01	1.59 ± 0.01	0.08	89
0.5 mg/mL	2.05 ± 0.01	1.56 ± 0.01	0.49	38
0.25 mg/mL	2.62 ± 0.12	1.86 ± 0.01	0.77	1.8

TABLE 3: **P**ercentage lipase inhibition of *Aframomum danielli* at different concentrations

Aframomum danielli	**T_0**	**T_{30}**	**$T_0 - T_{30}$**	**% inhibition**
1 mg/mL	1.92 ± 0.01	1.71± 0.01	0.21	73
0.5 mg/mL	1.75 ± 0.01	1.47 ± 0.03	0.28	64
0.25 mg/mL	1.63± 0.01	1.86 ± 0.01	0.41	47

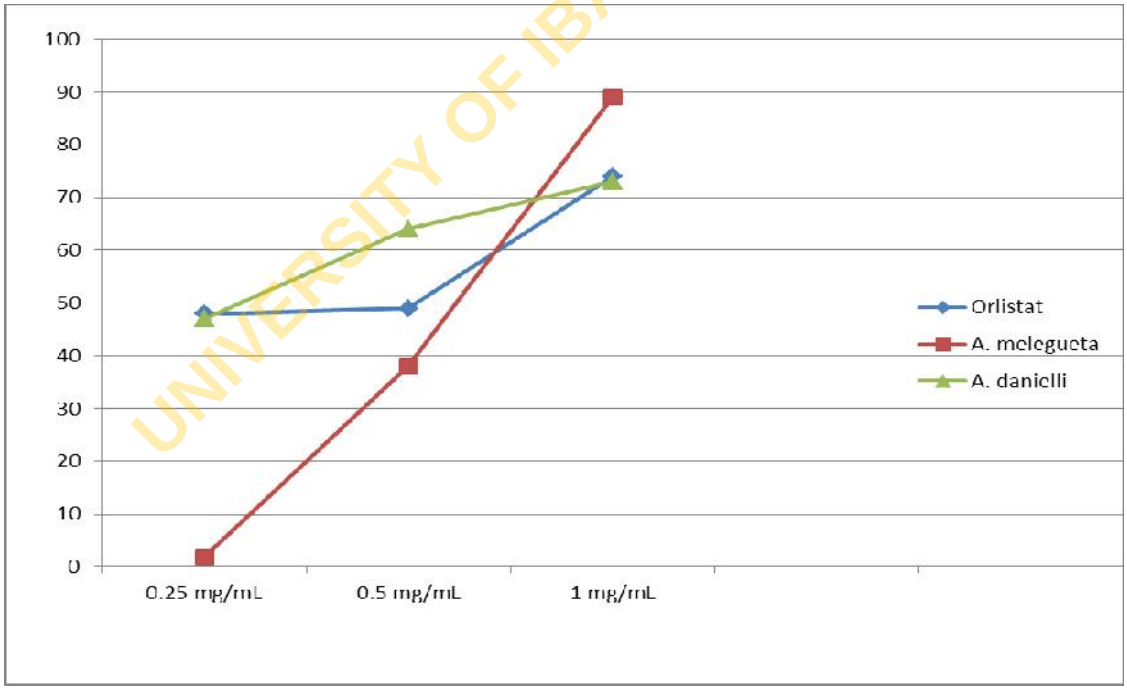

Figure 1: Graph showing the comparison of % inhibition of *A.danielli*, *A. melegueta* against Orlistat®

Aframomum melegueta revealed the highest percentage lipase inhibition of 89% while *Aframomum danielli* revealed a percentage lipase inhibition of 73% both at the highest concentration tested (1 mg/mL), comparable to the reference standard Orlistat® (74%) as shown in Figures 1 above**.**

Total phenolic content of extracts:

Table 4: Gallic acid absorbance for TPC

Concentration (µg/ml)	Absorbance ± S.E
200	1.919 ± 0.039
100	0.971 ± 0.008
50	0.599 ± 0.003
25	0.353 ± 0.005
12.5	0.243 ± 0.003
6.25	0.160 ± 0.008

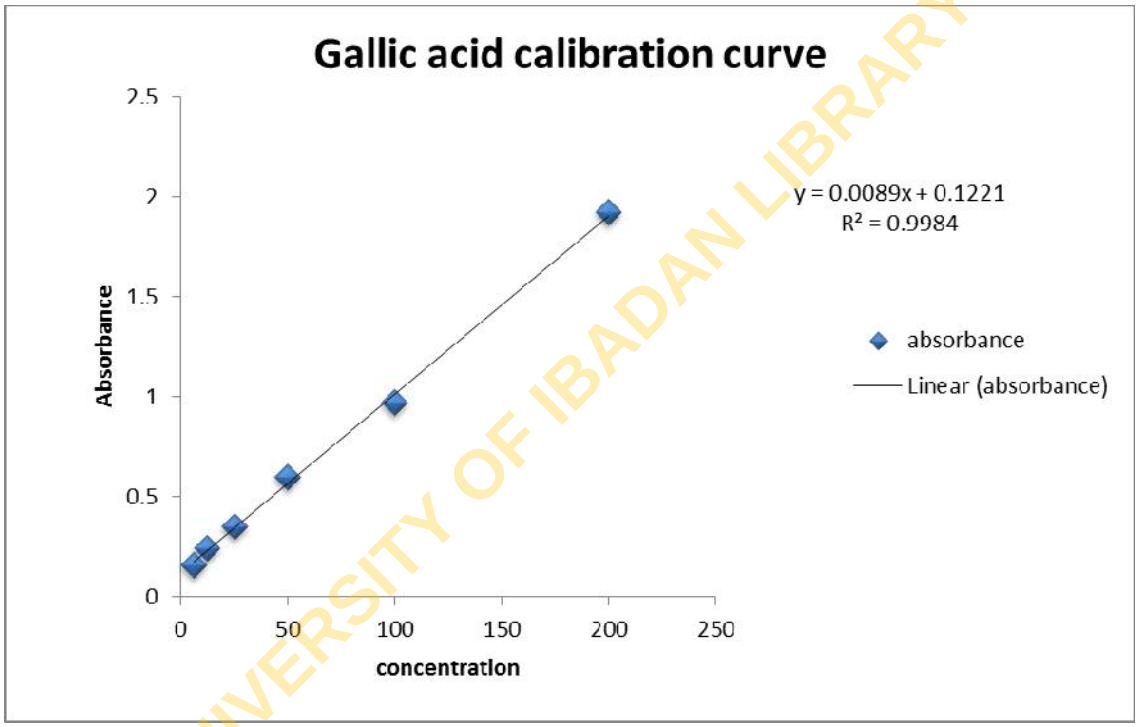

Figure 2: Gallic acid calibration curve

$y = 0.008x + 0.122$

y = absorbance of the extract

x = concentration

Phenols and polyphenolic compounds, such as flavonoids are widely found in food products derived from plant sources, and they have been reported to possess good antioxidant activities. Phenols are secondary metabolites in plants and are known to possess a wide range of therapeutic uses, such as antioxidant, antimutagenic, anticarcinogenic, free radical-scavenging activities and also decrease cardiovascular complication [34].

Table 5: Total phenolic content of extracts

Extract	Absorbance	TPC (µg/mL)	TPC(µgGAE/g)	Average TPC(µgGAE/g)	Average TPC ± S.E.(mgGAE/g)
Aframomum melegueta	0.593	58.875	58 875	60 375	60.375 ± 2.36
	0.580	57.25	57 250	-	-
	0.642	65.00	65 000		
Aframomum danielli	0.351	28.625	28 625	33 333	33.33 ± 2.71
	0.426	38.00	38 000	-	-
	0.389	33.375	33 375	-	-

In this study, total phenolic content (TPC) as determined by Folin-Ciocalteu method, and reported as mg/gallic acid equivalent/g of extract by reference to standard curve. The (TPC) of the crude ethanolic extracts of *Aframomum* was calculated from the gallic acid calibration curve using the formula.

$$TPC = \frac{\text{concentration of extract} \times \text{volume}}{\text{Weight of extract}}$$

Aframomum melegueta revealed the highest TPC of 60.375±2.36 mgGAE/g while *Aframomum danielli* revealed a TPC of 33.33 ± 2.71 mgGAE/g at 200µg/ml where GAE = gallic acid equivalent which suggests that these species also possess some antioxidant activity to different but appreciable degrees.

Antioxidant activity of extracts:

Table 6: Antioxidant activity of extracts at different concentrations determined by DPPH Method

Extract	Average antioxidant activity %				
	200µg/ml	100µg/ml	50µg/ml	25µg/ml	12.5µg/ml
A. melegueta	86.63 ±0.06	82.79±0.33	80.69±0.24	51.02±4.08	28.12±1.14
A. danielli	77.34±0.45	74.70±3.61	67.15±1.32	35.97±1.81	4.02±0.43
Gallic acid	ND	96.28±0.32	96.10±0.32	96.03±0.02	94.87±0.48

DPPH absorbance reading for sample (extract) = 0.556

DPPH absorbance reading for Gallic acid = 1.873

ND = Not determined (Activity was already very high at 100µg/ml)

The antioxidant potential of a compound can be attributed to its radical scavenging ability, and in order to evaluate the ability of the plant extracts to serve as antioxidants, their ability to scavenge DPPH radical was measured and the IC_{50} values (concentration of the extract that is able to scavenge half of the DPPH radical) calculated.

Aframomum melegueta revealed a scavenging activity with IC_{50} value of 42.16 and a concentration dependent antioxidant activity ranging from 86.63 ±0.06 to 28.12±1.14 at 200 µg/ml to12.5 µg/ml (Table 6). The Percentage antioxidant activity was calculated to be 86.63% using the formula;

Percentage antioxidant activity $= \frac{\text{Absorbance of DPPH} - \text{Absorbance of sample}}{\text{Absorbance of DPPH}}$

The highest IC_{50} value of 71.00 was obtained from *Aframomum danielli* when compared with the standard, gallic with IC_{50} of 15.1 suggesting that *A. danielli* has a lower scavenging activity when compared to *A. melegueta*. The concentration dependent antioxidant activity of *A. danielli* was found ranging from 77.34±0.45 to4.02±0.43 at 200 µg/ml to12.5 µg/ml as shown in Table 6 and the percentage antioxidant activity of *A. danielli* was calculated as 77.34%.

CONCLUSION

The result of this study validates the anti-lipase potential of these plants, and a support for the ethno medicinal claim in the treatment of obesity which is comparable to Orlistat®. However, further in vivo studies are recommended so as to validate these results.

Spectroscopic analysis on these plants is recommended in order to isolate, identify and characterize the bioactive constituents which could act as templates for future obesity drug development.

MATERIALS AND METHODS

Plant material:

The pods of *Aframomum melegueta* and *Aframomum danielli* were collected from Moniya village, Akinyele local Government Area of Ibadan and the leaves and pods were authenticated and voucher specimen numbered DPHUI 1183 and DPHUI 1460 respectively were deposited at the Department of Pharmacognosy herbarium.

Preparation of extracts:

The seeds of *Aframomum melegueta* and *Aframomum danielli* were separated from the pods and each of the seeds were weighed with an electric balance; 400 g of *Aframomum melegueta* seeds and 350 g of *Aframomum danielli* seeds were blended using an electric blender and the coarse powder obtained were extracted with ethanol by maceration for 72 h. The extracts were filtered separately and concentrated using the rotary evaporator under reduced pressure and kept for further use.

Determination of pancreatic lipase inhibition:

Measurement of pancreatic lipase activity in-vitro (spectrophotometric analysis):

Lipase activity was determined by using triolein as a substrate and measuring the rate of release of oleic acid from triolein. Inhibition of the enzyme lipase by the alcoholic extracts was determined by a standard modified assay [25] using orlistat® as the control. A suspension containing 1% (v/v) of triolein, and 1% (v/v) Tween 40 in 0.1 M phosphate buffer (pH 8) was prepared and emulsified. The assay was then initiated by adding 800 µl of the triolein emulsion to 200 µl of porcine pancreatic lipase (0.5 g pancreatin in 15 mL 0.1 M phosphate buffer at pH 8.0) and 200 µl of extract at different concentrations (1 mg/mL, 0.5 mg/mL, 0.25 mg/mL). The reduction in turbidity of triolein emulsion by porcine pancreatic lipase was determined spectrophotometrically at wavelength of 450nm. The contents were mixed and the absorbance measured immediately at 450nm designated as T_0. The test tubes were incubated at 37°C for 30 min and the absorbance at 450 nm was measured and designated as T_{30}. 0.1 M Phosphate buffer was used as a negative control while Orlistat® was used as a positive control; all measurements were made in triplicates.

The variation in absorbance = $[A_{450}\ (T_0) - A_{450}\ (T_{30})]$ was calculated for both control and the treatment and the percentage inhibition of the extracts were obtained using the formula:

$$\% \text{ inhibition} = \frac{([\text{“}A_{450\text{Control}} - \text{“}A_{450\text{Extract}}])\ \times\ 100}{(\text{“}A_{450\text{Control}})}$$

Determination of total phenolic content:

Total phenolic content of all the extracts was evaluated by a standard method [19]. Samples containing polyphenols are reduced by the Folin-Ciocalteu reagent thereby producing blue colored complex. The phenolic concentration of extracts was evaluated from a gallic acid calibration curve which was prepared by mixing 0.5 mL aliquots of 0, 50, 100, 150, 200, and 250 ¼g/mL methanolic gallic acid solutions with 2.5 mL Folin– Ciocalteu reagent (diluted ten-fold) and 2.5 mL (75 g/L) sodium carbonate. The mixture was then incubated at room temperature for 30 min and the quantitative phenolic estimation was determined at 765 nm using 752s spectrum lab UV/Visible spectrophotometer. The calibration curve was constructed by plotting the value of absorbance against concentration. The extracts (0.5 mL, 250¼g/mL) were also mixed with 2.5 mL Folin– Ciocalteu reagent (diluted ten-fold) and 2.5 mL (75 g/L) sodium carbonate. The mixture was incubated for 30 min and absorbance was measured in the same way as the standard (gallic acid). All determinations were performed in triplicates. Total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per g of extract.

DPPH radical scavenging activity:

The free radical scavenging activity of the extracts and gallic acid as positive control was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) by a standard method [28] with slight modifications. The extracts and control (2 mL) at various concentrations (100, 50, 25, 12.5, 6.25, 3.125 and 1.625 ¼g/mL) was added to 3 mL of freshly prepared DPPH solution (0.1 mM) in methanol. The mixture was incubated in the dark for 30 min at room temperature and absorbance was measured at 515 nm using HACH 4000 DU UV/Visible spectrophotometer. All experiments were repeated three times independently. The degree of decolorization of DPPH from purple to yellow indicates the scavenging efficiency of the extract. The percentage inhibition of DPPH free radical scavenging activity was calculated using the following equation:

$$\text{Percentage inhibition} = [(A_{DPPH} - A_{sample}) / A_{DPPH}] \times 100$$

Where:

A_{DPPH} = Absorbance of DPPH

A_{sample} = Absorbance of sample (extract/ascorbic acid)

The % inhibition data (antioxidant activity) was then plotted against log concentration fitted in a graph and IC_{50} (half-maximal inhibitory concentration) value was calculated by linear regression analysis.

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