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A STUDY OF THE PHYTOCHEMICAL, ANTI-OXIDANT AND ANTI-INFLAMATORY PROPERTIES OF CLOVES *(Syzygium Aromaticum)*.

by

Dr. Aloysius C. Ozuah

Department of Integrated Science

Nwafor Orizu College of Education, Nsugbe -Anambra State, Nigeria

Abstract

Syzygium aromaticum, commonly called clove, is a culinary spice with medical uses with medical uses and its main constituents possess antimicrobial, antioxidant, anti-inflammatory, analgesic, anticancer, and anesthetic effects. In this study, the antioxidant activity, anti-flammatory activity and phytochemical constituent of Syzygium aromaticum's flower bud were analyzed using three different extracts solvents (carbon tetrachloride, ethyl acetate and aqueous extract). The analyses were carried out using standard analytical methods. Phytochemical analysis was carried out using GC-FID while other parameter was assayed using spectrophotometer. The results showed that carbon tetrachloride extract possessed the highest phytochemical profile (22.311273ppm) while aqueous extract has the lowest phytochemical profile (9.8821797ppm). All the extract both in ABTS and H_2O_2 scavenging activity are significantly low ($p<0.05$) when compared to their standards and as a result may not be a good anti-oxidant agents. Aqueous extract showed a strong lipid peroxidation scavenging activity with an average valve of 0.2987 umol/ml while carbon tetrachloride has the least valve of 0.1328 umol/ml when compared with each other. The highest DPPH capacity was shown by Carbon tetrachloride extract with the average valve of 89.7966% and the least is 86.332% ethyl acetate extract when compared with each other. In anti-inflammatory activity, the result shows that aqueous and carbon tetrachloride extract are not significant ($p>0.05$) mean they are good albumin anti-inflammatory agents while ethyl acetate is significant low ($p<0.05$) mean not a good anti-inflammatory agent when compared with aspirin (standard). In Heat induced Hemolysis, ethyl acetate extract is the only good anti-inflammatory agent because it is the only not significant extract ($p>0.05$) when compared with the standard while the other are significant ($p<0.05$). Carbon tetrachloride extract showed a strong Anti-proteinase activity because it is the only not significant extracts ($p>0.05$) when compared with standard in this assay. Therefore, the study shows that all the extract of clove syzyium aromaticum may not possess the better anti-oxidant properties while in anti- inflammatory property carbon tetrachloride extract possess a better anti-inflammatory property.

Keywords: Phytochemical, Anti-oxidant, Anti-inflammatory, Properties & Clove

Introduction

Over the last decades, aromatic plants have been used traditionally as food preservative and for many therapeutic purposes (Shahid *et al*, 2019). Nowadays, the therapeutic properties of aromatic plants are used in the modern medicine; the volatile compound of those aromatic plant are the common

ingredients of pharmaceutical (Dorsaf *et al,* 2021). So, this plant is important to human everyday life (Yadav *et al*, 2020). There are different kinds of aromatic plants such as Cloves (Syzygium aromaticum), Peppermint (Mentha spp), Lavender (Lavandula spp), Lemon balm (Melissa officinalis) and Rosemary (Salvia rosmarinus). Antimicrobial and antioxidant properties are essential for food preservation and medicine which are found in Cloves (Syzygium aromaticum). Cloves (Syzygium aromaticum) is a dried flower bud which belongs to Myrtaceae family and the color is small brown (Batiha *et al,* 2020). It is evergreen tropical Myrtaceae family tree native to the island of Maluka in east Indonesia and it's widely distributed in tropical and subtropical areas of Asia, Africa, Madagascar and throughout Pacific and Oceanic region (Batiha *et al,* 2020). It was used as food preservatives, flavoring agents and nutritional additives, medicinal coloring agents (Cortes-Rojas *et al,* 2014).

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants which include saponins, alkaloids, tannins, flavonoid and phenolic compounds (Bacanli *et al*, 2019). The phytochemical analysis presence on the plant's extract reported the presence of eugenol, acetyl eugenol, Alpha and Beta-caryophyllene, vanillin, tannins in cloves. (Yusuf *et al,* 2021). Analysis of phytochemicals by GC-FID (Gas Chromatography-Flame Ionization Detector) is one of the modern techniques used to identify and isolate Phyto-constituents (Nwiloh *et al,* 2016). Cloves shows some pharmacological activities such as anti-oxidant, anti-cancer, anti-inflammatory, anti-pyretic, anti-viral, anti-diabetic, anesthetic, analgesic, anti-carcinogenic, antibacterial, antifungal, antibiotic (Yunusa *et al*, 2018).

Inflammation is a defense response of our body to hazardous stimuli such as allergens and/or injury to the tissues; on the other hand, uncontrolled inflammatory response is the main cause of a vast continuum of disorders including allergies, cardiovascular dysfunctions, metabolic syndrome, cancer, and autoimmune disease imposing a huge economic burden on individuals and consequently on the society. Inflammation is a comprehensive array of physiological response to a foreign organism including human pathogens, dust particles and viruses, (Arulselvan *et al,* 2016). Inflammation can be created by several different causes including a blood clot, an immune system disorder, a cancer, an infection, a chemical exposure, a physical injury or a neurological condition, such as Alzheimer's or depression (Roe, 2021). It can also arise as a result of production of free radicals from various sources due to an imbalance of natural antioxidants.

Antioxidants are molecules that inhibit or quench free radical reactions and delay or inhibit cellular damage (Young *et al,* 2001). Antioxidants compounds act as "free radical scavenger" by preventing and repairing damages caused reactive oxygen species (ROS) and therefore enhance the immune defense and lower the risk of cancer and degenerative disease (Valko *et al.,*2006). Free radical and ROS are generated by living cells during respiration and other cellular activities and endogenous antioxidants scavenge these free radicals/ROS to prevent them from attacking biomolecules and causing damaging effects in the cell. Oxidative damage to biological systems exceeds the scavenging/quenching capacity of the cell's endogenous antioxidants. These may result in DNA damage or degradation of protein and induction of lipid peroxidation. (Kola, 2019).

This study evaluates the phytochemical, anti-inflammatory and anti-oxidants properties of aqueous, ethyl acetate and carbon tetrachloride extracts that were extracted from Cloves (Syzygium aromaticum).

Aim of Study

The aim of this study is to investigate the phytochemical properties, anti-inflammatory properties and the anti-oxidants properties of different solvent extracts that were extracted from Syzygium aromaticum (cloves).

Objective of Study

1. To determine the phytochemical properties found in Syzygium aromaticum extracts
2. To determine the anti-inflammation properties of Syzigium aromaticium; the heat induced hemolysis, inhibitory action on albumin denaturation and the anti-proteinase activity on Syzigium aromaticum (clove) in each of the extracts.
3. To determine the anti-oxidant properties in Syzygium aromaticum; the ABTs scavenging effects, the hydrogen peroxide scavenging effects, assay of lipid peroxidation and DPPH scavenging effect in each of the extracts of Syzygium aromaticum (cloves).
4. To examine which among the three extracts has high phytochemical, anti-oxidant and anti-inflammatory properties
5. To examine if there is any significant different in the three extracts when compared to standard.

Materials

1. Spectrophotometer
2. Weighing balance
3. Centrifuge
4. Hot air oven
5. Beakers
6. Aluminum foils
7. Measuring cylinder
8. Heating mantle
9. Pasteur pipette
10. Reagent bottles
11. Test tubes and rack
12. Masking tape
13. Funnel
14. Cuvette

Reagents

1. ABTS Solution (7mM with 2.45mM ammonium persulfate)
2. Phosphate buffer (0.1M, pH 7.4)
3. H_2O_2(40mM) in phosphate buffer
4. TCA (10%)
5. TBA (0.1M)
6. Phosphate buffer (0.12M, pH 7.2)
7. DPPH – 2,2-diphenyl-2-picryl hydrazyl hydrate (0.3mM in methanol)
8. Methanol

Method of Sample Collection

The sample used for this project were bought at Eke-Awka market Awka, Anambra state and identified by a taxonomist in the department of Botany, Nnamdi Azikiwe University, Awka and it was bought in its dried formed after which it was grinded into powder ready for work

Method of Sample Preparation

600g of sample were weighed and macerated in 1500ml of methanol. The sample mixture was extracted for 72hrs. After 72hrs, it was filtered using mucelin cloth. The extract was filtered and the filtrate was finally dried at low room temperature (60^0c) under pressure in a rotary vacuum evaporator (Thermotech, buchi type model th-012). The extracts were concentrated, percentage yield calculated and then subjected to column chromatography and the product of column subjected to phytochemical screening using GC-FID, anti-oxidants and anti-inflammatory analysis. The dried extract was properly stored in the desiccators for further experiment and analysis.

Method of Analysis

Column chromatography

1. 50g of Silica gel G was heated in an oven at 130^0c for 4hrs and was transferred to a 250ml size beaker and placed in a desiccator.
2. 10g of silica gel was package into the conventional size chromatography column and a glass wool was plugged in the bottom. The column has a clamp to stop the flow of the solvent. And 10 ml of ethanol was mixed with 10 g silica to obtain slurry. The column was filled with the slurry until alumina settles down to 4-5 cm height.
3. Some ethanol was kept above the silica's top and the silica was not allowed to be dry. Ethanol was about 1 mm at the top of the column and a stopper at the bottom of the column with a hose clamp.
4. Crude extract was eluted with a step gradient of n-hexane, ethyl acetate and

methanol. N-hexane was first added to the crude extract and was properly mixed and then transferred to the column; The process was repeated until all the component of the extract soluble in n-hexane were extracted.

5. The process was repeated with ethyl acetate and finally with methanol.
6. Eluent collected was been evaporated using rotary evaporator and the eluent was weighted

The extracted was further subjected to GC-FID analysis

Extraction of Phytochemicals

0.1g of Clove (Syzgium aromaticum) extract was weighed and transferred in a test tube and 15ml ethanol and 10ml of 50%m/v potassium hydroxide was added. The test tube was allowed to react in a water bath at 60^0c for 3hrsmins. After the reaction time, the reaction product contained in the test tube was transferred to a separatory funnel. The tube was washed successfully with 20ml of ethanol, 10ml of cold water, 10ml of hot water and 3ml of hexane, which was all transferred to the funnel. The Clove (Syzgium aromaticum) extract was combined and washed three times with 10ml of 10%v/v ethanol aqueous solution. The ethanol solvent was evaporated. The sample was solubilized in 1000ul of pyridine of which 200ul was transferred to a vial for analysis.

Quantification by GC - FID

The analysis of phytochemical was performed on a BUCK M910 Gas chromatography equipped with a flame ionization detector. A RESTEK 15meter MXT-1 column (15m x 250um x 0.15um) was used. The injector temperature was 280^{o}C with spitless injection of 2ul of sample and a linear velocity of 30cms^{-1}, Helium 5.0pas was the carrier gas with a flow rate of 40 mlmin^{-1}. The oven operated initially at 200^0c, it was heated to 330^0c at a rate of 3^0c min^{-1} and was kept at this temperature for 5min. the detector operated at a temperature of 320^0c. Phytochemicals were determined by the ratio between the area and mass of internal standard and the area of the identified phytochemicals. The concentration of the different phytochemicals expresses in ug/g.

Assessment of in Vitro Anti-Oxidant Activity

ABTS Scavenging Effects

Method

The antioxidant effect of the clove was studied using ABTS (2, 2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid) radical cation decolorization assay according to the method of Shirwaikar *et al*. (2006).

Principle

Anti-oxidant effect of the clove extract was studied using (2, 2'-azino-bis- 3-ethyl benzthiazoline-6-sulphonic acid) radical cation decolorization assay. The ABTs assay measures the relative ability of anti-oxidant to scavenge the ABTS radical cations (ABTS+) which has a dark blue color is reduced by an antioxidant to give a colorless ABTs product having absorption maxima at 745nm.

Procedure

ABTS radical cations (ABTS+) techniques were produced by reacting ABTS solution (7mM) with 2.45mM ammonium persulphate. The mixture was allowed to stand in the dark at room temperature for 12-16 hours before use. Aliquots (100mg, 200mg and 300mg) of the different samples extract were added to 0.3ml of ABTs solution and final volume was made up to 1000mg with ethanol. The absorbance was read at 745nm in a spectrophotometer (Genesys 10-S, USA) and the per cent scavenging activity was calculated using the formula:

$$\text{Scavenging activity (\%)} = \frac{\text{(Control – test)}}{\text{Control}} \times 100$$

Hydrogen Peroxide Scavenging Effects

Method

The ability of the clove (Syzgium aromaticum) to scavenge hydrogen peroxide was assessed by the method of Ruch *et al*. (1989).

Principle
The ability of clove to scavenge hydrogen peroxide. It was determined by measuring decrement of H_2O_2 in an incubation system containing H_2O_2 and the scavenging activity using classical UV-method at 230nm.

Procedure
A solution of H_2O_2 (40mM) was prepared in phosphate buffer. Clove (Syzgium aromaticum) extracts at the concentration of (100mg, 200mg and 300mg) were added to a test-tube and the volume was made up to 1000mgrespectively. And, H_2O_2 solution (0.6ml) added and the total volume was made up to 3ml by adding 2.3ml of distilled water. The absorbance of the reaction mixture was recorded at 230nm in a spectrophotometer (Genesys 10-S, USA). A blank solution containing phosphate buffer, without H_2O_2 was prepared. The extent of H_2O_2scavenging of the sample samples was calculated as:

$$\% \text{ scavenging of hydrogen peroxide} = \frac{(A0 - A1)}{A0} \times 100$$

A0 - Absorbance of control
A1 - Absorbance in the presence of sample

Assay of Lipid Peroxidation
Method

The extent of lipid peroxidation was estimated according to the method of Okhawa *et al.* (1979).

Principle
Malondialdehyde (MDA) formed from the breakdown of polyunsaturated fatty acids serves as a convenient index for the determination of the extent of peroxidation reaction. MDA, a product of lipid peroxidation reacts with TBA (thiobarbituric acid) to give a pink colored product having absorption maxima at 535nm.

Procedure
Different concentration of the extract (100mg, 200mg and 300mg) were prepared in phosphate buffer A 20% liver homogenate was prepared in phosphate buffer (pH 7.2). 0.5ml of the homogenate of the extracts and 0.12M phosphate buffer to make up 1000 mg), 1.0ml of TCA and 1.0ml of TBA were added and mixed thoroughly. The mixture was heated in a boiling water bath for 20 minutes. The tubes were centrifuged at 1000g for 20 minutes and the absorbance was read at 535nm in a spectrophotometer (Genesys 10-S, USA) against a blank containing all the reagents except the homogenate. The MDA equivalents of the samples were calculated using the extinction coefficient 1.56 × 105 M-1cm-1.

DPPH Spectrophotometric Assay
Method
The scavenging ability of the natural antioxidants of the sample towards the stable free radical DPPH was measured by the method of Mensor *et al*. (2001).

Principle
DPPH free radical method is an antioxidant assay based on electron-transfer that produces solution in ethanol. This free radical, stable at room temperature is reduced in the presence of san anti-oxidant molecule, giving rise to colorless ethanol solution.

Procedure
Clove (Syzgium aromaticum) extracts at the concentration of (100mg, 200mg and 300mg) were added to a test-tube and the volume was made up to 1000mgrespectively. And 0.5ml of 0.1mM methanolic solution of DPPH and 0.48ml of methanol was added. The mixture was allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol, without the clove (Syzgium aromaticum) extract, served as the positive control while butylated hydroxytoluene (BHT) served as reference. After 30 minutes of incubation, the discoloration of the purple color was measured at 518nm in a spectrophotometer (Genesys 10-S, USA). The radical scavenging activity was calculated as follows:

$$\text{Scavenging activity \%} = 100 - \frac{A518 \text{ (sample)} - A518 \text{ (blank)}}{A518 \text{ (blank)}} \times 100$$

Assessment of *in Vitro* Anti-Inflammatory Activity

Inhibition of Albumin Denaturation

Method

The anti-inflammatory activity of Clove (Syzgium aromaticum) was studied by using inhibition of albumin denaturation technique which was studied according to Mizushima et al [1968] and Sakat et al [2010] followed with minor modifications.

Principle
The egg albumin denaturation assay is based on the idea that substance with anti-inflammatory properties has the ability to stabilize protein structure and prevent denaturation which is frequently linked to inflammation and tissue damage.

Procedure
The reaction mixture was consisting of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount of 1N HCl. The homogenate (0.1ml, 0.2ml, 0.3ml of the extracts and distilled water to make up 1 ml) and 1ml of 1% aqueous solution of bovine albumin fraction was added and incubated at 37 ºC for 20 min and then heated to 51 º C for 20 min, after cooling the samples the turbidity was measured at 660nm. (UV Visible Spectrophotometer Model 371, Elico India Ltd). The Percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition = (Abs Control –Abs Sample) X 100/ Abs control.

Heat Induced Haemolysis
Method
According to the method by Sakat *et* al, 2010.

Principle
It involved stabilization of human red blood membrane by hypo tonicity induced membrane lysis.

Procedure
The reaction mixture (2ml) consisted of test sample of different concentrations (100mg, 200mg and 300mg of the extracts and distilled water to make up 1000mg) and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56 ºC for 30min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm.

The Percentage inhibition of Hemolysis was calculated as follows:

Percentage inhibition = (Abs control –Abs sample) X 100/ Abs control

Anti-Proteinase Activity

Method
The test was performed according to the modified method of Oyedepo et al, 2012 and Sakat et al 2010

Principle
It is a method that measured proteinase inhibitors ability to reduce tissue damage by proteinase that are released by dead or dying inflammatory cells.

Procedure
The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml 20 mM Tris HCl buffer (pH 7.4) and 1 ml test sample of different concentrations (100mg, 200mg and 300mg of the extracts and distilled water to make up 1000mg). The mixture was incubated at 37oC for 5 min and then 1 ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. 2 ml of 70% perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The experiment was performed in triplicate. The percentage inhibition of proteinase inhibitory activity was calculated.
Percentage inhibition = (Abs control –Abs sample) X 100/ Abs control.

Results

Extraction of Phytochemicals

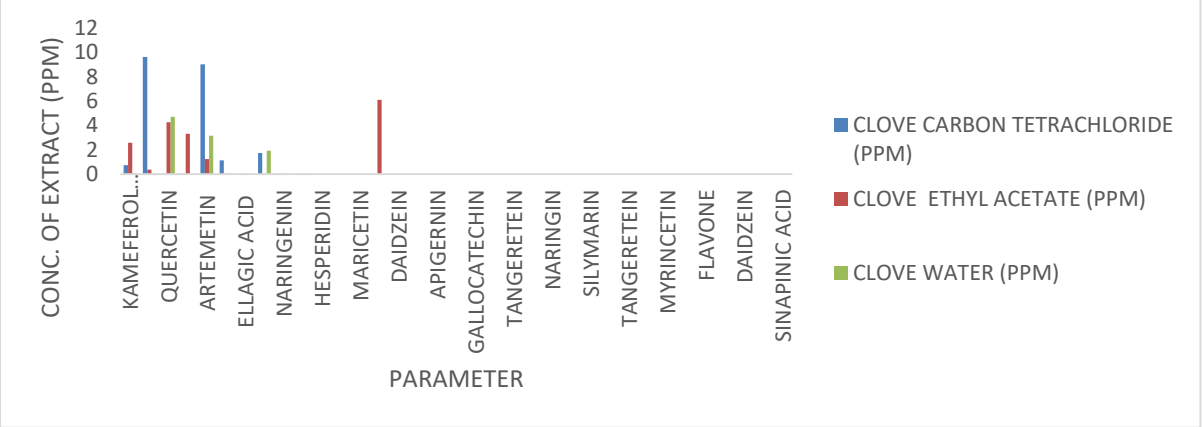

Fig.1: Phytochemical Profile of all the Extracts

This result show that catechin has the highest concentration in carbon tetrachloride, followed by artementin in carbon tetrachloride extracts and epicatechin in ethyl acetate extract with the concentration of 9.61826ppm, 9.01830ppm and 6.08818ppm respectively while naringenin in ethyl acetate, retusin in ethyl acetate and catechin in aqueous extracts has the lowest concentration of phytochemical profile with the concentration of 0.0177608ppm,0.0181873ppm and 0.0182906ppm respectively when compared with each other. However, when compared with each other carbon tetrachloride extract has the highest phytochemical composition with the total concentration of 22.311273ppm while aqueous extracts have the lowest phytochemical composition with total concentration of 9.8821797ppm when compared to each other.

Assessment of *in Vitro* Anti-Oxidant Activity

ABTS Scavenging Effect

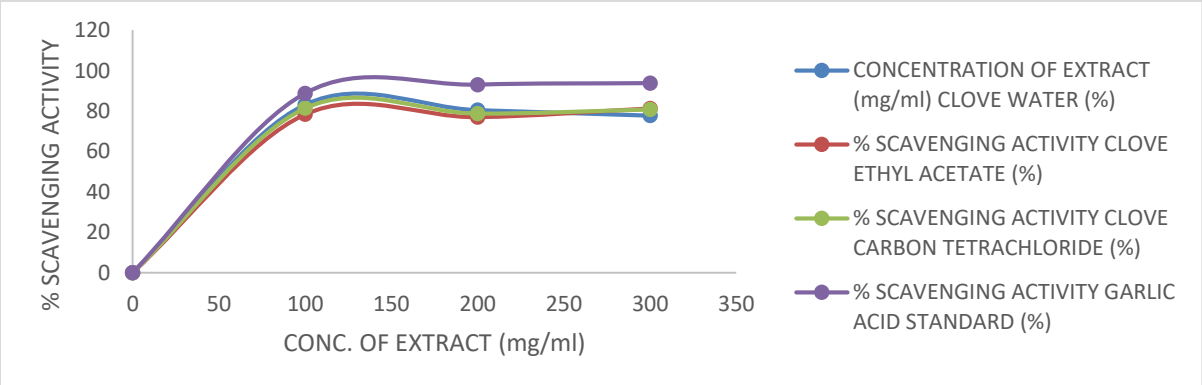

Fig.2: ABTs Percentage Scavenging Effect of all the Extract with Garlic Acid as Standard

The result above shows that there is high percentage scavenging activity of aqueous extract when compared with ethyl acetate and carbon tetrachloride of clove ex tract. The average percentage scavenger for aqueous extract is 80.44% while carbon tetrachloride

extract is 80.312% and ethyl acetate extract is 78.86% when compared with standard of 91.83%. As the concentration increases, the result shows that there is increase in the percentage scavenging activity of ethyl acetate and carbon tetrachloride while there is decrease in the concentration of the aqueous extract. The results also show that all the extracts are significant statistically ($p < 0.05$) when compared with standard.

Mean of ABTs Scavenging Effect

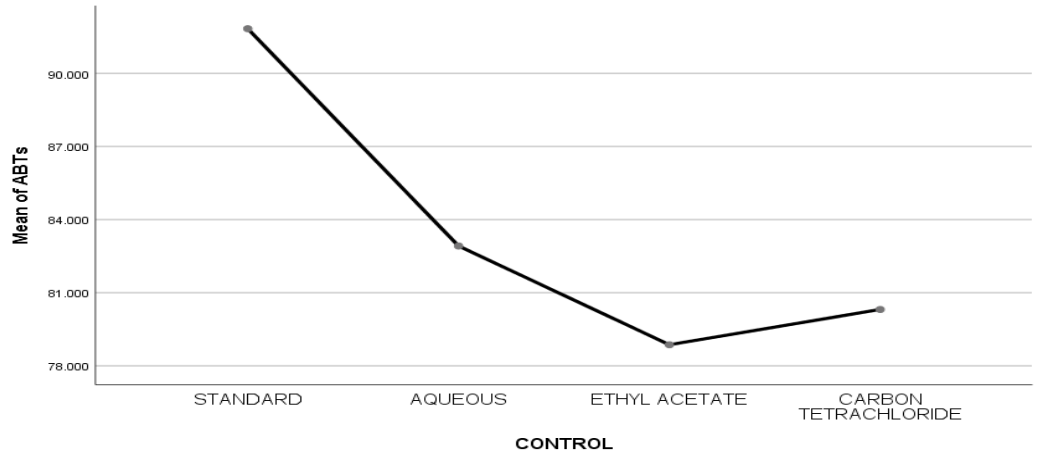

Fig.3: Mean of ABTs Scavenging Effect of Standard, Aqueous, Ethyl Acetate and Carbon Tetrachloride Extracts

The graph shows that all the extracts are significantly low when compared to standard ($p > 0.05$)

Hydrogen Peroxide Scavenging Effects

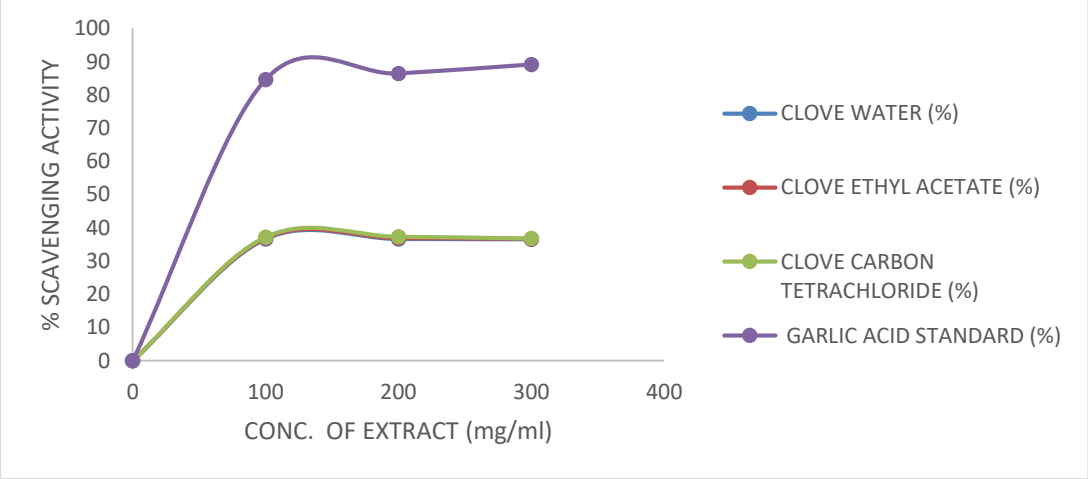

Fig.4: Hydrogen Peroxide Percentage Scavenging Activity of all the Extract with Garlic Acid as Standard.

The fig above shows that carbon tetrachloride extract has the highest percentage scavenging activity when compared to ethyl acetate and clove aqueous extracts. Carbon tetrachloride extracts has the highest average percentage scavenging activity of 37.096% while ethyl acetate extracts have 36.804% and aqueous extract has 36.623% when compared to standard of 86.732%. As the concentration increase, the result shows that all the extracts

slightly increase in the %scavenging activity having no significant differences. And it also shows that all the extracts vary significant ($p < 0.05$) when compared with standard.

Means of Hydrogen Peroxide Scavenging Effect

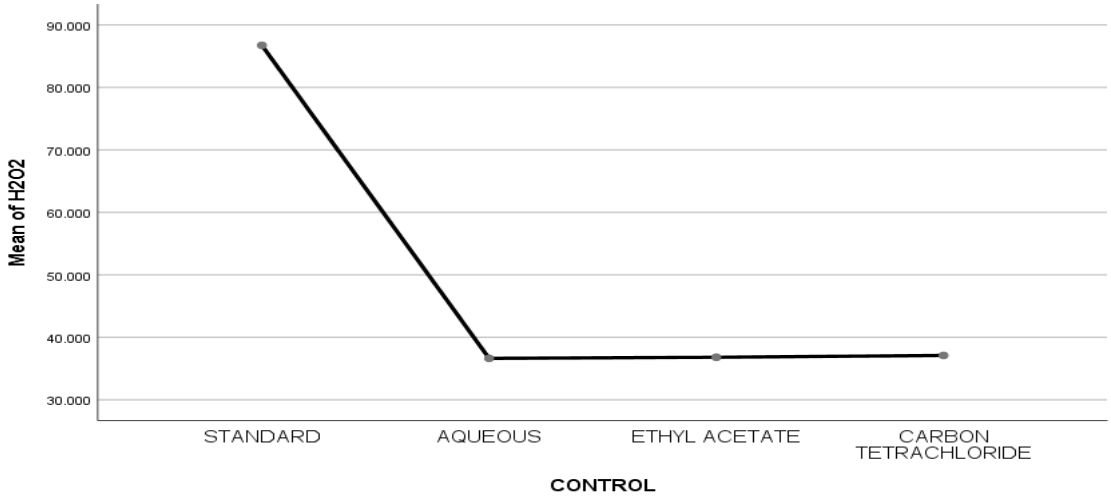

Fig.5: Mean of Hydrogen Peroxide Scavenging Effect of Standard, Aqueous, Ethyl Acetate and Carbon Tetrachloride Extracts

This shows that all the extracts are significantly low when compared to the standard ($p < 0.05$).

Assay of Lipid Peroxidation

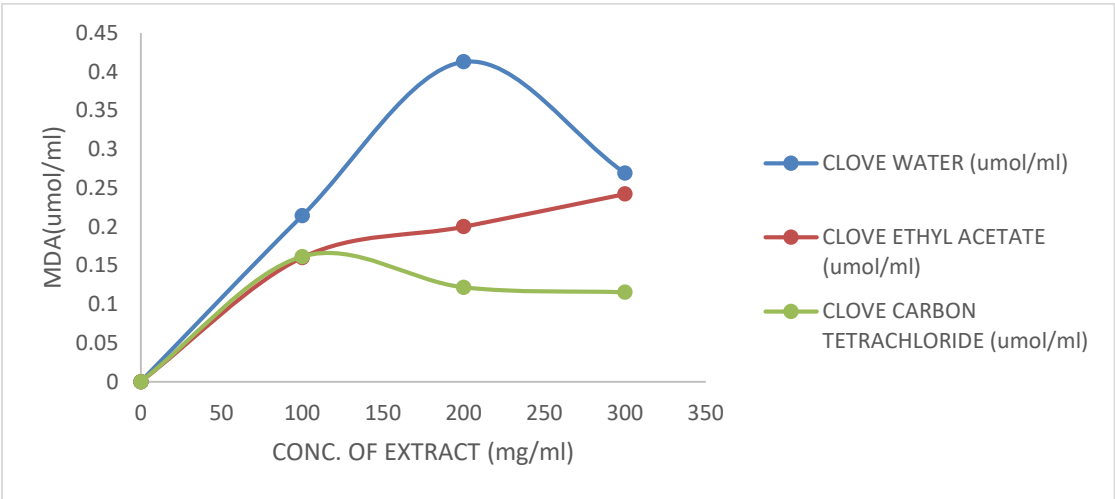

Fig.6: Lipid Peroxidation of All the Extracts

This result shows that aqueous extract has the highest MDA activity when compared to ethyl acetate and carbon tetrachloride extracts. The average MDA activity of aqueous extract is0.2987 umol/ml while ethyl acetates extract is 0.2009umol/ml and carbon tetrachloride extract is 0.1328umol/ml when compared to each other. There is decrease in MDA activity of carbon tetrachloride extract, while there is increases in MDA activity of ethyl acetate

extract as the concentration of extract increases. Also, there is an increase and decrease of aqueous extract as the concentration of extract exceed 100mg/ml.

DPPH Spectrophotometric Assay

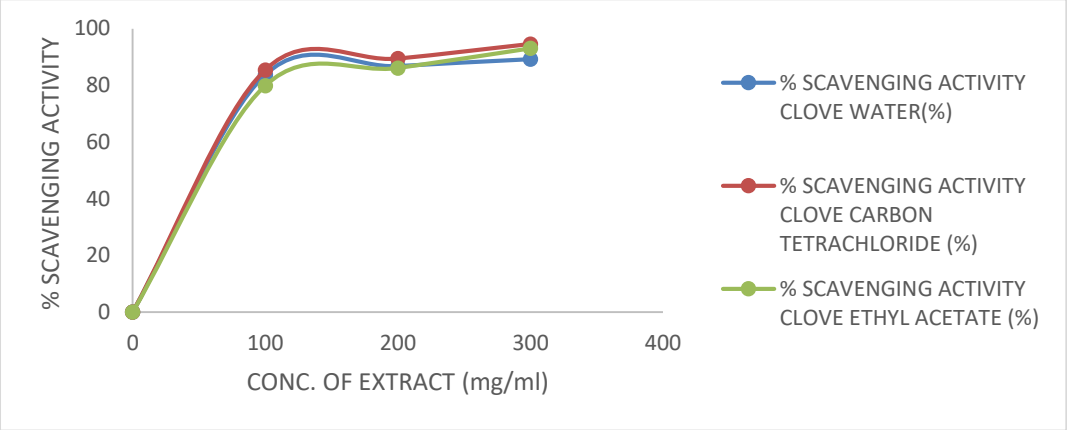

Fig.7: DPPH Spectrophotometric Assay of All the Extract.

The fig above shows that carbon tetrachloride extract has the highest % scavenging activity while ethyl acetates extract has the lowest % scavenging activity when compared to each other. Carbon tetrachloride extract has the average percentage scavenging activity of 89.7966% while aqueous extract of 86.556% and ethyl acetates extract of 86.332% as the concentration of extract increases. It also shows that there is increase in DPPH % scavenging activity of both ethyl acetate and carbon tetrachloride extracts while aqueous has a decrease in the % scavenging activity as the concentration of the extract increase.

Assessment of *in Vitro* Anti-Inflammatory Activity
Inhibition of Albumin Denaturation

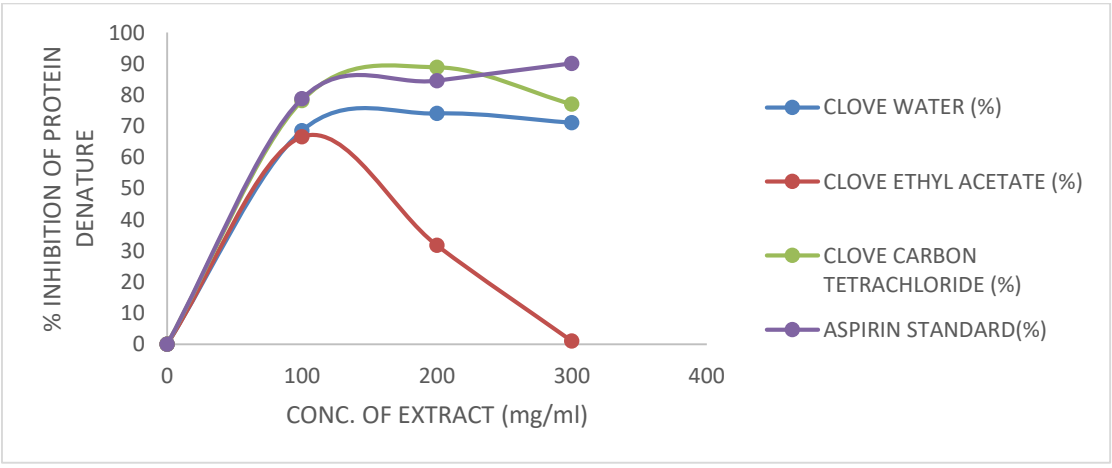

Fig.8: % Inhibition of Albumin Denaturation of all the Extracts with Aspirin as Standard

This graph above shows that carbon tetrachloride extract has the highest concentration of % inhibition while ethyl acetate extract has the lowest % inhibition of albumin denaturation when compared to standard. The average % inhibition of carbon tetrachloride extract is 81.3785% while aqueous extract is 71.33% and ethyl acetate

extract is 33.11% when compared to standard of 84.4727%. It also shows that % inhibition of albumin denaturation of carbon tetrachloride and ethyl acetate decrease as the concentration of extracts exceed 200mg/ml while clove aqueous decreases in % inhibition as the concentration of extract increases. However, the result also shows that aqueous and carbon tetrachloride extract are not significant statistically ($p< 0.05$) while ethyl acetate extract is significant ($p>0.05$) when compared to standard.

Mean of %Inhibition of Albumin Denaturation

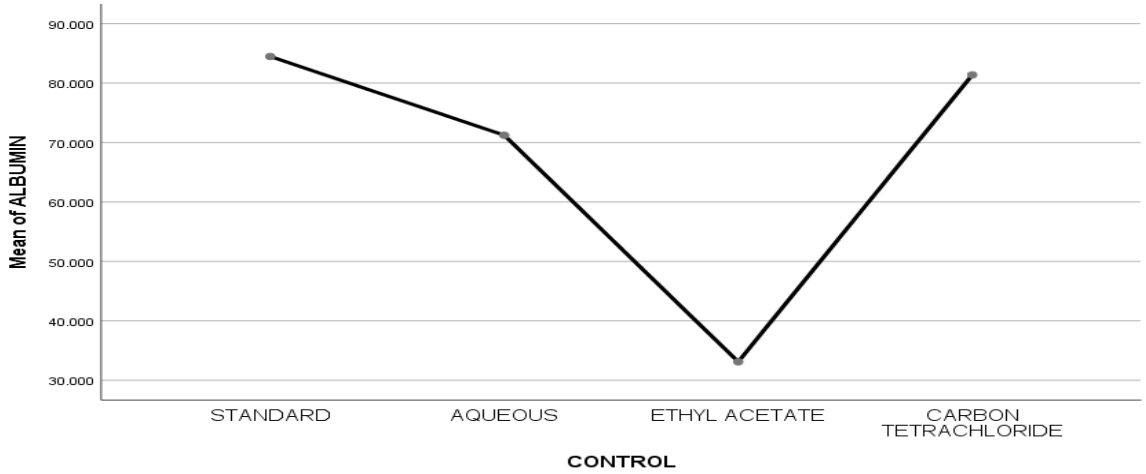

Fig.9: Mean of albumin denaturation inhibition of standard, aqueous, ethyl acetate and carbon tetrachloride extracts

The graph shows that carbon tetrachloride and aqueous extracts are not significantly ($p>0.05$) while ethyl acetate extract is significantly low ($p< 0.05$) when compared to standard.

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Heat Induced Haemolysis

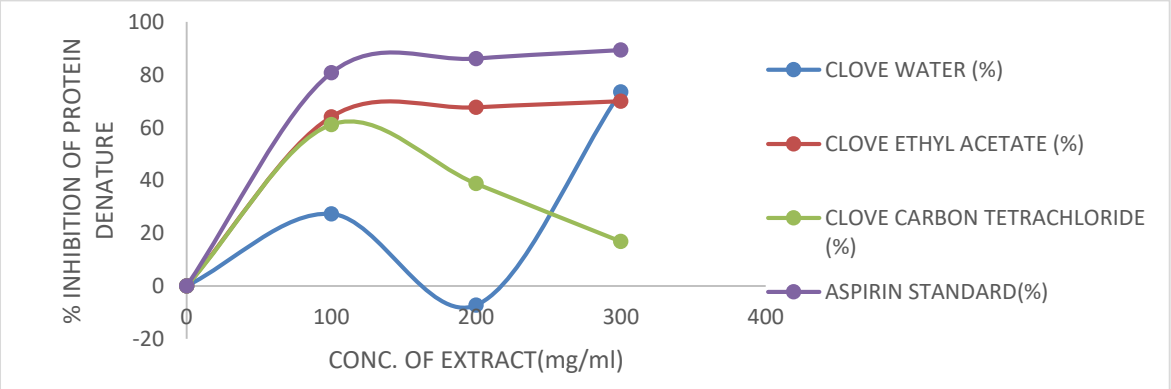

Fig.10: % Inhibition of Heat Induced Heamolysis of All the Extract with Aspirin as Standard

The result show that % inhibition of ethyl acetate extract is high while the % inhibition of aqueous extract is low when compared to standard. It also evaluates that there is an increase in ethyl acetate extract and decrease in % inhibition of carbon tetrachloride extract while aqueous extract show increase and decreases in % inhibition as the concentration of extract increases when compared to standard. The average % inhibition of ethyl

acetate extract is 67.4368% while carbon tetrachloride extract is 38.9419% and aqueous extract is 31.205% when compared to standard of 85.4415%. However, the result also shows that aqueous and carbon tetrachloride extract are significant statistically ($p<0.05$) while ethyl acetate extract is not significant ($p>0.05$) when compared to standard

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Mean of Heat Induced Haemolysis

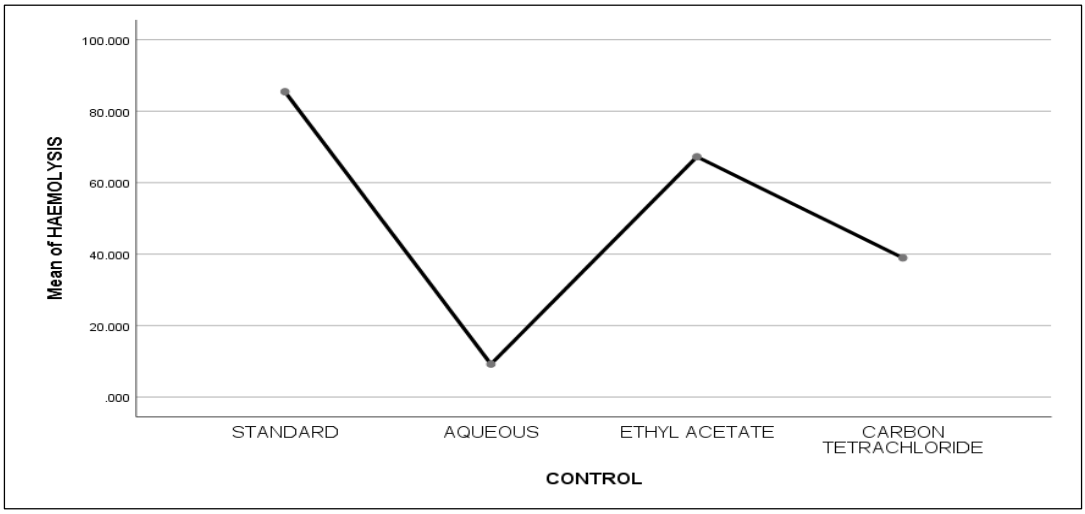


Fig.11: Mean of Heat Induced Heamolysis Inhibition of Standard, Aqueous, Ethyl Acetate and Carbon Tetrachloride

The graph shows that ethyl acetate extract is not significantly ($p>0.05$) while carbon tetrachloride and aqueous extract are significantly low ($p<0.05$) when compared to standard.

Anti-Proteinase Activity

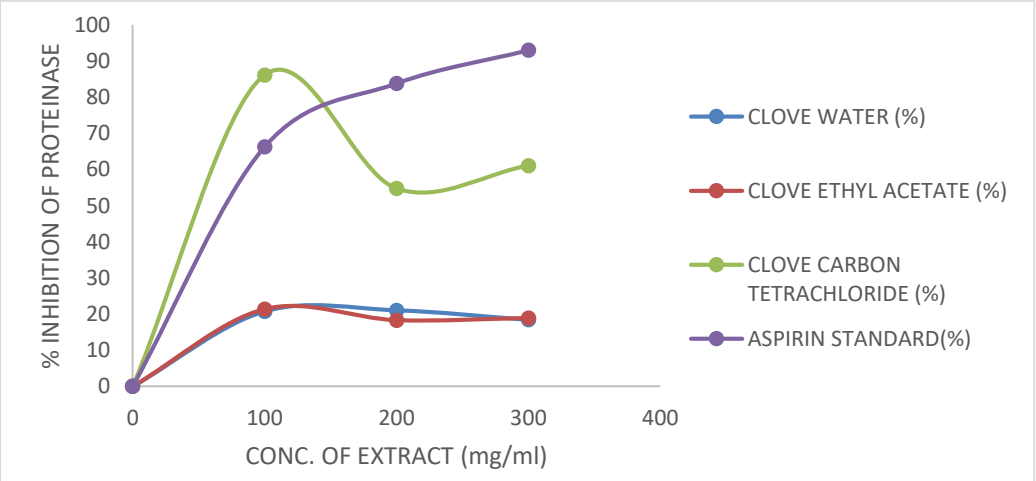
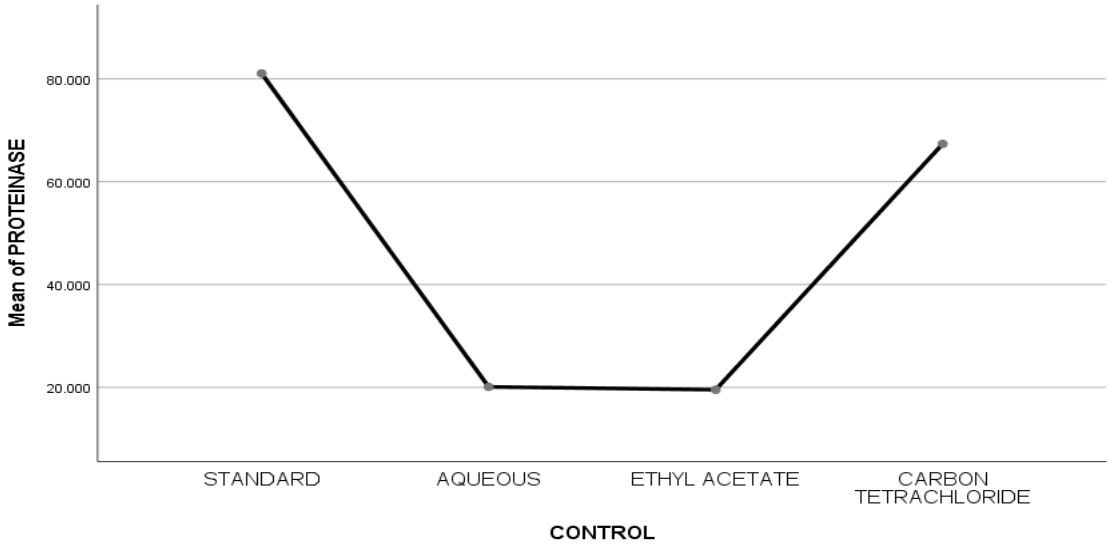


Fig. 12: % Inhibition of Anti-Proteinase Activity of All the Extract with Aspirin as Standard.

The graph above shows that carbon tetrachloride extract has a high % inhibition while ethyl acetate extract has a low % inhibition when compared to standard. Carbon tetrachloride extract show an average % inhibition of 67.3429% and aqueous extract of 20.0875% and ethyl acetates extract of 19.5306% average inhibition when compared to standard of 81.0439%. As the concentration increases, there is increases

and decreases of % inhibition of all the extract. Moreover, the result also shows that aqueous and ethyl acetate extract are significant (p<0.05) while carbon tetrachloride is not significant (p>0.05) when compared to standard.

Mean of Anti-Proteinase Activity



F*ig*. 13 Mean of Anti-Proteinase Inhibition of Standard, Aqueous, Ethyl Acetate and Carbon Tetrachloride

The graph shows that clove carbon tetrachloride is not significantly (p>0.05) while ethyl acetate and aqueous are significantly low statistically (p< 0.05) when compared to standard.

Conclusion

The investigation into the biochemical activities of clove (*Syzygium aromaticum*) extracts reveals both diverse and promising properties. The anti-proteinase activity, lipid peroxidation, hydrogen peroxide scavenging, ABTS scavenging, and DPPH scavenging activities showcase the potential therapeutic applications of clove. The extracts exhibit varied inhibition levels in anti-proteinase activity, distinct antioxidant patterns in lipid peroxidation, and reliable potential in neutralizing hydrogen peroxide radicals. The ABTS and DPPH scavenging activities demonstrate commendable antioxidant potential, emphasizing clove's role as a natural source for the development of antioxidant-rich formulations and therapeutic interventions. The nuanced variations observed underscore the need for further research to elucidate specific compounds responsible for these activities and optimize extraction methods for enhanced efficacy. Overall, clove emerges as a promising natural resource with multifaceted bioactive potential.

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