ISSN Print: 2664-6536
ISSN Online: 2664-6544
Impact Factor: RJIF **5.4**
IJBB 2024; 6(2): 15-18
www.biosciencejournal.net
Received: 16-05-2024
Accepted: 23-06-2024

Osime EC
Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

Onoagbe IO
Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

Akpa CP
Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

Corresponding Author:
Osime EC
Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

Evaluation of proximate and phytochemical analysis on *Mangifera indica* Linn (Mango) stem bark obtained from crude oil-polluted and crude oil free environments

Osime EC, Onoagbe IO and Akpa CP

DOI: https://dx.doi.org/10.33545/26646536.2024.v6.i2a.75

Abstract
Concerns about the impact of crude oil spills on food safety are increasingly driven by the associated health risks. In Nigeria, rural areas that serve as key sites for crude oil exploration are also the main producers of food and cash crops. This study investigated the proximate and phytochemical composition of *Mangifera indica* stem bark. Plant samples were collected from a crude oil-polluted environment, labeled (C), in Umuechem, Ikwere Local Government Area, which is designated as an oil-contaminated area. The results were compared to those of identical *Mangifera indica* stem bark harvested from farmland along Oba Market Road in Oredo Local Government Area, Edo State, a designated unpolluted environment, labeled (F). Quantitative analysis revealed that the crude ash, protein, fiber, dry matter, moisture, and carbohydrate contents of samples from the polluted environment (C) differed significantly ($p< 0.05$) from those obtained in the unpolluted environment (F). However, the crude fat content showed no significant difference ($p> 0.05$) between the two environments. Additionally, the levels of phytochemicals, such as terpenoids and flavonoids, were significantly higher ($p> 0.05$) in samples from the unpolluted area compared to those from the crude oil-polluted area. This suggests that pollutants from crude oil spills can adversely affect the phytochemical components of *Mangifera indica* stem bark, reducing its potential therapeutic value.

Keywords: *Mangifera indica*, tannins, flavonoids and wistar albino rats

Introduction
Petroleum crude oil is a complex mixture of hydrocarbons, both aliphatic and aromatic, and exists as a liquid in its natural state (Atlas and Bartha, 1973) [13]. It is inherently toxic to various forms of biomass. Accidental and intentional crude oil spills have long been, and continue to be, a major source of environmental pollution, posing serious threats due to the potential contamination of air, water, and soil (Trindade *et al*., 2005) [14]. The appearance and composition of crude oil can vary significantly between different types (Craig, 2003) [15]. Since the discovery of oil in Nigeria's Niger Delta region, agricultural land has suffered extensively from oil spills, pipeline vandalism, and the transportation of crude oil and its derivatives. An unavoidable consequence of these transport activities is the frequent accidental spillage of oil into both land and water (Craig, 2003) [15]. Crude oil pollution has been reported to negatively affect plant germination and seedling growth primarily by creating conditions that make essential nutrients, such as nitrogen and oxygen, unavailable for plant growth (Ogbo *et al*., 2009) [1]. Studies have shown that crude oil contamination can reduce the protein, dry matter, and crude fiber content in crops like cassava (Ogbuehi *et al*., 2010) [2]. Additionally, crude oil on farmlands has been found to decrease growth parameters and crop yield in plants such as *Arachis hypogaea* (Peanuts) and *Zea mays* (maize). Given that humans have historically relied on natural products from plants to treat diseases, especially from their immediate environments (Cragg and Newman, 2001) [8], it is crucial to further investigate how crude oil pollution affects the bioactive constituents and proximate composition of plants. This study, therefore, aims to explore the impact of crude oil contamination on the bioactive compounds and proximate composition of plant stem bark, using *Mangifera indica* as a case study.

Materials and Methods

Chemicals: All the chemicals and reagents used were of analytical grade obtained from m/s Merck India, Ltd. Bombay. Distilled water and acid washed glassware were used throughout the analysis.

Collection of plant: The stem bark of *Mangifera indica* (C) was collected from an oil-polluted farmland in a community in Umuechem, located in the Ikwere Local Government Area of Rivers State. In contrast, crude oil-free *Mangifera indica* stem bark (F) was sourced from a farmland at No. 24 Oba Market Road, Oredo Local Government Area, Edo State, Nigeria, which is an uncontaminated environment. Both samples were sealed, labeled, and sent to the Department of Plant Biology and Biotechnology for identification. There, Dr. Akinnibosun Henry provided a voucher number: UBHM 0249.

Preparation of crude drug powder

The preparation of crude drug powder from both the crude oil-free and contaminated *Mangifera indica* stem barks involved cutting the barks into smaller pieces, thoroughly cleaning and washing them with tap water. The pieces were then air-dried indoors in a well-ventilated area for three weeks. After drying, they were ground using a milling machine in the Department of Pharmacy at the University of Benin. The resulting powdered materials were subsequently used for phytochemical and proximate analyses.

Preparation of Aqueous and Ethanolic Extract

Preparation of Aqueous Extract: A 100 g portion of the powdered herb was extracted by soaking it in 1000 ml of distilled water, using the water as the solvent. The mixture was stirred and left at room temperature for 72 hours. Afterward, the solution was filtered through sintered glass to remove cellulose fibers, with the filtrate being collected. This filtrate was then concentrated using a rotary evaporator and subsequently freeze-dried with a freeze dryer. The resulting extract was stored in clean plastic bottles and refrigerated at 4 °C.

Phytochemical and proximate analyses

1. **Proximate analysis:** The proximate analysis of the powder samples was performed in triplicate using the standard method described by the Association of Official Analytical Chemists (A.O.A.C, 1990) [4] at the Department of Biochemistry, University of Benin. This analysis provides information about the class of food present in the sample, such as protein, fat, fiber, ash content, and dry matter.
2. **Phytochemical analysis**
   The phytochemicals in the powdered samples were determined using the standard phytochemical methods of testing. The dry powdered samples were boiled for 20 minutes, filtered, and the filtrate used to test for Tannins, Flavonoids, Saponins, Alkaloids, Steroids, Terpenoids, Glycoside, Anthraquinones, and Carbohydrates.

- **Test for Tannins:** The determination of tannins was done using the method of Evans *et al*. (2002) [9].
- **Test for Flavonoids:** The presence of flavonoids in the sample can be determined using the method of Harborne (1998) [5].
- **Test for Saponins:** The presence of saponins in the sample can be determined by the frothing test using the method of Evans *et al*. (2002) [9].
- **Test for Alkaloids:** The presence of alkaloids in the sample can be determined using the method of Harborne (1998) [5].
- **Test for Steroids:** The presence of steroids in the sample can be determined using the method of Sofowora (1993) [3].
- **Test for Terpenoids:** The presence of terpenoids in the sample can be determined using the method of Sofowora (1993) [3].
- **Test for Glycosides:** The Glycoside test was carried out using the method of Sofowora (1993) [3].
- **Test for Anthraquinones:** A phytochemical test for anthraquinones was carried out using the method of Harborne (1998) [5].
- **Test for Carbohydrate:** The test for carbohydrates was carried out using the method of Evans *et al*., 2002 [9].

3. **Statistical Analysis**
   All data obtained were subjected to statistical analysis using Student's t-test using Statistical Package for Social Sciences (SPSS for windows, version 12.0). Data were expressed as mean ± standard error of mean (SEM). Values of $p < 0.05$ were considered significant.

Results

Qualitative phytochemical screening as shown in table 1.0 revealed the presence of vital bioactive compounds such as tannins, flavonoids, saponins, alkaloids, steroids, terpenoids, glycosides, and anthraquinones. Among these compounds, the crude oil free plant material exhibited relatively higher abundance of flavonoids, steroids, terpenoids, and anthraquinones compared to the crude oil-contaminated plant ($p < 0.05$). Quantitative proximate analysis as shown in table 2.0 also showed a significant increase in the levels of crude protein, total ash, crude fiber, dry matter, and carbohydrates in plants obtained from crude oil-contaminated environments, in comparison to plants obtained from crude oil free environments at $p<0.05$. Conversely, the moisture content and crude fat levels in plants from crude oil-contaminated environments were significantly lower than those in plants from crude oil free environments at $p<0.05$.

Table 1: Phytochemical composition of *Mangifera indica* Stem bark

Phytochemical	*Mangifera indica* (F)	*Mangifera indica* (C)
Tannins	++	++
Flavonoids	+	++
Saponins	+++	+++
Alkaloids	+	+
Steroids	+	++
Terpenoids	+	++
Glycosides	+	+
Anthraquinones	+	++
Carbohydrates	+	+

Key: +=Present ++= Much present +++= Abundant.

Table 2: Proximate Composition of *Mangifera indica* stem bark

Proximate constituents	*Mangifera indica* (F)	*Mangifera indica* (C)
Crude proteins	2.63±0.00115[a]	9.23±0.00058[b]
Total ash	0.83±0.00115 [a]	2.10±0.00200 [b]
Crude fat	4.33±0.00577 [a]	4.00±0.03464 [b]
Crude fibre	24.77±0.00058 [a]	35.53±0.00115 [b]
Dry matter content	40.32±0.01414 [a]	67.52±0.00707 [b]
Moisture content	59.70±0.03055 [a]	32.47±0.01155 [b]
Carbohydrates	11.80±0.01528 [a]	24.22±0.01528 [b]

Key: Means with distinct letters within row indicate a significant difference ($p \leq 0.05$).
F= *Mangifera indica* obtained from an uncontaminated environment
C= *Mangifera indica* obtained from a crude oil-contaminated environment

Discussion

Some phytochemicals, such as flavonoids and polyphenols, play a crucial role in alleviating oxidative stress, serving as secondary metabolites that plants use for defense and protection. In Table 1.0, the observed reduction in certain phytochemicals, such as flavonoids, in *Mangifera indica* samples from the crude oil-polluted environment (C), compared to those from the unpolluted environment (F), may be due to a weakened ability of the plant's antioxidant defense systems to neutralize harmful molecules like hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), hydroxyl radicals (OH), and lipid peroxides absorbed during crude oil contamination. This reduction likely results from the plant's antioxidant enzymes being overwhelmed by the excessive production of free radicals. This finding aligns with the work of Okolie *et al*. (2018) [11], who reported that crude oil contamination can lead to significant decreases in the concentrations of phenolics, flavonoids, alkaloids, and terpenoids.

The significant reduction ($p < 0.05$) in moisture and crude fat content in sample C compared to sample F, as shown in Table 2.0, may be caused by water stress induced by the presence of crude oil in the soil, impairing water conduction through the xylem tissue and causing physiological drought. Additionally, lipid catabolism likely coincides with sugar accumulation, possibly due to the conversion of lipid molecules to carbohydrates during germination and growth. The decreased fat content in sample C relative to F may also be a result of the damaging effects of reactive oxygen species, leading to cellular lipid depletion and the peroxidation of polyunsaturated fatty acids. These findings are consistent with the results of Diyaolu *et al*. (2021) [7]. The significant increase ($p < 0.05$) in carbohydrate and fiber content in sample C compared to F could be attributed to the plant's conversion of degraded fat molecules into carbohydrates, which is a physiological response to stress. This rise in carbohydrate content may also be due to alterations in the amyloplast membrane and the redistribution or translocation of assimilates within the plant to support growth under stress conditions like crude oil pollution. This finding is also consistent with the results of Diyaolu *et al*. (2021) [7]. Furthermore, the plant's stress response may impair the photosynthetic apparatus due to crude oil exposure. The significant increase ($p < 0.05$) in dry matter and total ash content in sample C compared to F suggests higher levels of inorganic residues and detoxification of nitrogen and sulfur oxides, possibly through protein synthesis. These results are in line with the findings of Idowu *et al*. (2017) [10], who reported significant decreases in protein and lipid content in crude oil-contaminated plants compared to control samples.

Conclusion

The findings of this study, in conjunction with other research, suggest that hydrocarbons found in crude oil can have a substantial impact on the health and growth of living organisms, like plants.

Acknowledgments

I would like to express my sincere gratitude to Prof. I.O. Onoagbe for his invaluable guidance and support throughout the completion of this research. I am also deeply appreciative of my incredibly supportive family, the entire team of laboratory technicians, and my colleagues, including Dr. Abu Osahon from the Department of Biochemistry, for their unwavering assistance. Additionally, we have no conflicts of interest to declare.

Reference

1. Ogbo EM. Effect of diesel fuel contamination on seed germination of four crop plants-Arachis hypogea, *Vigna unguiculata*, *Sorghum bicolor* and *Zea mays*. Afr. J Biotechnol. 2009;8(2):250-253.
2. Ogbuehi HC, Ezeibekwe IO, Agbakwuru U. Assessment of crude oil pollution on the proximate composition and macro elements of cassava crop in Owerri, Imo State. Int Sci Res. 2010;2:62-65.
3. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. 2nd ed. Ibadan, Nigeria: Spectrum Books Ltd; c1993. p. 134-156.
4. AOAC. Official Method of Analysis. 15th ed. Washington DC: AOAC International; c1990.
5. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. London: Chapman & Hall; c1998. p. 67.
6. Atlas RM, Bertha R. Biodegradation of petroleum in sea water at low temperatures. Can J Microbiol. 1972;18:1851-1855.
7. Diyaolu OA, Attah AF, Oluwabusola ET, Moody JO, Jaspars M, Ebel R. Heavy metals, proximate analysis, and brine shrimp lethality of *Vernonia amygdalina* and *Ocimum gratissimum* growing in crude oil-rich Delta State, Nigeria. Foods. 2021;10:2913.
8. Cragg GM, Newman DJ. Natural products drug discovery in the next millennium. Pharm Biol. 2001;39:8-17.
9. Evans WC. Trease and Evans Pharmacognosy. 15th ed. India: Elsevier; c2002. p. 27, 46, 183-4, 289-91, 411-3, 434, 485-6.
10. Idowu MA, Adeola A, Olaniyan DJ, Oke KE, Omoniyi SA. Quality evaluation of cocoyam-cowpea flour blends and sensory attributes of their cooked paste (Amala). Ann Food Sci Technol. 2017, 18(2).
11. Okolie NP, Uwakwe AA, Adimonyemma CI, Orisakwe OE. The effect of crude oil on the immune function of Wistar rats. Environ Sci. Pollut. Res. Int. 2018;25(9):8922-8929.
12. Trinder P. Determination of serum cholesterol by enzymatic colorimetric method. Ann Clin Biochem. 1969;6:24-27.

13. Atlas RM, Bartha R. Stimulated biodegradation of oil slicks using oleophilic fertilizers. Environmental Science & Technology. 1973 Jun 1;7(6):538-541.
14. Trindade PV, Sobral LG, Rizzo AC, Leite SG, Soriano AU. Bioremediation of a weathered and a recently oil-contaminated soils from Brazil: A comparison study. Chemosphere. 2005 Jan 1;58(4):515-522.
15. Craig AD. Interoception: The sense of the physiological condition of the body. Current opinion in neurobiology. 2003 Aug;13(4):500-505.