FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 8 No. 2, April, 2024, pp 113 - 117
DOI: https://doi.org/10.33003/fjs-2024-0802-2239

EFFECT OF WASTE ENGINE OIL ON PROXIMATE, PHYTOCHEMICAL, AND ANTIBACTERIAL CONSTITUENTS OF *Teffairia Occidentalis* (PUMPKIN LEAF)

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ABSTRACT

The improper disposal of waste engine oil effluent onto gutters, open lands, and cultivated farm lands is a common practice in Nigeria, especially among mechanics. These effluents contain substances derived from hydrocarbons that can diminish soil fertility and also influence the properties of plants. This study was consequently undertaken to examine the effects of waste engine oil on the nutritional, phytochemical, and antibacterial constituents of *Telfairia occidentalis* (Pumpkin leaf). Various levels (0ml, 100ml, 200ml, 300ml, and 400ml) of diesel oil contamination were mixed with 2 kilograms (kg) of soil, with each treatment replicated three times. Plants were watered daily for four months, and after 16 weeks, leaf samples were collected for laboratory analysis. The nutritional composition, phytochemical properties, and antibacterial components of the leaves were examined. The results indicated a significant reduction ($P<0.05$) in protein (12±0.02), fats (7±0.02), ash (15±0.25), fiber (10±0.03), and carbohydrate (10±0.25) content in the 400ml waste oil treatment compared to the control (0ml) values (protein: 38±0.01, crude fat: 25±0.13, total ash: 45±0.07, crude fiber: 36±0.15, carbohydrate: 42±0.04). Phytochemical analysis demonstrated a noteworthy decrease in alkaloids, saponins, flavonoids, and phenols as waste oil levels increased in the soil, compared to the control (0ml). The antibacterial activity screening revealed that among the various concentrations (80mg/ml, and 20mg/100 ml) of extracts studied, 80g/ 100 ml showed the highest degree of inhibition on all the test organisms. However, there was a notable reduction in inhibition as the level of waste oil pollution increased in the soil, compared to the control experiment (0ml). It is therefore recommended that proper disposal of waste oil should be encouraged as to prevent pollution of arable land meant for agriculture and already contaminated soil should undergo remediation before engaging in any agricultural activities.

Keywords: Spent engine Oil, Proximate compositions, Phytochemicals, Antibacterial constituents, *Telfairia occidentalis*

INTRODUCTION

The improper disposal of spent engine oil (SEO) is a prevalent practice in Nigeria, particularly among motor mechanics, who often discard it into gutters, water drains, open vacant plots, and farms. This oil, also known as spent lubricant or waste engine oil, is typically collected after servicing and draining from automobile and generator engines (Anoliefo and Vwioko, 2001). The used oil contains substantial amounts of hydrocarbons, including the highly toxic polycyclic aromatic hydrocarbons (Wang *et al*., 2011). Industrial activities, particularly automotive maintenance and repair, frequently contribute to the release of engine oil effluents into the environment **(**Nkwoada et al., 2018**).**

These effluents, containing various pollutants such as heavy metals and hydrocarbons, have the potential to contaminate soil and water resources (Ayotammo *et al*., 2006). The alteration of soil properties resulting from the presence of petroleum-derived substances can cause a significant decrease in organic carbon content, total nitrogen, and available phosphorus in the soil (Wyszokowska and Kucharski, 2000). Soil contamination can also restrict the photosynthetic process in plants, diminish fertility, disrupt metabolic activity, and adversely affect the proximate compositions and mineral elements of plants (Onyeri, 1998; Wyszokowiski *et al*., 2004). *Telfairia occidentalis,* commonly known as fluted pumpkin, is prevalent in the forest zones of West and Central Africa, notably in Benin, Nigeria, and Cameroon (Kayode and Kayode, 2011). Widely recognized in Nigeria, this vegetable is esteemed for its rich protein and amino acid content, making it a preferred dietary choice over blood tonic supplement tablets (Martinez *et al.,* 2008). Additionally, pumpkins are believed to house antioxidant vitamins such as carotenoids and tocopherols (Vitamin E) (Gayatri *et al.*, 2014). The tender leaves and young seeds are consumed as vegetables. (Oluba *et al*., 2008; Jacob *et al*., 2015).

Numerous investigations have highlighted the medicinal properties of *Telfairia occidentalis*. In Nigeria, the leaf juice is employed by women after childbirth, pregnant women, and individuals with anemia to boost blood strength and replenish lost blood (Dina *et al*., 2006; Kayode and Kayode, 2011). Moreover, the leaf extract alone is known for its efficacy in managing hypercholesterolemia, liver problems, and compromised immune systems (Eseyin *et al.,* 2005). Extracts from *Telfairia occidentalis* exhibit inhibitory effects on the growth of certain bacteria (Oboh *et al*., 2006), and they also demonstrate antiplasmodial potential (Okokon *et al*., 2007). Since indigenous vegetables like pumpkin (*Telfairia occidentalis)* are exposed to these commonly disposed waste engine oil contaminants, comprehending their impact on plant properties is crucial for ensuring food safety. Therefore, the aim of this study is to assess the impact of waste engine oil on the proximate, phytochemical, and antibacterial constituents of pumpkin leaves.

MATERIALS AND METHOS

Sample collection

The Pumpkin (*Telfairia occidentalis*) seeds were acquired from Premium Seeds Nigeria Ltd. in Benin City, Edo State. The uppermost layer of humus soil samples was collected from a farm situated within the Faculty of Agriculture, University of Benin. Collected within the depth range of 0-10cm, the soil was subsequently sieved, dried, and weighed. Waste engine oil was sourced from an automotive mechanic workshop opposite S and T barracks in Benin City. Bacterial strains were obtained from the Medical Microbiology Laboratory at the University of Benin Teaching Hospital, Benin City, Nigeria

Experimental setup
Five (5) plastic buckets with perforations at the base were utilized, each filled with 2kg of soil sample blended with varying levels (0, 100ml, 200ml, 300ml, and 400ml) of waste engine oil. Four bowls represented soil contaminated with waste engine oil, while one bowl served as a control (0ml). The mixture was left undisturbed for 12 days to ensure a thorough blending of diesel oil and soil, maintaining a consistent mixture. *Telfairia occidentalis* seeds, soaked in water for three hours to ensure viability, were then planted at a depth of 2-3cm, with five seeds per pot in the waste engine oil-contaminated soil. The pots were watered daily with distilled water to sustain a soil moisture level of 200 ml for four weeks, after which the plants were harvested for further analysis.

Plant preparation
The harvested plant sample was cut into smaller piece and air dried under shade for weeks at ambient temperature. The air-dried plant was latter oven dried at 45 °C to disintegrate the lipid wax and then prepared for grinding. The dried plant samples were then pulverized using a Thomas Willey Milling Machine in the Department of Pharmacognosy, University of Benin. The powdered sample was weighed and stored in an air tight container separately for further use.

Extraction of sample in aqueous solvent
One hundred grams (100 g) of the pulverized sample was weighed separately into an Extracting Bottle and was extracted in 500 ml of hot distilled water for twenty-four hours with occasional shaking at intervals. At the end of the experiment, it was filtered with sieve cloth to collect the filtrate and concentrated with water bath at 40 °C.

Proximate analysis
The proximate analysis of the leaves of *Telfairia occidentalis* for crude fibre, crude protein and fat contents were determined using the methods described by Pearson (1976).
Total ash content was determined by Furnace incineration using the method of James (1995).
Moisture and carbohydrate contents were determined using the method described by AOAC (20-00)

Phytochemical properties of *Telfairia occidentalis* treated plant
Alkaloids
A standard alkaloid compound (e.g., caffeine) was used to create a calibration curve. Add a few drops of ferric chloride to the extract. spectrophotometric analysis was done at 650nm wavelength.

Flavonoids
A standard solution of a flavonoid (e.g., quercetin) was prepared. Add concentrated hydrochloric acid to the extract, followed by a few drops of concentrated sulfuric acid. Spectrophotometer was used to measure the absorbance of the standard and sample at 650nm wavelength.

Saponins
The calibration curve was established using a standard saponin solution. The absorbance of the sample was measured at 550nm wavelength.

Phenols
A calibration curve was created using a standard phenolic compound (e.g., gallic acid). Few drops of ferric chloride were added to the extract and the absorbance was measured at 650nm.

Determination of antimicrobial activity of aqueous extract of *Telfairia occidentalis* treated plant on bacterial isolates
The agar diffusion method was employed. Two level concentrations (20 and 80 mg/ml) of the plant extract were prepared for this test. A loopful of each bacterial strain (*Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Streptococcus* sp) was collected into 15 ml of distilled water in a clinical bottle to activate the organisms. The activated organisms were streaked on the surface of nutrient agar using a sterile cotton swab stick. Then, 10mm diameter wells were aseptically made in the plates using sterile cork-borer. Into the wells were introduced separately each concentration of the plant extract. The plates were then incubated at 37^{C} for 24 hours. Antibacterial activity was measured as the diameter of inhibition zones.

Statistical Analysis
Analysis of variance (ANOVA) and Dunnet's method were employed for data evaluation; $p<0.05$ was taken as statistically significant. The software package, Graph pad prism 5 was used for data analysis.

RESULTS AND DISCUSSION
The impact of diesel oil pollution on the carbohydrate and moisture content of *Telfairia occidentalis* leaves is detailed in Table 1. The findings demonstrate a gradual decrease in both carbohydrate and moisture content of *Telfairia occidentalis* leaves with an increasing level of diesel oil pollution. The control treatment (0ml) exhibited the highest values for both carbohydrate and moisture content in *Telfairia occidentalis* leaves.
For crude protein, fat, and ash content, the leaves of pumpkins grown in 0ml of the spent oil showed the highest values, recording 38.0, 25.0, and 45.0, respectively. These values displayed a significant difference ($p<0.05$) when compared to the other treatments. Pumpkin plants cultivated in soils polluted with 400ml of spent oil exhibited the lowest protein (12.0), fat (7.0), and ash (15.0) content in their leaves (Table 1).

Table 1: The proximate composition of *Telfairia occidentalis* (Pumpkin leaf) treated plant

Parameters	Treatment (spent engine oil)				
	100ml	200ml	300ml	400ml	0ml(Control)
Moisture %	25	19	15	10	36
Ash%	37	31	22	15	45
Protein (mg/kg)	23	20	17	12	38
Carbohydrate (mg/kg)	25	20	16	10	42
Lipid %	11	10	9	7	25

The effect of diesel oil pollution on phytochemical properties in the leaves of *Telfairia occidentalis* is presented in table 2. The result showed that there were significant differences among the various treatments of diesel oil pollution in the soil. The alkaloids content ranged from 1.6-4.5mg/kg, saponins (1.0-4.0 mg/kg), flavonoids (2.1-6.1 mg/kg) and phenols (1.4-8.3 mg/kg). however, the highest values of alkaloids (7.3), saponins (7.5), flavonoids (8.3) and phenols (10.0) content were seen in plant samples grown in the control experiment (0ml).

Table 2: Phytochemical properties of *Telfairia occidentalis* (Pumpkin leaf) treated plant

Parameters	Treatment (spent engine oil)				
	100ml	**200ml**	**300ml**	**400ml**	**0ml(Control)**
Alkaloids (mg/g)	4.5	3.2	2.3	1.6	7.3
Saponin (mg/g)	4.0	3.5	2.9	1.0	7.5
Flavonoids (mg/g)	6.1	4.6	2.8	2.1	8.3
Phenols (mg/g)	8.3	5.6	3.7	1.4	10.0

Table 3 shows the antimicrobial activities of aqueous extract of *Telfairia occidentalis* (Pumpkin leaf) treated plant on bacterial isolates at 80g/ 100 ml concentration. At a concentration of 80g/100 ml the inhibitory effect of the tested plants against *E. coil* ranged from 12.30±2.51-20.40±3.43, *S. aureus* (12.05±2.76-26.40±3.28), *Streptococcus* sp. (12.00±2.29-22.90±2.33), *P. aeruginosa* (12.02±2.46 - 24.60±2.40), *P. mirabilis* (12.07±2.53- 18.90±1.72) and *K. pneumonia* (12.00±2.39 -20.90±0.89). The highest antibacterial activity was seen in the control (0ml) experiment.

Table 3: Antimicrobial activities of aqueous extract of *Telfairia occidentalis* treated plant on bacterial isolates at 80g/ 100 ml concentration

Isolates	Treatment (spent engine oil)				
	100ml	**200ml**	**300ml**	**400ml**	**0ml(Control)**
E. coli	16.33± 2.38	14.30 ±2.51	11.37± 2.62	9.40±2.41	23.40 ±3.43
S. aureus	20.10± 2.63	15.07± 2.44	11.05 ± 2.76	10.30± 2.48	29.40± 3.28
Streptococcus sp	17.15 ±2.47	15.00± 2.29	9.00 ±2.60	5.00± 2.52	25.90 ±2.33
P. aeruginosa	20.02 ±2.46	17.07 ±2.72	14.20 ±2.81	10.33 ±2.29	27.60± 2.40
P. mirabilis	13.07 ±2.53	11.20± 2.42	8.20± 2.70	7.33 ±2.31	21.90 ± 1.72
K. pneumonia	14.05± 2.57	10.00 ±2.48	8.00± 2.39	5.22± 2.61	23.90± 0.89

Table 4: shows the antimicrobial activities of aqueous extract of *Telfairia occidentalis* treated plant on bacterial isolates at 20 g/ 100 ml concentration. At a concentration of 20 g/100 ml the inhibitory effect of the tested plants against *E. coil* ranged from 8.00±0.38 -10.40±1.52, *S. aureus* (8.00±0.39-12.20±1.16), *Streptococcus* sp. (8.00±0.36 14.50±1.03), *P. aeruginosa* (8.00±0.44-11.40±1.34), *P. mirabilis* (8.00±0.26-10.30±1.58) and *K. pneumonia* (8.00±0.1 - 12.10±1.02). However, there was a gradual decrease in the antibacterial activities as the concentration of the spent engine oil increase. Consequently, the highest antibacterial activity was recorded in the control experiment (0ml).

Table 4: Antimicrobial activities of aqueous extract of *Telfairia occidentalis* treated plant on bacterial isolates at 20 g/ 100 ml concentration

Isolates	Treatment (spent engine oil)				
	100ml	**200ml**	**300ml**	**400ml**	**0ml(Control)**
E. coli	8.00±0.52	7.00±0.38	5.00±0.83	3.00±0.62	12.40±1.52
S. aureus	11.10±0.48	9 00±0.39	8 00±0.67	6.05±0.49	16.20±1.16
Streptococcus sp	8.00±0.72	7.00±0.41	5.00±0.58	3.00±0.36	15.50±1.03
P. aeruginosa	12.00±0.44	8.10±0.71	7.00±0.82	5.00±0.50	16.40±1.34
P. mirabilis	7.00±0.60	6.00±0.83	4.00±0.26	2.00±0.38	12.30±1.58
K. pneumonia	10.00±0.28	8.00±0.31	7.00±0.11	6.10±0.43	14.10±1.02

Discussion

From the findings, the decrease in moisture content in the leaf samples of *Telfairia occidentalis*, resulting from spent oil application compared to the (0ml) control experiment, may be attributed to water stress caused by the existence of waste engine oil in the soil. This could hinder water transport along the xylem tissue, resulting in physiological drought. This finding is consistent with the report by Adenipekun and Kassim (2006), indicating that engine oil has an impact on the moisture content in *Celosia argentea*.

The observed decrease in carbohydrate content as the waste oil concentration levels increased could suggest a physiological response to stress induced by the impact of the waste engine oil pollution on the photosynthetic processes of the plant. This is similar to the findings of Ekpo et al. (2014), who noted a reduced carbohydrate content in the leaves of *Ocimum gratissimum* in diesel oil-polluted soil. However, this contradicts the findings of Ogbuehi et al. (2010), which indicated an increase in carbohydrate content in *Manihot esculentus* due to crude oil pollution.

The decrease in proximate composition, as indicated in Table 1, may result from the diminished essential macro and micro elements in the soil necessary for synthesizing crucial nutrients in the plant's leaf samples. Additionally, it could be linked to the impact of spent diesel oil on the photosynthetic process and, consequently, the production of photosynthetic products. A comparable reduction in proximate composition has been documented by Agbogidi et al. (2007), who observed a decline in protein, moisture, and fat contents in *Zea mays*, possibly attributed to the impairment of photosynthetic activities through cell injury, disruption in cellular membrane activities, or other stress-inducing properties of crude oil, leading to anatomical aberrations in plants.

Phytochemicals, as identified by various researchers, play significant roles in plant extracts therapeutic properties and potential health benefits (Khanna and Tosh, 2014). Jensen *et al*. (2014) reported that bioactive substances, such as carotenoids, flavonoids, terpenoids, saponins, phytosterols, and phenols, are natural compounds in plants with various biological functions in humans. The *Telferaira occidentalis* plants in the 0ml spent oil exhibited higher levels of alkaloids, saponins, flavonoids, and phenols in their leaves. Conversely, these constituents decreased progressively with an increasing concentration of the waste engine oil. This decrease could be ascribed to the development of a hydrophobic layer covering the roots impacted by the waste engine oil, restricting the absorption of the water and nutrients essential for the production of alkaloids, saponins, flavonoids, and phenols in the leaf samples. The elevated levels of these compounds in the control (0ml) experiment align with Roozbeh et al. (2013) findings on phytochemical compounds in plant extracts used in Benin City.

The Antibacterial efficacy was observed to be greater in *Telferaira occidentalis* leaves samples from plant treated with 0ml of the waste engine oil (control) compared with *Telferaira occidentalis* leaves samples from plant treated with the waste engine oil. However, all the different plant extract concentrations exhibited antibacterial potency against the tested bacteria. The exact basis for the antibacterial activity of the extracts cannot be definitively stated; nevertheless, it could result from secondary metabolites such as saponins, tannins, flavonoids, and alkaloids present in the plant. These secondary metabolites have been reported to possess antimicrobial and therapeutic activities (Urbano *et al*., 2000). This aligns with the findings of Talalay and Talalay (2011), who observed that antimicrobial components of plant extracts, including terpenoids, alkaloids, and phenolic compounds, interact with enzymes and proteins of the microbial cell membrane, causing its disruption and inducing cell death or inhibiting enzymes necessary for amino acids biosynthesis. Additionally, Sampath and Vasanthi (2012) reported that the hydrophobic character of plant extracts has an inhibitory effect, interacting with proteins of microbial cell membranes and mitochondria, disrupting their structures and altering their permeability

CONCLUSION

This study has demonstrated that soil contaminated with spent oil significantly affects the proximate, phytochemical, and antibacterial constituents of *Telfairia occidentalis* plants. It is recommended that environmental agencies conduct public sensitization on the detrimental effects of improperly discarding spent engine oil on arable land designated for agriculture. Additionally, lands polluted with spent engine oil should undergo remediation before any agricultural activities are undertaken on those lands

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