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Research Paper

Antioxidant Activity of Water, Ethanol and Diethyl Ether Extracts of *Monodora Myristica* and *Syzygium Aromaticum*

George, Betty Omenebelle and *Okpoghono, Joel

Department of Biochemistry, Faculty of Science, Delta State University, Abraka, Delta State, Nigeria.
*Corresponding author E-mail: okpoghono@gmail.com

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This study reports *in vitro* antioxidant activities of *Monodora myristica* (Africa nutmeg) and *Syzygium aromaticum* (clove) in water extract (WE), ethanol extract (EE) and diethyl ether extract (DEE). *S. aromaticum* and *M. myristica* were sun-dried for two weeks to obtain constant weight and then crushed into fine particles using electric blender. Extraction was carried out using water (60°C), ethanol (95% v/v) and diethyl ether (95% v/v). The extracts were set aside for the antioxidant analysis. The results obtained showed that there was significant ($p < 0.05$) increase in the reducing power of *S. aromaticum* in DEE, EE and WE when compare with *M. myristica.* The reducing power level in butylatedhydroxyanisole (BHA) was significantly ($p < 0.05$) higher when compare with *S.aromaticum* and *M. myristica* in DEE, EE and WE. A significant increase were also observed in the total phenolic content, flavonoids, reduced glutathione (GSH), ascorbate oxidase activities and total antioxidant levels of *S. aromaticum* when compare with *M. myristica* in DEE, EE and WE. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) level at the concentration of 25 µg/ml of *S. aromaticum* extracts was significantly ($p<0.05$) higher when compare with *M.myristica* extracts. BHA has significantly ($p<0.05$) higher DPPH level when compare with *S. aromaticum* extracts and *M. myristica* extracts. As the concentration of the spices increased, the DPPH levels in the spices also increased (25µg/ml <50 µg/ml <100 µg/ml). In this study, it is concluded that DEE, EE and WE of *S. aromaticum* and *M. myristica* have good antioxidant properties. DEE is more effective as compared to EE and WE. The extracts of *S. aromaticum* showed relatively higher antioxidant activities than that of *M. myristica.*

Key words: *Monodora myristica, Syzygium aromaticum,* extraction, antioxidant

INTRODUCTION

Oxidative stress is an imbalance between production of oxidants and antioxidant defences (Betteridge, 2000). Oxidative stress can result to inflammation (Maeda and Omata, 2008), type 2 diabetes and obesity (Sell and Eckel, 2009) and cardiovascular diseases (Montecucco et al., 2011). Antioxidant can be natural or synthetic. Currently, synthetic antioxidants, such as butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ) are of important in the food industry. The intake of synthetic antioxidants can be toxic to liver and carcinogenic (Badu et al., 2013). However, the use of natural antioxidants in the preservation of food is preferred over synthetic compounds (George and Osioma, 2011). Antioxidants possess the ability to protect the body by fighting against oxidative damage (Ozsoy et al., 2008) by scavenging the free radicals or inhibition of peroxidation. Phenolic compounds are plant secondary metabolites which constitute a crucial category of antioxidant metabolites. They have at least one aromatic ring in their molecule and may exist in the form of glycosides. Manach et al. (2004) reported that phenolic compounds protect plants against the attack of microorganisms and harmful environmental conditions. *Syzygium aromaticum* (Cloves) are dried unopened floral buds of an evergreen tree 10-20 m in height with aromatic smell. They belong to the family Myrtaceae (Muhson and Mashkor, 2015) and indigenous to Nigeria, India, Indonesia, Zanzibar and

Ceylon. Cloves have many therapeutic uses such as anti-inflammatory, antioxidant and antifungal (Shafi et al., 2002).*Monodora myristica* is an edible plant of the family Annonaceae. It is found commonly in the forests of West Africa. The common names are African nutmeg and calabash nutmeg (Burabai et al., 2007). Koudou et al. (2007) reported the medicinal use of *M. myristica* and stated that the stem bark is used in the treatments of stomach ache, fever pains and eye diseases. The seeds are used in treating headache (Uwakwe and Nwaoguikpe, 2008). The aim of this study was to assess the antioxidant activity of *M.myristica* and *S. aromaticum* in water, ethanol and diethyl ether extracts.

MATERIALS AND METHODS

Chemicals and reagents

The chemicals and reagents used for this study were of analytical grade. Folin-Ciocalteu phenol reagent, Aluminiumtrichloride, Methanol, 2, 2-diphenyl- 1 – picrylhydrazy l (DPPH) solution, reduced glutathione and Gallic acid were purchase from Sigma-Aldrich, Germany. Potassium ferricyanide, Ascorbic acid, anhydrous sodium carbonate, Trichioroacetic acid, anhydrous ferric chloride and all other chemicals were procured from BDH Chemical Laboratory, England, United Kingdom.

Plant materials

African nutmeg and cloves were purchased from a local market in Abraka, Delta State, Nigeria. They were identified at the Department of Botany, Delta State University, Abraka, Delta State.

Preparation of the spice extracts

The extract of *M. myristica* and *S. aromaticum* were obtained using the extraction technique as previously described by George et al. (2012). The spices were sundried to constant weight for two weeks and then crushed into fine particles using electric blender at high speed. One hundred grams each of the powdered spice was extracted with five hundred milliliters (ml) of the respective solvent (hot water (60°C), ethanol (95% v/v), and diethyl ether, 95 % v/v) and allowed to stand for 48h. The mixture was then filtered using a clean muslin cloth. Thereafter, the filtrate was used for the biochemical analysis.

Biochemical analysis

The determination of total phenolic content was carried out according to the method described by Liu and Yao (2007). Total flavonoid of the spice was determined with colorimetric aluminum chloride methods as described by Ebrahimzadeh et al. (2008). The free radical scavenging ability of the extracts against DPPH free radical was estimated using the method described by Ursini et al. (1994). The assay of ascorbate oxidase activity was carried out using the method of Vines and Oberbacher (1965). Reduced glutathione concentration was estimated using the method of Eliman (1959). The total antioxidant capacity and reducing power was evaluated using the method described by Prieto et al.(1999) and Oyaizu, (1986) respectively.

Statistical analysis

The results were expressed as mean bars. The significant differences between groups were analyzed using one way analysis of variance (ANOVA) and least significant difference (LSD). A threshold of $p< 0.05$ was regarded statistically significant.

RESULTS AND DISCUSSION

This study elucidated the antioxidant properties of *Monodora myristica* (African nutmeg), *Syzygium aromaticum* (Clove). A significant ($p<0.05$) increase were observed in the reducing power (units/g dry weight) of S. *aromaticum* in water extract (WE), ethanol extract (EE) and diethyl ether extract (DEE) when compare with *M myristica.* The reducing power level in BHA was significantly ($p <0.05$) higher when compare with S. *aromaticum* and *M. myristica* in DEE, EE and WE (Figure 1). The results indicated that the *S. aromaticum* and *M. myristica* extracts have reducing ability to act as antioxidant. This is in line with the statement of Duh et al. (1999) that the reducing ability of a compound depends on the presence of reductants which have been exhibiting antioxidative potential by breaking the free radical chain through the donation of a hydrogen atom**.** A significant ($p <0.05$) increase were observed in the total phenolic content (units/g dry weight) and flavonoids (units/g dry weight) (Figure 2) of *S. aromaticum* when compare with *M. myristica* in DEE, EE and WE. The results indicated that *S. aromaticum* have high levels of total phenol and flavonoid contents than *M. myristica.* Phenolics antioxidant activity may be due to their redox properties (Ademiluyi and Oboh, 2008), by allowing them to act as free radical quenchers, metal chelators and also as reducing agents (Erukainure et al., 2012). The results of reducing power, phenolic contents and flavonoids observed in *M. myristica* extracts were similar with that of the previous study conducted by George and Osioma, (2011) who reported the phenolic content and total antioxidant capacity of some local spices in Nigeria. Figure 3 shows that the 2,2 -dipheny -1 – picryihydrazyl (DPPH) level at the concentration of 25 µg/ml of *S. aromaticum* extracts was significantly ($p< 0.05$) higher when compare with *M. myristica* extracts. The DPPH

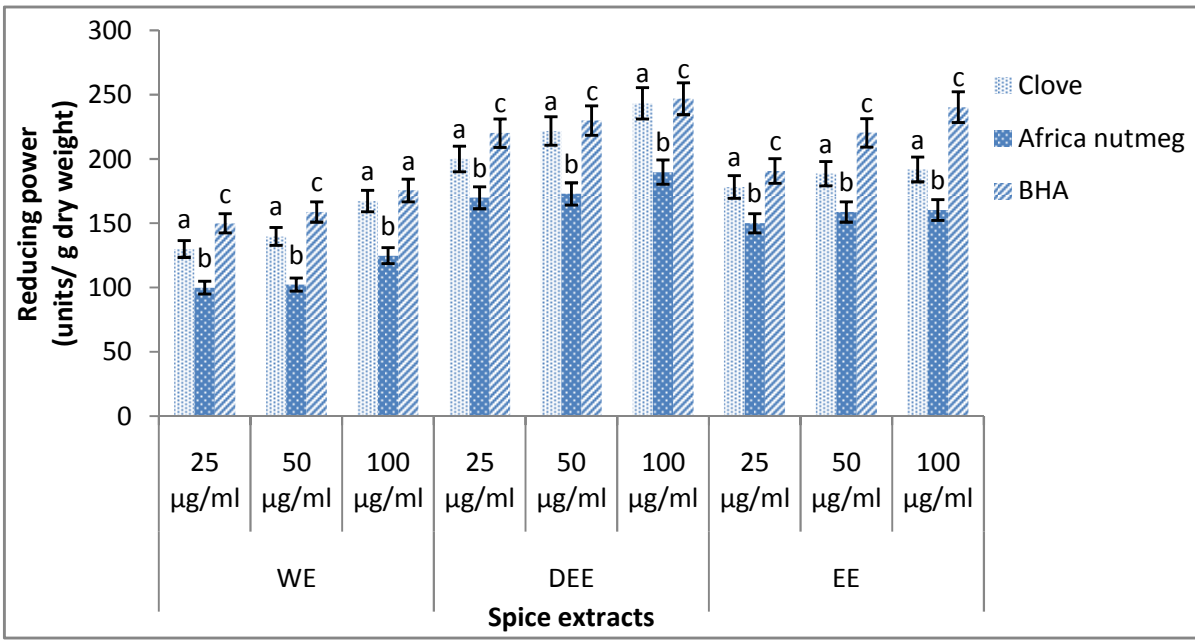

Figure 1. Reducing power level of clove and Africa nutmeg extracts. Bars represent mean of triplicates values. Bars with different superscript letter differ significantly at $p<0.05$. Water Extract (WE); Diethyl Ether Extract (DEE); Ethanol Extract (EE).

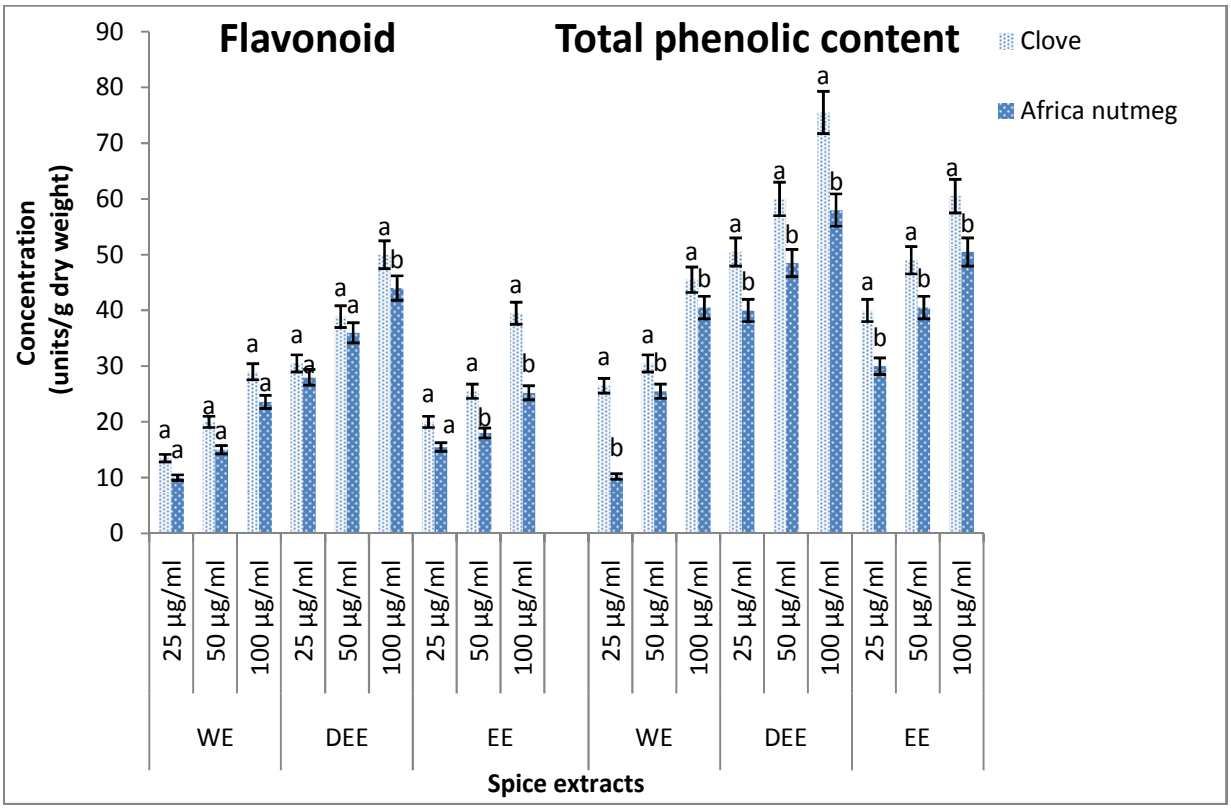

Figure 2. Flavonoid and phenolic content of clove and Africa nutmeg extracts. Bars represent mean of triplicates values. Bars with different superscript letter differ significantly at $p<0.05$. Water Extract (WE); Diethyl Ether Extract (DEE); Ethanol Extract (EE).

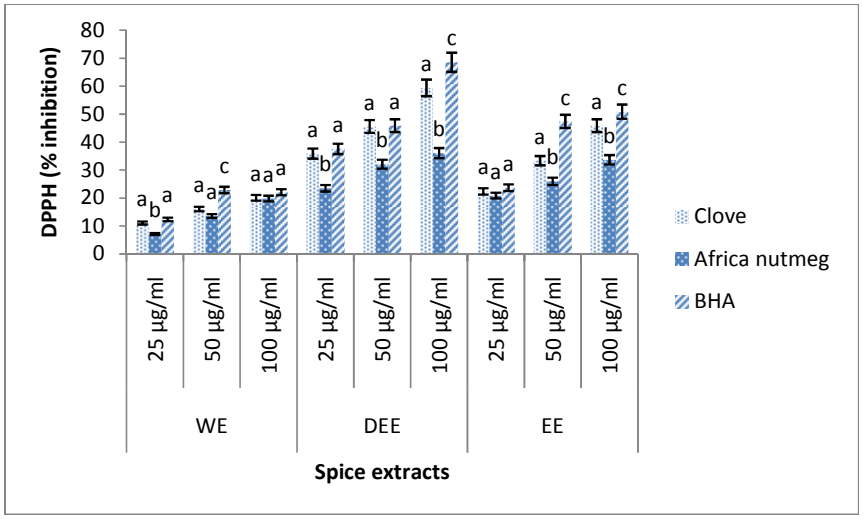

Figure 3. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity of clove and Africa nutmeg extracts. Bars represent mean of triplicates values. Bars with different superscript letter differ significantly at p<0.05. Water Extract (WE); Diethyl Ether Extract (DEE); Ethanol Extract (EE).

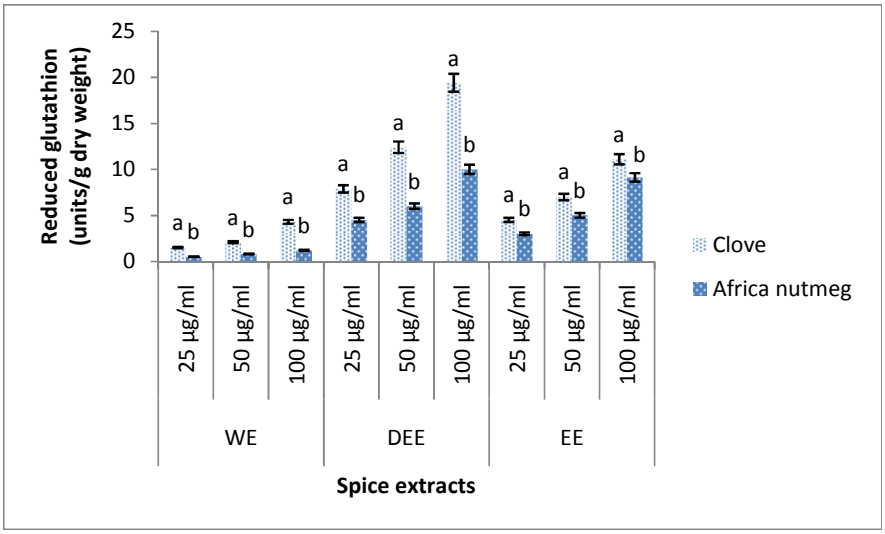

Figure 4. Reduced glutathione level of clove and Africa nutmeg extracts. Bars represent mean of triplicates values. Bars with different superscript letter differ significantly at p<0.05. Water Extract (WE); Diethyl Ether Extract (DEE); Ethanol Extract (EE).

level in BHA was significantly (p< 0.05) higher when compare with *S. aromaticum* extracts and *M.myristica* extracts respectively. DPPH has been used in evaluation of radical scavenging ability of antioxidants (Brad-Williams et al., 1995; Zhou and Yu, 2006). *S. aromaticum* extracts and *M. myristica* extracts possess high scavenging activity this could be due to the observed the high phenolic and flavonoid contents. At the concentration (25 -100 µg/ml) WE, EE and DEE of *S. aromaticum* and *M. myristica,* the DPPH level was as follows; BHA> S. *aromaticum*> *M. myristica.* As the concentration of the spices increases, the DPPH levels in the spices increases (25 µg/ml < 50g/ml < 100 µg/ml). This is in line with the previous study conducted by Badu et al., (2013) which stated that DPPH is very stable and the radical-scavenging activities of all the extracts of *Tetrapleura tetraptera* and *Panda biglobosa* increased with increasing concentration.

The reduced glutathione (units/g dry weight) (Figure 4), ascorbate activities (units/g dry weight) (Figure 5) and total antioxidant activities (units/g dry weight) (Figure 6) of *S. aromaticum* was significantly higher when compare with that of *M. myristica* in DEE, EE and WE. The reducing power, total phenolic content, DPPH radical

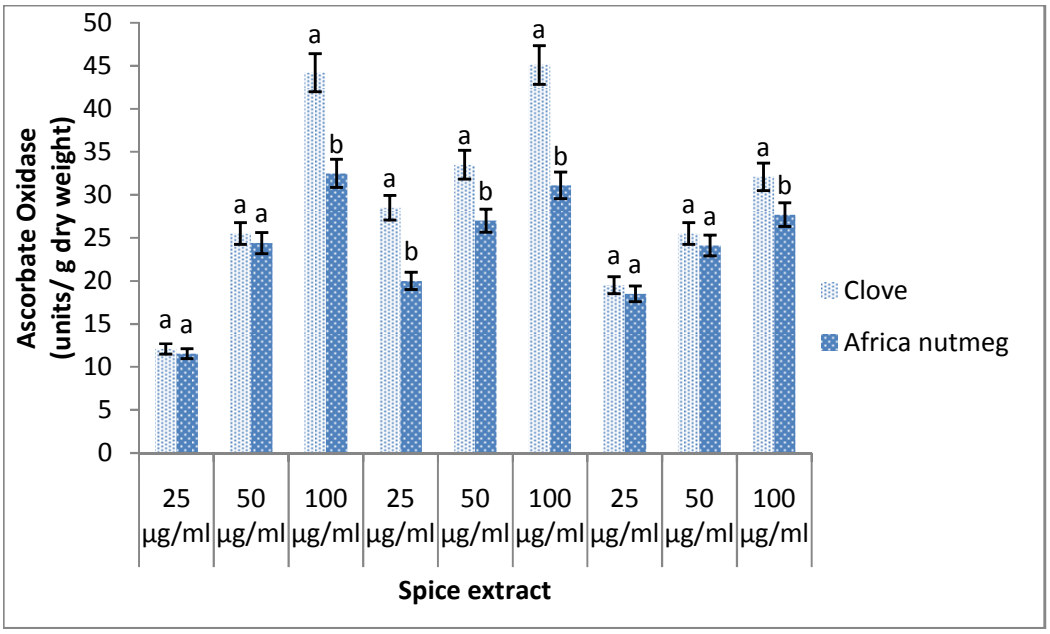

Figure 5. Ascorbate oxidase activity of clove and Africa nutmeg extracts. Bars represent mean of triplicates values. Bars with different superscript letter differ significantly at $p<0.05$. Water Extract (WE); Diethyl Ether Extract (DEE); Ethanol Extract (EE).

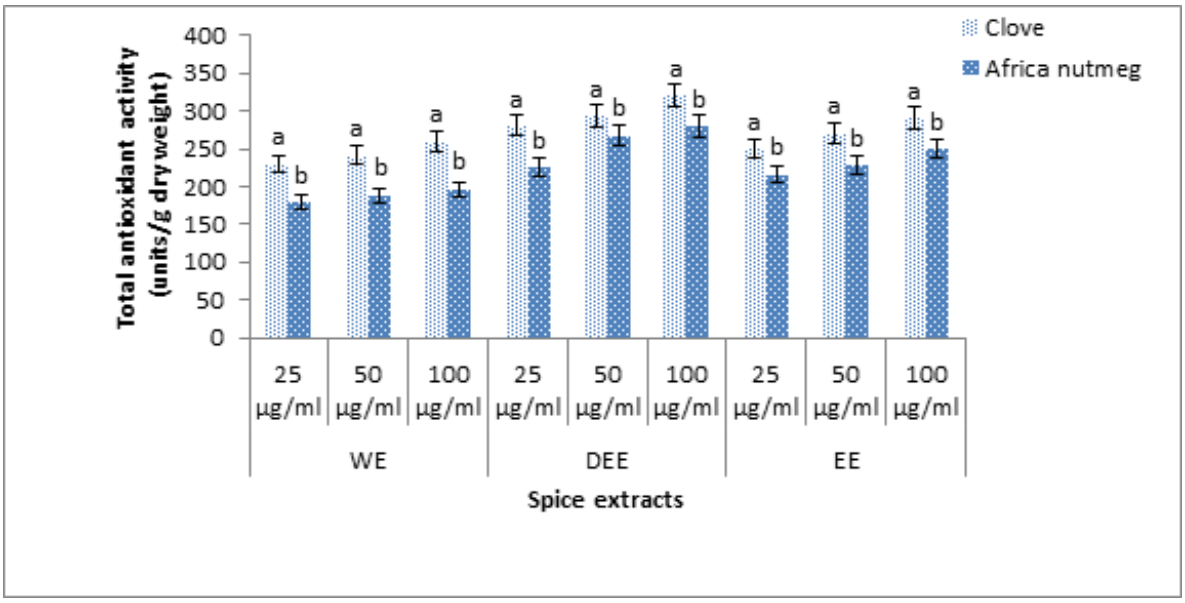

Figure 6. Total antioxidant activity of clove and Africa nutmeg extracts. Bars represent mean of triplicates values. Bars with different superscript letter differ significantly at $p<0.05$. Water Extract (WE); Diethyl Ether Extract (DEE); Ethanol Extract (EE).

scavenging activities, total flavonoid, reduced glutathione, ascorbate oxidase activities and total antioxidant of S. *aromaticum* and *M. myristica* extracts were as follows; DEE > EE > WE. Reduced glutathione plays an essential role in the antioxidant effects, nutrient metabolism and regulation of various cellular processes (Wu and Cederbaum, 2003). Ascorbate oxidase is an enzyme that employs one cofactor, copper (Pignocchi et al*.,* 2003) and plays an important role in cell growth by modulating the reduction/oxidation in plants.The enzyme also play a potential role in plant defence protein against insect herbivory (Felton and Summers, 1993). Antioxidant capacity of *S. aromaticum* and *M. myristica* extracts may be due to their polyphenol content, as polyphenols plays

an essential role as antioxidants in living systems.

Conclusion

In this study, it is concluded that DEE, EE and WE of *S. aromaticum* and *M. myristica* have good antioxidant property and could be attributed to their reducing power, flavonoids, phenolic content and reduced glutathione effects. As the concentration of the *S. aromaticum* and *M myristica* extracts increases, the antioxidant activity increases. The extracts of *S. aromaticum* showed relatively higher antioxidant activities than that of *M. myristica.* The results of this study show that the extracts of *S. aromaticum* and *M. myristica* can be of use as an easily accessible source of natural antioxidants. Therefore, it is suggested that further work could be done on the isolation and identification of the antioxidative components in *S. aromaticum* and *M. myristica*.

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