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Evaluation of phytochemicals, antioxidant contents and *in vitro* antioxidant activities of a combined ethanol extract of *Spermacoce radiata* and *Hypselodelphys poggeana* leaves

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ABSTRACT

Background & Aim: Oxidative stress causes lipid peroxidation, damages various macromolecules, tissues and organs, and is involved in the pathogenesis of several diseases. Antioxidant supplementation could help scavenge free radicals and prevent oxidative stress. This study evaluated the phytochemicals, antioxidant compositions and *in vitro* antioxidant activities of a combined extract of *Spermacoce radiata* and *Hypselodelphy spoggeana* leaves (CEESH).

Experimental: This study determined phytochemicals and antioxidant vitamins contained in CEESH. The i*n vitro* antioxidant activities of CEESH comprising DPPH (1,1-diphenyl-2-picrylhydrazyl), nitric oxide, ferric reducing antioxidant power (FRAP), total antioxidant capacity (TAC) and thiobarbituric acid reactive substances (TBARS) were assayed.

Results: The phytochemical results indicated substantial amounts of total phenols, alkaloids, and terpenoids, along with moderate amounts of tannins, flavonoids, and steroids in CEESH with a low glycoside concentration, but saponin was not detected. The plant extract also showed substantial concentrations of vitamins A, C, and E.The DPPH, nitric oxide, FRAP, TAC and TBARS for the CEESH indicated that the extract has substantial free radical scavenging activities. The CEESH demonstrated a dose-dependent significant increase in the nitric oxide, TAC, and TBARS scavenging activities, while the CEESH showed dose-dependent marked increase in FRAP within a concentration range of 40–640 µg/mL. The half-optimal effective concentration (EC_{50}) values of the CEESH for DPPH, nitric oxide, and TBARS were 7.23, 39.40, and 63.41 µg/mL, respectively.

Recommended applications/industries: These findings suggest that CEESH contains abundant phytochemicals and antioxidant vitamins and possesses high free radical scavenging potential. Therefore, CEESH could be a viable therapeutic agent for managing oxidative stress.

1. Introduction

Medicinal plants have proven indispensable in managing several diseases and various medical conditions due to bioactive phytochemicals, including antioxidant compounds, minerals and vitamins. A literature survey showed that oxidative stress is involved in the pathogenesis of numerous diseases. The

insufficient antioxidants to counter the damaging effects of free radicals, including reactive oxygen and nitrogen species, are the significant reasons for oxidative stress, which is implicated in the pathogenesis of numerous diseases (Banothua *et al.*, 2017;Uroko *et al.*, 2019). The oxidative stress usually occurs in the presence of free radicals, pro-oxidants, and few antioxidants to scavenge them, thereby causing lipid peroxidation, injury to tissues and organs, and damage to proteins, lipids, membranes and nucleic acids (Liguori *et al.*, 2018). Most reactive free radicals are generated from the incomplete transfer of electrons to the final electron acceptor, the oxygen in the electron transport chain. Free radicals could be generated during the breakdown of various macromolecules, including drugs and xenobiotics (Pizzino *et al.*, 2017).

The prominent groups of free radicals are the reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are reactive free radicals and non-radicals of oxygen and nitrogen origins, respectively, which mostly contain unpaired electrons (Liguori *et al.*, 2018). Some free radicals positively affect the immunological responses against foreign antigens and mediation of signal transmission, but their numerous adverse health effects have masked their health benefits (Genestra, 2007). Oxidative stress occurs mainly due to a deficiency in the number of circulating antioxidants and thus could be managed via antioxidant supplementation to scavenge or quench free radicals and prevent harmful effects associated with oxidative stress (Pirbalouti *et al.*, 2013). Oxidative stress contributes in numerous ways to the pathogenesis of several diseases, including cancer, chronic inflammation, cardiovascular diseases, neurological diseases, respiratory disorders, renal diseases, and retarded sexual development (Valavanidis *et al.*, 2013; Singh *et al.*, 2004; Samuel *et al.*, 2011; Pizzino *et al.*, 2017). It has been reported that a decline or excessive physical activity could increase free radical generation and oxidative stress. Still, moderate physical activities reduce the level of circulating free radicals and avert oxidative stress to a greater extent (Golbidi *et al.*, 2011). Supplementation with antioxidants or medicinal plants rich in phytochemicals with high antioxidant activities, including phenols, flavonoids, alkaloids, and vitamins A, C and E, could attenuate oxidative stress and reduce the risk of the adverse health effects associated with it (Uroko *et al.*, 2020).

Species of *Spermacoce radiata* (DC.) and *Hypselodephy spoggeana*are very potent medicinal plants from *Marantaceae* and *Rubiaceae* families whose combined leaf extracts are commonly employed as a therapeutic agent against several diseases, including benign prostatic hyperplasia in traditional medicine. The combined *S.radiata* and *H. poggeana* leaf extracts are used for treating urinary tract infections, gallstones, diarrhoea, sore throat, headache and managing body weight (Vinayak *et al.*, 2013; Urokoet *al.*, 2021). The combined extract has been shown to possess antihyperlipidaemic effects, renal protection and shrinking of an enlarged prostate to average size (Uroko *et al.*, 2021; Urokoet *al.*, 2022). Despite the numerous medicinal uses of the combined extract of *S.radiata* and *H. poggeana* leaves, a literature survey showed that there is rare information on the phytoconstituents of the combined extract. This study evaluates the contents of phytochemicals, antioxidant vitamins, and in vitro antioxidant activities of a combined extract of *Spermacoce radiata* and *Hypselodephy spoggeana* leaves (CEESH).

2. Materials and Methods

2.1. Plant materials

The *Spermacoce radiata* and *Hypselodelphys poggeana* leaves were collected from forests around Agbama-Olokoro, Umuahia South, Local Government Area, Abia State. Taxonomists at the Herbarium Unit of the Department of Forestry, College of Forestry and Environmental Sciences, Michael Okpara University identified the plants as *Spermacoce radiata* and *Hypselodephys poggeana* and assigned specimen voucher numbers MOUAU/DF/BCH/03712 and MOUAU/DF/BCH/03713, respectively to the plants.

2.2. Chemicals and reagents

All chemicals and reagents used in this study were of analytical grades. Ethanol, ascorbic acid, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxyl toluene (BHT), Folin-Ciocalteu reagent, andthiobarbituric acid (TBA) were sourced from Sigma-Aldrich Chemicals (USA). At the same time, the ferric chloride ($FeCl_3$) was obtained from Merck company (Germany).

2.3. Preparation of CEESH

The *S. radiata* and *H. Poggeana* leaves were handpicked and rinsed in running tap water, followed

by slicing into smaller pieces to increase their surface areas for easy drying. The sliced leaves were dried under a shade till a constant dry weight was obtained after twenty-one days and pulverized into a coarse powder with a mechanized grinder. A quantity of 350 g of the coarse powders of *S. radiata* and *H. Poggeana* leaves corresponding to 700 g of the combined plant sampled were weighed into a clean, dry and sterile container and 2.5 L of absolute ethanol solvent was added. It was concentrated with a rotary evaporator, followed by weighing the concentrated CEESH which gave a yield of 23.52 g, corresponding to a percentage yield of 3.36 %.

2.4. Phytochemical screening

The qualitative screening for alkaloids, total phenols, tannins, flavonoids, glycosides, steroids and saponins was carried out by Harborne (1998).

2.5. Quantitative phytochemical analyses

The phytochemicals present in the CEESH from the qualitative analyses were quantified according to the procedures outlined by Harborne (1998).

2.6. Quantitative analyses of antioxidant vitamins

The phytochemical analyses of antioxidant vitamins present in the CEESH were determined using the methods described by Pearson (1976).

2.7. In vitro antioxidant assay of CEESH

The *in vitro* DPPH (1,1-diphenyl-2-picrylhydrazyl), nitric oxide ($NO^{\cdot}$), and TBARS (thiobarbituric acid reactive substances) radicals scavenging activities of CEESH were assayed following the methods described by Gyamfi *et al*. (1999); Marcocci *et al*. (1994) and Banerjee *et al*. (2005), respectively. The ferric reducing antioxidant power (FRAP) and the total antioxidant capacity (TAC) of the CEESH were determined in line with the methods of Yen and Chen (1995) and El-hashash *et al*. (2010), respectively.

2.8. Statistical analysis

The data from the study were subjected to a one-way analysisof variance and paired sample t-test, respectively, using Statistical Products and Service Solutions (SPSS) version 22. The statistically significant difference was obtained 95 % confidence level ($P<0.05$). The results were presented as mean ± standard deviation of the triplicate determination (n=3).

3. Results and discussion

Increased generation of free radicals under low antioxidant status triggers oxidative stress, which causes lipid peroxidation, injury to tissues and organs, DNA damage, and several diseases. Antioxidant supplementation via dietary intake of fruits and vegetables rich in antioxidant-phytochemicals and vitamins could counter oxidative stress and prevent complications (Yazdanparas *et al.*, 2008). This study evaluated phytochemicals, antioxidant contents, and *in vitro* antioxidant activities of a combined ethanol extract of *Spermacoce radiata* and *Hypselodelphy spoggeana* leaves (CEESH) with the view of ascertaining its antioxidant potential. The findings of our study indicated high phytochemicals, antioxidant compositions, and in vitro free radicals scavenging activities of CEESH, suggesting it could be of high medicinal value in the management of oxidative stress and associated disease conditions.

3.1. Preliminary phytochemical screening result of CEESH

The qualitative phytochemical results in Table 1 showed that CEESH contained total phenol, alkaloids, and terpenoids in high amounts. The CEESH also had a moderate concentration of tannins, flavonoids, and steroids, along with a low concentration of glycosides, while saponins were not detected.

The absence of saponins in CEESH showed that other phytochemicals present in CEESH are responsible for its pharmacological properties rather than saponins which aligns with Maruthupandian and Mohan (2011). Thus, suggesting there was no need for the qualitative evaluation of saponins content in CEESH.

Table 1. Qualitative phytochemicals contents in CEESH.

Phytochemicals	Bioavailability
Total phenols	+++
Alkaloids	+++
Tannins	++
Flavonoids	++
Terpenoids	+++
Glycoside	+
Steroids	++
Saponins	ND

Keys: ND = Not detected; +++ = Present in high proportion; ++ = Present in moderate proportion; + = Present in low proportion.

3.2. Concentrations of phytochemicals in CEESH

Table 2 shows that total phenols were the most abundant phytochemical in CEESH. At the same time, steroids were the least available phytochemical in the CEESH. The trend of the phytochemical contents in the CEESH showed total phenols> alkaloids> terpenoids > flavonoids > glycoside> tannins> steroids.

Table 2. Phytochemical content in the CEESH.

Phytochemicals	Quantities (mg/100 g)
Total phenols	2924.95±7.36
Alkaloids	1205.56±6.64
Tannins	55.11±2.34
Flavonoids	156.13±2.66
Terpenoids	580.07±5.16
Glycoside	104.14±3.90
Steroids	39.97±2.09

The values in table depict the mean ± standard deviation (n = 3).

The rich phytochemical contents in the CEESH showed that the therapeutic activities exhibited by the combined plant samples might be attributed to these bioactive phytoconstituents. Plant extracts rich in alkaloids have been reported to possess comprehensive pharmacological properties, including cardioprotective, anti-inflammatory, and anaesthetic properties linked to the alkaloids in the extract (Heinrich *et al.*, 2021). The potency of rich alkaloid therapeutic agents against many diseases, including Parkinson's, hepatitis, venereal diseases, rheumatism, and neuralgia, showed that CEESH could play a crucial medicinal role in managing various conditions due to its high alkaloid content in line with Amirkia *et al*. (2014). In like manner, the flavonoids found in the CEESH have been shown to mediate medicinal and therapeutic activities, making it an effective anticancer agent, antioxidative stress, anti-inflammatory, antiviral, cardioprotective, renoprotective and neuroprotective agent (Ullah *et al.*, 2020). Total phenols are bioactive phytoconstituents with potent antioxidant activities that are therapeutic and effective against many oxidative stress-related diseases, and its high concentration in CEESH is indicative that CEESH could counter reactive free radicals and prevents conditions such as oxidative stress, DNA damage, inflammation, and repress carcinogenesis in line with Huang *et al.* (2010). The CEESH possesses high therapeutic potential in the management of ailment and tropical disease because many studies have reported that plant extracts with high tannins content contain increased antioxidant potentials, antimicrobial activities, anti-inflammatory, antidiarrhea, antimalaria, antiulcer, wound healing, as an antidote for heavy metal poisoning (Subhan *et al.*, 2018). The numerous pharmacological activities of terpenoids, including anticancer, antimalaria, antioxidative stress, anti-inflammation, and diuretic property, further suggest that CEESH has high therapeutic potential due to its rich terpenoid content, which agrees with Cox-Georgian *et al*. (2019). Most phytomedicines like CEESH contain varying glycosides, and pharmacological properties like renal protection, anti-benign prostatic hyperplasia, anti-inflammation, antidiabetics, antioxidants, and anticancer have partly been attributed to their glycoside content (Xiao *et al.*, 2016). The moderate steroid level in the CEESH suggests that its consumption could be beneficial against asthma, dermatological reactions, and inflammation. Still, steroids could compromise the immune system if ingested excessively over a long period (Ericson-Neilsen and Kaye, 2014). Due to the overlapping pharmacological activities exhibited by the various phytochemicals in CEESH, it is apparent that they may not act in isolation; instead, they could work in synergy to achieve pharmacological effects that would be difficult for any of them to achieve alone.

3.3. Concentrations of antioxidant vitamins in the CEESH

Table 3 indicated antioxidant vitamins in the CEESH, with vitamin E as the most abundant vitamin in the CEESH, followed by vitamin C and then vitamin A.

Table 3. Contents of antioxidant vitamins in CEESH.

Vitamins	Quantities (mg/100 g)
Vitamin A	0.14±0.00
Vitamin C	1.96±0.03
Vitamin E	61.35±0.52

Values are displayed as mean ± standard deviation (n = 3)

Vitamins C is a water-soluble vitamin regarded as a potent antioxidant because of its ability to neutralize circulating reactive free radicals and prevent oxidative stress and lipid peroxidation, including damage to macromolecules, tissues, and organs in the body. Plant extracts rich in antioxidant vitamins, including vitamins A, C, and E in the CEESH, could scavenge reactive free radicals, including reactive oxygen species (ROS) and reactive nitrogen species (RNS), from both the aqueous and lipophilic environments to a greater extent

and avert oxidative stress and associated cellular damage and lipid peroxidation according to Traber *et al*. (2011). The high antioxidant vitamins in the CEESH showed that it has sufficient antioxidant compositions capable of quenching the oxidative attack of reactive free radicals, in line with the report of Akbari *et al*. (2016). The substantial amounts of vitamins C and E in the CEESH suggest it could scavenge ROS and RNS and shield proteins and DNA molecules from the adverse effects of lipid peroxidation, damage, and any other structural alteration that could impair cellular functions, which are in agreement with Traber *et al*. (2011). Vitamin A, a fat-soluble vitamin, can easily permeate the lipophilic medium and mediate antioxidant activity, including scavenging peroxyl radicals and singlet oxygen, primarily due to its polyene chain. The substantial amount of vitamins A, C, and E in the CEESH are indicative that this extract, when administered in the right amount, could provide sufficient antioxidants that drastically scavenge most reactive radicals like hydroxyl, alkoxyl, peroxyl and reactive radicals in both the aqueous and lipophilic environment which would damage to the cellular environment which align with Lu *et al*. (2010). Considering other biochemical functions of the vitamins, including the role of vitamin C in metabolism via its actions as a coenzyme and the roles of vitamins A and E in the stimulation of immunological response, it was evident that CEESH has promising medicinal potential.

3.4. Effects of CEESH on DPPH radical concentrations

The DPPH radicals scavenging activities of CEESH in Figure 1 indicated a considerable dose-dependent increase in activities when 10– 80 µg/mL of CEESH was tested but showed a dose-dependent decline in DPPH radical scavenging activities when 160-640 µg/mL of the CEESH was tested. The CEESH demonstrated considerable DPPH free-radical scavenging activities at all the concentrations tested compared to vitamin C, which had high DPPH radical scavenging activities at all the concentrations. The CEESH exhibited the most elevated and minor DPPH free-radical scavenging activities at 80 and 640 µg/mL, contrary to vitamin C, which showed the highest and least activity at 40 and 640 µg/ml, respectively. The half optimum effective concentrations (EC_{50}) of CEESH and vitamin C required to scavenge half of DPPH radicals were 7.23 and 5.36 µg/mL, respectively.

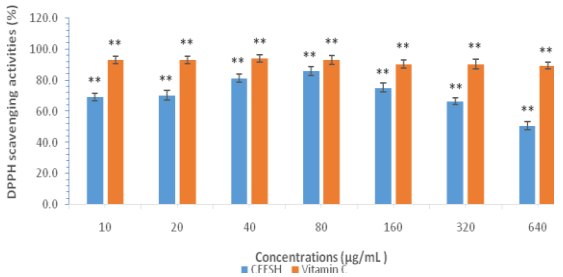

Fig. 1. DPPH radical scavenging activities of CEESH. Bars are displayed as the mean ± standard deviation of three replicates, and bars with a double asterisk (**) are significantly ($P<0.05$) different from the paired mean.

The DPPH radical scavenging activity of a medicinal plant extract measures its antioxidant activities via donating a hydrogen atom to the circulating reactive free radicals, making them stable and non-reactive. The dose-dependent increase in the DPPH scavenging activity of the CEESH within the concentration range of 10–80 µg/mL demonstrated by the rapid decolourization of the reacting methanol-DPPH solution showed the high antioxidant activity of CEESH, which agrees with Rahman *et al*. (2015). The highest DPPH radical scavenging of CEESH observed at 80 µg/mL suggests that 80 µg/mL of CEESH possesses the optimal antioxidant property required to manage oxidative stress. Conversely, the decrease in the DPPH radical scavenging activity of CEESH when the concentration of the extract was increased above the optimum dose of 80 µg/mL showed that CEESH is a potent and desirable antioxidant agent. The high DPPH radical scavenging activity of CEESH could be attributed to its rich phytochemical composition, including phenols, flavonoids, terpenoids, alkaloids and tannins, and vitamins A, C, and E, which possess significant antioxidant properties in line with Lalhminghlui and Jagetia, (2018). However, the decline in the DPPH radical scavenging activity of CEESH at increased concentrations suggests that CEESH might contain pro-oxidants and possible phytoconstituents that could interfere with antioxidant activity at higher concentrations. Plant extracts with higher antioxidant activities at lower doses are preferable to those with lower antioxidant activities at lower concentrations because of the reduced dose to be ingested, cost, and possible toxicity associated with the consumption of excess doses.

3.5. Effects of CEESH on nitric oxide radical concentrations

Figure 2 shows lower nitric oxide radical scavenging activities of CEESH compared to vitamin C within a lower concentration range of 10– 80 µg/mL. However, the CEESH exhibited a dose-dependent increase in the nitric oxide radical scavenging activities compared to vitamin C within an increased concentration range of 160– 640 µg/mL.The CEESH had an EC_{50} value of 39.40 µg/mL, while the EC_{50} of vitamin C was 6. 05 µg/mL.

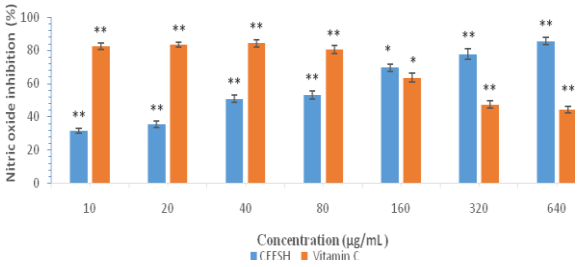

Fig. 2. Nitric oxide radical scavenging activities of CEESH. Bars are displayed as the mean± standard deviation of three replicates, and bars with a double asterisk (**) are significantly (P<0.05) different from the paired mean.

Nitric oxide mediates animals' essential biochemical and physiological functions, including inflammatory responses, vasodilation, and immunological responses. Still, its excessive production could elicit some adverse reactions and several disease conditions, including diabetes and ulcer, because it can quickly react with superoxide radicals to generate very toxic reactive peroxynitrite anions (Hazra *et al.*, 2008). The substantially dose-dependent increase in the nitric oxide scavenging activities of CEESH could be attributed to the antioxidants in the extract, which caused a decline in nitric oxide generation from the reaction of oxygen with sodium nitroprusside. The antioxidants in CEESH could have diminished the nitric oxide level by reducing the available oxygen level that would have reacted with the sodium nitroprusside to generate nitric oxide, which might have inevitably slowed down the production of nitric oxide. The study demonstrated that while vitamin C inhibited nitric oxide significantly than CEESH at lower concentrations, CEESH possesses increased nitric oxide scavenging activity at higher concentrations than vitamin C, suggesting that CEESH could be a viable therapeutic agent in the management of oxidative stress and related conditions in line Venkatachalam and Muthukrishnan (2012).

3.6. FRAP of CEESH

Figure 3 indicated that the FRAP of CEESH at 40 and 80 µg/mL were lower than the FRAP value of CEESH at 20 µg/mL. The FRAP of CEESH at 320 and 640 were substantially high compared to the FRAP values observed in every other concentration.

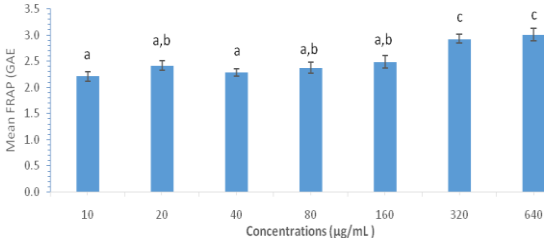

Fig. 3. Ferric reducing antioxidant power (FRAP) of CEESH. Bars are displayed as the mean ± standard deviation of three replicates, and bars with a different letters are significantly (P<0.05) different.

The high FRAP exhibited by CEESH showed that it has the antioxidant capacity to scavenge excess free radicals via electron transfer to the unpaired Fe^{3+}, thereby reducing it to a stable Fe^{2+} which is not a free radical. The drastically increased FRAP activity of CEESH at the concentrations of 320– 640 µg/mL suggests that CEESH possesses more substantial antioxidant potential at higher doses and could be a potent antioxidative agent at these higher concentration ranges compared to the lower concentrations. The high FRAP, DPPH, and nitric oxide activities of the CEESH showed that it contains rich antioxidant compounds capable of scavenging or quenching free radical reactions due to the ability of CEESH to prevent oxidative attack via the donation of a hydrogen atom and transfer of electrons. The high FRAP activity demonstrated by CEESH aligns with Venkatachalam and Muthukrishnan (2012), who reported increased FRAP activity by the *Desmodium gangeticum* extract.

3.7. TAC exhibited by CEESH

Figure 4 shows a dose-dependent increase in the TAC activity of CEESH. A minor TAC activity (0.26± 0.04 AAE) was recorded at the lowest tested concentration (10 µg/mL). The highest TAC activity of CEESH (1.10 ± 0.07 AAE) occurred at the highest tested concentration of the CEESH (160 µg/mL).

Total antioxidant capacity (TAC) activity measures the totality of the antioxidant potential of medicinal

plant extracts, food, and therapeutic agents. I*n vitro* evaluation in medicinal plant, extract indicates the antioxidant potential of non-enzymatic antioxidants, including vitamins, total phenols, flavonoids, and alkaloids contained in the extract.

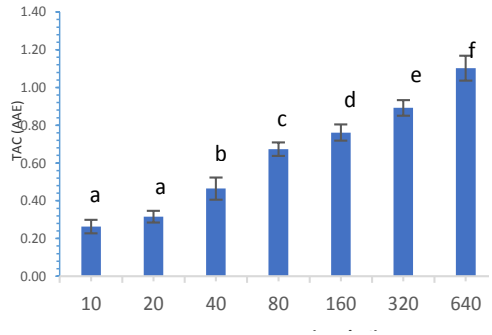

Fig. 4. Total antioxidant capacity of CEESH. Bars are displayed as the mean ± standard deviation of three replicates, and bars with different letters are significantly (P<0.05) different.

The dose-dependent increase in the TAC activity of CEESH also showed that it possesses high antioxidant potential, as earlier indicated by the DPPH, nitric oxide, and FRAP activities. It aligns with Banothua*et al*. (2017). The high TAC of CEESH at increased concentrations showed that a high concentration of CEESH should be ingested to elicit significant antioxidant activity that could sufficiently scavenge or quench free radicals to prevent oxidative stress.

3.8. Effect of CEESH on TBARS

Figure 5 indicated a dose-dependent TBARS inhibitory activity of CEESH comparable to the effect of BHT on TBARS at higher concentrations. Conversely, the TBARS inhibitory effects of CEESH at lower concentrations were substantially higher than BHT. The EC_{50} of CEESH and BHT were 63.41 and 60.85 µg/mL, respectively.

The biochemical evaluation of the concentrations of thiobarbituric acid reactive substances (TBARS) generated in a medium is one of the acceptable methods of assessing the degree of lipid peroxidation, which is inversely related to the antioxidant status of the medium. The levels of TBARS increase with the increasing level of lipid peroxidation due to a decline in antioxidant activity and vice versa. The dose-dependent increase in the percentage inhibition of the concentrations of TBARS by CEESH could be attributed to the antioxidant activity exhibited by the extract, which inhibited lipid peroxidation and caused a decline in the level of TBARS released.

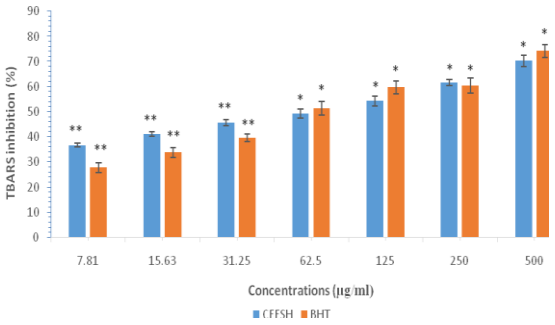

Fig. 5. TBARS inhibitory activities of CEESH. Bars are displayed as the mean ± standard deviation of three replicates, and bars with a double asterisk (**) are significantly (P<0.05) different from the paired mean.

The higher percentage of TBARS inhibition of the CEESH at lower concentrations than the BHT could serve as a preferable antioxidant agent at these reduced concentrations. The percentage of TBARS inhibition by CEESH at an increased concentration comparable to that of the BHT showed that CEESH has high antioxidant potential, which, when administered rightly, could effectively prevent oxidative stress and lipid peroxidation. The marked increased inhibitory effect of CEESH on the levels of TBARS generated due to a reduction in lipid peroxidation aligns with previous findings that plant extracts rich in antioxidant phytochemicals and vitamins inhibit lipid peroxidation (Dissanayak *et al.*, 2009).

4. Conclusion

The findings from our study showed that CEESH contains substantial concentrations of biochemically and physiologically essential phytochemicals and antioxidants vitamins with significant in vitro antioxidant activity as demonstrated by the DPPH, nitric oxide, TBARS, TAC and FRAP radicals scavenging mechanisms. The high antioxidant potential of CEESH suggests that it could play a significant therapeutic role in managing oxidative stress and its related disease conditions.

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