UJMR, Vol. 8 No. 1, June, 2023, pp 80 - 83. ***E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668***

https://doi.org/10.47430/ujmr.2381.010

Received: April 20, 2023 **Accepted:** June 26, 2023

Effect of Oil Pollution on the Antioxidant Activity of the Methanolic Extracts of Polluted and Unpolluted Leaves of *Hyptis suaveolens* (L)

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Abstract

Hyptis suaveolens (L.) Poit, an aromatic weed known as pignut or daddoya-ta-daji in the Hausa language, has a longstanding history of usage as food and medicine in Kaduna state. This plant is interestingly found thriving in environments with or without oil pollution. In our modern industrial society, it's practically inevitable that these medicinal plants come in contact with oil contamination. This is largely because oil pipelines, often aged and susceptible to corrosion or illicit activities leading to leakages, are scattered across urban and rural settlements, influencing the plants' phytochemical composition. This study is focused on examining if oil pollution alters the antioxidant activity of this plant. An evaluation of the antioxidant properties of methanol extracts from Hyptis suaveolens (L.) leaves, both from polluted and unpolluted areas, was carried out using the 2,2-Diphenyl-1-Picrylhydrazyl-hydrate (DPPH) free radical scavenging assay. The outcomes showed that the extracts have significant ($p < 0.05$) concentration-dependent antioxidant activity. Yet, the unpolluted H. suaveolens leaves' methanol extract exhibited superior DPPH radical scavenging action compared to the extract from the polluted leaves. The median inhibitory concentrations (IC50) for the polluted and unpolluted leaves' methanol extracts were 35 µg/ml and 20 µg/ml respectively. Therefore, the contamination had a detrimental impact on the plant extract's antioxidant capacity.

Key Words: Hyptis suaveolens, Anti-oxidants, DPPH, Pollutants

INTRODUCTION

Hyptis suaveolens (L.) is a plant known for its aromatic properties and its use in traditional medicine. The plant's medicinal effectiveness can be attributed to the presence of several phytochemicals, including tannins, saponins, flavonoids, phenols, steroids, and terpenoids. These bioactive compounds transform it into a potent medicinal resource with a range of uses. It is commonly used as a carminative, stimulant, sudorific, wound healing agent, and also has antimicrobial, antioxidant, and anti-inflammatory properties (Raju *et al.*, 2019; Oumarou *et al.*, 2018).

When hydrocarbon oil pollution occurs on land, heavy metals of health concern such as mercury, arsenic, cadmium, lead and so on are released into the environment. These toxic metals negatively affect crops and plants by altering their physiology, biochemical processes and plant growth, thereby affecting the phytoconstituents (Al Mayyahi 2018).

Free radicals are important in the body tissues as they help in biological processes like gene expression and so on. But when they are in excess; these free radicals cause oxidative stress leading to cardiovascular diseases, cancer, blood sugar, arthritis and inflammatory diseases (Guchu *et al.*, 2020; Moriasi *et al.*, 2021). The human body is provided with antioxidant to combat these free radicals but when in excess, the body's defense mechanism is normally not enough; therefore, external antioxidant is required to argument (Shekhar and Anju, 2014; Guchu *et al.*, 2020).

Medicinal plants provide both anti-inflammatory and antioxidant sources. This has made herbal plants (traditional medicine) gain more popularity since there is little or no side effect; it is readily available, natural and cheaper than conventional medicine (Nakalembe *et al.*, 2019). Consequently, the objective of this study is to assess the antioxidant capabilities of methanol extracts from the leaves of Hyptis suaveolens (L.), both from polluted and unpolluted environments.

MATERIALS AND METHODS

Sample collection and Preparation

Leaves of Hyptis suaveolens, both from polluted and unpolluted environments, were gathered for this study. Leaves exposed to pollution were obtained from an oil-contaminated site at the Kaduna Refining and Petrochemical Company. In contrast, unpolluted leaves (used as control) were gathered from Murtala Mohammed Square, located in Kaduna city, within Kaduna State. The goal was to compare the extracts derived from these two contrasting environments. The collected leaves were then air-dried at ambient temperature and pulverized into powder.

Preparation of Crude Extract

In order to create the plant extract, 1.8 kg of the pulverized leaf sample was soaked in 5 liters of methanol (at a concentration of 60% v/v) for a duration of 96 hours, periodically agitating the flask. Subsequently, the mixture was strained through a funnel lined with Whatman No. 1 filter paper. The liquid obtained, or filtrate, was then condensed in-vacuo using a rotary evaporator at a temperature of 40°C, and later reduced to a dry state using a water bath. The resulting extract was kept at 4°C until further use (Cacique *et al.*, 2020).

Antioxidant Assay

The antioxidant potential of the methanolic extracts from the leaves of Hyptis suaveolens was evaluated through the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, as per the methods delineated by Runde *et al.* (2015) and Yahaya *et al.* (2018). A 0.1 mM solution of DPPH in methanol was prepared, to which 2.4 ml was combined with 1.6 ml of the leaf extract in methanol at varying concentrations (10-50 µg/ml). This resultant blend was thoroughly vortexed and allowed to stand in a dark, room temperature environment (25°C) for 30 minutes. Absorbance readings of the various mixtures were then acquired using a spectrophotometer set to a wavelength of 517 nm. Butylated hydroxytoluene (BHT) served as the reference standard. The percentage of DPPH radical scavenging activity was then calculated using the following formula:

$$\% \text{ RSA} = \{(Abs0 - Abs1 / Abs0) \text{ X } 100\}$$

Where Abs_0 = absorbance of blank; Abs_1= absorbance of test sample.

The concentration of the plant extract or BHT that resulted in 50% inhibition of DPPH activity (EC50) was identified through the plotting of inhibitory response against the logarithmic concentration. This experiment was executed in triplicate (Runde *et al.*, 2015).

For the statistical analysis:

- The method of analysis of variance (ANOVA) was employed, utilizing the PROC MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA).
- The gathered data was represented as mean plus or minus the standard error of the mean (mean ± SEM).
- A p-value less than 0.5 or 0.01 was interpreted as significant, whereas p-values greater than 0.05 or 0.01 were deemed as non-significant.

RESULTS

DPPH Radical Scavenging Activity

The antioxidant capabilities of the methanolic leaf extracts of Hyptis suaveolens demonstrated variability depending on the concentration of the extract. For each tested concentration, the unpolluted leaf extract of *Hyptis suaveolens* exhibited notably superior ($p < 0.05$) radical scavenging activity in comparison to the polluted extract (refer to Table 1). The IC50 values recorded for the methanol extracts from the polluted and unpolluted leaves of *Hyptis suaveolens* were 35 and 20 µg/ml, respectively (as depicted in Figure 1).

Table 1: Antioxidants activity of methanol extracts of polluted and unpolluted leaves of *Hyptis suaveolens*

Concentration (µg/ml)	DPPH Scavenging Activity (%)	
	Polluted leaf Methanol extract	Unpolluted leaf methanol extract
10	14.6±2.40	39.7±1.74
20	27.2±2.40	50.3±1.73
30	44.3±2.40	60.9±1.79
40	58.2±2.40	71.5±1.73
50	74.8±2.40	84.1±1.79

- Keyword : mean + Standard error of the mean (mean + SEM)
- DPPH : 2,2-Diphenyl-1- picrylhydrazyl-hydrate

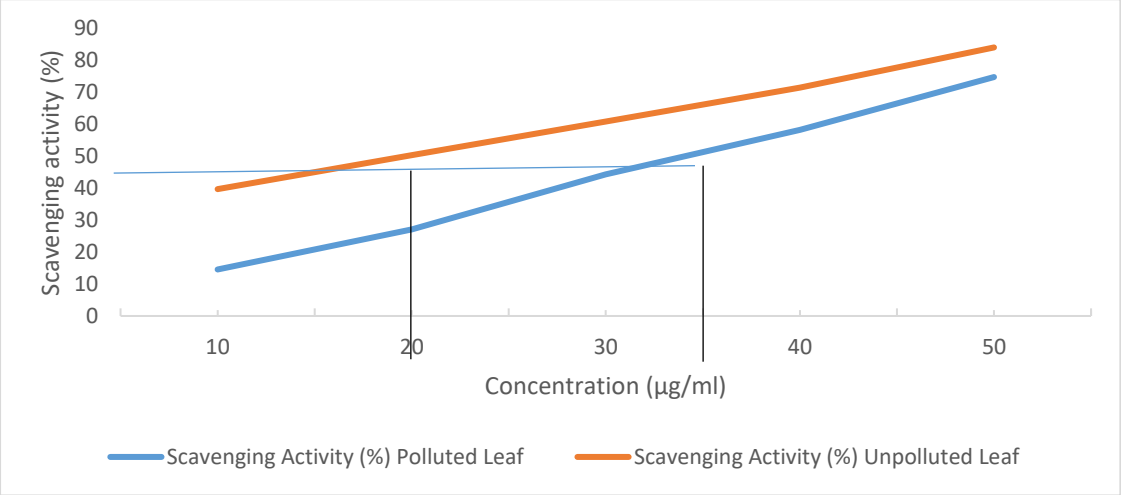

Figure 1: Antioxidant activity of polluted and unpolluted *Hyptis suaveolens* showing the IC_{50} for polluted leaf = 35 µg/ml; and IC_{50} for unpolluted leaf = 20 µg/ml

DISCUSSION

Inflammation and oxidative damage to biological molecules can instigate or worsen numerous human diseases, including but not limited to cancer, diabetes, neurodegenerative, and heart-related disorders (León *et al.*, 2018; Akimat *et al.*, 2021). Nonetheless, many societies, particularly those in developing economies, rely on plant-based treatments for managing inflammation and oxidative stress (Guchu *et al.*, 2020). Phenols and flavonoids in plant extracts are believed to mediate their antioxidant actions, by neutralizing lipid free radicals and curtailing the formation of reactive oxygen species from hydroperoxides (Moriasi *et al.*, 2021; Kozłowska *et al.*, 2022).

Current findings show that the methanolic leaf extracts of Hyptis suaveolens displayed notable ($p < 0.05$) radical scavenging activity, with median DPPH inhibitory activities (IC50) of 35 and 20 µg/ml for the polluted and unpolluted samples of H. suaveolus respectively. This aligns with the reports of Nakalembe *et al.* (2019), Guchu *et al.* (2020), and Rodríguez-Yoldi (2021), which highlighted significant in vitro antioxidant properties in solvent extracts of plant materials. Furthermore, our results revealed that the DPPH radical scavenging activities of H. suaveolens' methanolic leaf extracts exhibited a concentration-dependent pattern, with superior inhibitory activity noted at higher extract concentrations. This mirrors Guchu *et al.* (2020)'s findings concerning the concentration-dependent radical scavenging activity of the methanolic extracts of plants such as Caesalpinia volkensii Harms., Vernonia lasiopus (O. Hoffm), and Acacia hockii.

The differential DPPH radical scavenging activity observed between polluted and unpolluted samples of H. suaveolus (IC50 of 35 and 20 µg/ml, respectively) concurs with other scholarly assertions that environmental pollutants can adversely impact plant antioxidant properties (Ukom *et al.*, 2019). These pollutants reportedly increase the production of reactive oxygen species (Gjorgieva *et al.*, 2013) and cause modifications in the presence and quantities of bioactive constituents in affected plants (Radwan *et al.*, 2018).

CONCLUSION

In conclusion, the methanol extract derived from H. suaveolens demonstrated substantial DPPH radical scavenging potential. Nevertheless, the antioxidant capacity of the unpolluted leaf extract surpassed that of the polluted leaf methanol extract considerably, possibly resulting from the influence of oil pollution.

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