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RESEARCH ARTICLE

Phytochemical composition and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities of some essential oils

Opeyemi Adewumi Adediran[1*], Abdulhafeez Opeyemi Hamzat[1], Tolulope Oreoluwa Faniyi[2], Chika Ahaiwe[3], Olutola Busola Jegede[4]

[1]Department of Animal Science, University of Ibadan, Ibadan, Nigeria.
[2]Department of Crop and Animal Science, Ajayi Crowther University, Oyo, Nigeria.
[3]Department of Animal Science, University of Calabar, Calabar, Nigeria.
[4]Department of Agriculture and Agricultural Technology, Bamidele Olumilua University of Education, Science, and Technology, Ikere-Ekiti, Nigeria

* **Corresponding author:** dimu4ever@yahoo.com

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Conflict of interest

The authors declare no conflict of interest.

Abstract

Essential oils, derived from aromatic plants are known for their antioxidant and bioactive properties. They have shown promise in inhibiting lipid oxidation and conferring functional properties to food products. This research was carried out at the Animal Products and Processing Laboratory, Department of Animal Science, University of Ibadan; it was designed to examine the phytochemical composition and radical scavenging activities of selected essential oils as potential bioactive and antioxidant additives in edible products.

Four different essential oils namely, Cinnamon bark essential oil (CEO), Oregano essential oil (OEO), Thyme essential oil (TEO) and Lemon essential oil (LEO) were selected for this study and obtained from a reputable processor. The selected oils were subjected to evaluation of phytochemical composition and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities using standard procedures.

Phytochemical analysis revealed the presence of saponin, alkaloid, flavonoid, tannin and reducing sugar in the oils. Tannin and alkaloid were highest in Cinnamon oil and lowest in Thyme oil. While flavonoid was highest in Lemon oil and lowest in Cinnamon oil. Reducing sugar was highest (7.28) in Thyme oil and lowest in Oregano oil. DPPH was highest (75.07%) in Thyme essential oil and lowest in Cinnamon bark essential oil (70.31%) while values for oregano essential oils and lemon essential oils are (73.42% and 68.29%).

Cinnamon essential oil had the highest tannin, alkaloid, and saponin content (which play key roles in antioxidation). DPPH analysis however showed that thyme essential oil as potentially stronger in inhibiting lipid oxidation, among the evaluated oils. Thus, it is concluded that thyme essential oil and cinnamon essential oil are more likely to reduce lipid oxidation and improve food functionality. Researchers in food processing could focus on the synergistic effects of thyme and cinnamon essential oil in reducing lipid oxidation and improving functional properties of food.

KEYWORDS: *Essential oils, Thyme, Lemon, Oregano, Cinnamon Bark, Phytochemical*

1.0 Introduction

The use of essential oils in food processing has gained significant attention due to their natural origin and multifunctional properties. Essential oils, extracted from various parts of plants, are known for their strong aromatic characteristics and bioactive compounds, which have been extensively studied for their antimicrobial and antioxidant activities (Hyldgaard *et al.,* 2012; Bag and Chattopadhyay, 2015; da Silva *et al.,* 2021). These properties make Essential oils promising candidates for enhancing food functionality, food safety and extending the shelf life of food products, particularly by inhibiting microbial growth and lipid oxidation (Gutiérrez *et al.,* 2008). The increasing consumer demand for natural food preservatives over synthetic additives further supports the exploration of essential oils in this context (Bakkali *et al.,* 2008; Falleh *et al.,* 2020).

Previous research has identified several key characteristics of essential oils that contribute to their efficacy as microbial and lipid oxidation inhibitors. Their complex mixtures of terpenes, phenolic compounds, aldehydes, and alcohols play a crucial role in disrupting microbial cell membranes, leading to the inhibition of bacterial and fungal growth (Raut and Karuppayil, 2014; Rao *et al.,* 2019). Additionally, the antioxidant properties of essential oils are linked to their ability to scavenge free radicals, thereby preventing lipid oxidation, a major cause of food spoilage (Ruberto and Baratta, 2000). Cinnamon and clove oils, rich in eugenol, have demonstrated significant antioxidative activities, making them effective in preserving the quality of fats and oils in food products (Viuda-Martos *et al.,* 2010).

Furthermore, the variability in the chemical composition of essential oils, influenced by factors such as plant species, geographical origin, and extraction methods, impacts their potency and applicability in food systems (Bakkali *et al.,* 2008). Numerous studies have confirmed that the synergistic effects of essential oil components enhance their overall efficacy, which can be optimized by careful selection and combination of oils tailored to specific food matrices (Pateiro *et al.,* 2021). Consequently, the integration of essential oils into food processing not only offers a natural solution for microbial control and lipid stabilization but also aligns with the growing trend towards clean-label products (Hyldgaard *et al.,* 2012; Falowo *et al.,* 2014; Adediran and Omojola, 2022).

This research was therefore carried out to examine the phytochemical composition and radical scavenging activities of Cinnamon bark essential oil (CEO), Oregano essential oil (OEO), Thyme essential oil (TEO) and Lemon essential oil (LEO) as potential bioactive and antioxidant additives in edible products.

2.0 Materials and Methods

2.1 Study Site

The study was carried out at the Animal Products and Processing Laboratory, Department of Animal Science, University of Ibadan, Ibadan, Nigeria. This laboratory was selected for use because of its location in Nigeria's premier University, and its reputation for scientific accuracy.

2.2 Sourcing of Essential Oils

Thyme, Lemon, Oregano and Cinnamon Bark Essential oils used for the experiment were obtained from Blomera oils, Lagos State, Nigeria.

2.3 Screening of Essential Oils

2.3.1 *Qualitative and quantitative phytochemical screening of essential oils*

The initial qualitative assessment of the phytochemical content involved screening the essential oils to ascertain the presence of secondary metabolites, following the methodology outlined by Harborne (1998) and Parekh and Chanda (2007). Alkaloids were identified qualitatively using dragendroff reagent (potassium bismuth iodide), flavonoids were detected with Benedict's solutions (Adamu *et al.,* 1970), saponins were determined via a frothing test, tannins were assessed using wohler's test, and phenols were detected using ferric chloride solution, as detailed by Ajuru *et al.,* (2017). Subsequent quantitative analysis of certain phytochemicals involved determining phytate levels according to AOAC (1990) protocols, saponins were quantified using the double solvent extraction gravimetric method outlined by Harborne (1973), phenols were quantified using the Folin Ciocalteu reagent method (McDonald *et al.,* 2001), alkaloids were quantified following the alkaline precipitation gravimetric method described by Harborne (1973), tannins were quantified using the Folin-Denis method (Polshettiwar *et al.,* 2007), and flavonoids were quantified using the procedure outlined by Harborne (1973).

2.3.2 DPPH activities of essential oils

The radical scavenging activity was assessed using a spectrophotometric technique based on the reduction of a methanol solution containing 2,2-diphenyl-1-picrylhydrazyl (DPPH), following the method described by Blois, 1958. Briefly, one milliliter of varying concentrations of each essential oil, dissolved in methanol, was mixed with an equal volume of a 0.2mM methanol solution of DPPH. After vigorous shaking, the mixture was allowed to incubate at room temperature in the dark for 30 minutes. Subsequently, the absorbance was measured at 517nm against a blank using a spectrophotometer (Shimadzu, Tokyo, Japan), with absolute methanol serving as the zero point for the instrument. The DPPH solution was freshly prepared, stored in a darkened flask covered with aluminum foil, and refrigerated at 4°C until further use. The inhibition of free radicals, expressed as a percentage (I%), was determined using the formula:

$$I\% = (Ablank - Asample) / Ablank \times 100$$

where Ablank represents the absorbance of the control reaction containing all reagents except the test compound, and Asample denotes the absorbance of the test compound. The concentration of the extract required to achieve 50% inhibition (IC50) was determined from a graph plotting inhibition percentage against extract concentration. A lower IC50 value indicates higher antioxidant activity. All experiments were conducted in triplicate.

2.4 Statistical analysis

The data generated from the study was subjected to one-way analysis of variance (ANOVA) and significant differences ($P<0.05$) between means was determined by Scheffe multiple comparison test using SPSS 16.0.1 for Windows.

3.0 RESULTS

3.1 Qualitative phytochemical composition of selected essential oils

Presented on table 1 is the qualitative phytochemical composition of the selected essential oils. The essential oils used in this study contained saponin, alkaloid, flavonoid, tannin and reducing sugars.

3.2 Quantitative phytochemical composition of selected essential oils

The quantitative composition of some of the phytochemicals present in the analysed essential oils is displayed on table (2). The Tannin and Alkaloid contents were significantly ($p<0.05$) highest in Cinnamon oil and lowest in Thyme oil. The flavonoid was highest in Lemon oil and lowest in Cinnamon oil. Reducing sugar was significantly ($p<0.05$) highest (7.28) in Thyme oil and lowest in Oregano oil.

3.3 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities of selected essential oils

Displayed on table 3, is the DPPH radical scavenging activities of different essential oils. The DPPH was significantly ($p<0.05$) highest (75.07%) in Thyme essential oil and lowest in Cinnamon bark essential oil (70.31%). Values for oregano essential oils and lemon essential oils are (73.42% and 68.29%).

4.0 DISCUSSION

Phytochemicals, which are bioactive compounds, found in plants, play a significant role in essential oils, contributing to their antioxidative effects which helps in preserving food quality when used as additives (Abugri *et al.,* 2013; Tongnuanchan and Benjakul, 2014 da Silva *et al.,* 2021). Essential oils are rich in phytochemicals like phenols, flavonoids, and aromatic terpenes, and therefore impart distinct flavours and aromas to food, improving sensory appeal while also exhibiting antimicrobial properties that can help inhibit the growth of spoilage microorganisms. Phytochemicals can enhance the taste and appearance of edible products while also offering health benefits (Burt, 2004; Gonçalves *et al.,* 2021). Higher tannin and alkaloid contents in Cinnamon oil compared to Thyme oil may be attributed to the unique phytochemical profiles of these essential oils, as supported by research indicating that different plant species produce varying levels of secondary metabolites (Singh *et al.,* 2007). The highest flavonoid content in Lemon oil and the lowest in Cinnamon oil however align with some previous reports that citrus oils are particularly rich in flavonoids, which contribute to their strong antioxidant properties (Benavente-García *et al.,* 1997; Ullah *et al.,* 2020). The significantly higher reducing sugar content in Thyme oil compared to Oregano oil can be linked to the specific carbohydrate profiles of these herbs, as documented in studies showing that Thymus species tend to have higher sugar concentrations (Nickavar *et al.,* 2008).

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a widely used method to measure the antioxidant activity of compounds (Gerasimova *et al.,* 2022), including essential oils. Essential oils have shown significant DPPH radical scavenging activity, which indicates their strong potential in neutralizing free radicals and preventing oxidative damage (Mossa *et al*,. 2011; Rao *et al.,* 2019; Da Silva *et al.,* 2021). Thyme essential oil exhibited the highest radical scavenging activity (75.07%), likely due to its high content of thymol and carvacrol, compounds known for their potent antioxidant properties (Singh *et al.,* 2005; Falleh *et al.,* 2020). Cinnamon bark essential oil on the other hand showed the lowest radical scavenging activity (70.31%) contrary to observations by (Lin *et al.,* 2020), possibly due to variations in its phytochemical composition, and tested concentrations which might affect its antioxidant efficacy. Oregano (73.42%) and lemon essential oils (68.29%) also demonstrated considerable DPPH radical scavenging activities, reflecting their diverse bioactive compounds that contribute to their overall antioxidant potential which was also observed by Rajaei *et al.,* 2010.

Conclusion

Cinnamon essential oil had the highest tannin, alkaloid, and saponin content (which play key roles in antioxidation). DPPH analysis however showed that thyme essential oil is likely to be more effective in inhibiting lipid oxidation. Thus, it is inferred that thyme essential oil and cinnamon essential oil are more likely to reduce lipid oxidation and microbial proliferation in food systems.

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APPENDICES: TABLES

Table 1: Qualitative phytochemical composition of selected essential oils

Essential Oil	Tannin	Saponin	Alkaloid	Reducing sugar	Flavonoid
CEO	+++	++	++	+	++
OEO	++	++	++	+	++
TEO	++	++	++	+	++
LEO	++	++	++	+	++

Remarks key:

(++++) Present in an abundant amount, (+++) Present in an appreciable amount

(++) Present in a moderate amount, (+) Present in a traceable amount, (-) completely absent

CEO: Cinnamon Bark Essential Oil, OEO: Oregano Essential Oil

TEO: Thyme Essential Oil, LEO: Lemon Essential Oil

Table 2: Quantitative phytochemical composition of selected essential oils

Essential Oil	Tannin (mg/100g)	Saponin (mg/100g)	Alkaloid (mg/100g)	Reducing sugar (mg/100g)	Flavonoid (mg/100g)
CEO	72.55±0.08^{a}	44.69±0.04^{a}	29.63±0.03^{a}	6.49±0.04^{b}	23.56±0.98^{d}
OEO	65.83±0.06^{b}	39.35±0.07^{b}	25.22±0.05^{b}	5.75±0.05^{c}	26.38±0.06^{c}
TEO	60.3±0.10^{c}	36.21±0.04^{c}	19.88±0.04^{c}	7.28±0.20^{a}	30.05±0.02^{b}
LEO	56.44±0.03^{d}	31.90±0.04^{d}	15.29±0.09^{d}	7.23±0.02^{a}	34.67±0.03^{a}

$^{a, b, c, d}$: means with different superscripts on the same row are significantly different ($p < 0.05$)

Cinnamon Bark Essential Oil, OEO: Oregano Essential Oil, TEO: Thyme Essential Oil,

LEO: Lemon Essential Oil

Table 3: DPPH radical scavenging activities of selected essential oils.

PARAMETERS	CEO	OEO	TEO	LEO
DPPH	70.31±0.05^{c}	73.42±0.04^{b}	75.07±0.05^{a}	68.29±0.05^{d}
SEM	0.03	0.02	0.03	0.03

$^{a, b, c, d}$: means with different superscripts on the same row are significantly different ($p < 0.05$)

CEO: Cinnamon Bark Essential Oil, OEO: Oregano Essential Oil, TEO: Thyme Essential Oil, LEO: Lemon Essential Oil

SEM: Standard Error of Mean