Evaluation of Potential Biodegradation of Used Engine Oil by Extracts of some Trees belong to Family Meliacea*e*

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ABSTRACT

Petroleum hydrocarbons pose a major environmental concern due to their widespread distribution and harmful effects on human health. Biodegradation is considered an effective remediation strategy for breaking down hydrocarbons from spilled petroleum. In this study, the potential of indigenous mahogany leaf extracts to degrade petroleum *in vitro* was investigated. Crude engine oil was isolated and analyzed by GC-MS without any prior treatment. The oil was then incubated for 7 days with leaf extracts from three mahogany species: *Swietenia macrophylla*, *Swietenia mahagoni*, and *Khaya senegalensis*. At the end of the incubation period, various assays were performed, including oil displacement, drop collapse, rhamnose content analysis, and tests for foaming and emulsifying properties. The present study also aimed to screen the genetic diversity of three *Swietenia mahagoni* genotypes using simple sequence repeat (ISSR) markers. The genotypes were obtained from the Timber Tree Department Orchard, Horticultural Research Institute, Giza Governorate. Molecular characterization revealed that *Swietenia mahagoni* was the most effective extract for crude oil degradation. Inter-simple sequence repeat (ISSR) analysis was conducted to further assess genetic variation. Three ISSR primers produced distinct banding patterns. Out of 62 amplified ISSR fragments, 27 were polymorphic, and 28 were identified as cultivar-specific markers. Overall, *Swietenia mahagoni* extracts-both from leaves and roots-demonstrated the highest efficiency in degrading crude oil.

Keywords: *Swietenia mahogany*-Hydrocarbons- GC-MS- ISSR assay.

INTRODUCTION

Petroleum is a complex, heterogeneous mixture of hydrocarbons including aliphatic (n-alkanes), alicyclic, and aromatic compounds such as polycyclic aromatic hydrocarbons (PAHs) whose composition and physical properties vary depending on the origin of the reservoir (Hamme et al., 2003). These hydrocarbons are organic compounds composed primarily of carbon and hydrogen and are notably insoluble in water. Microorganisms can either degrade or produce hydrocarbons, depending on the presence of specific metabolic pathways and environmental conditions (Ehrlich et al., 1995). Anthropogenic activities, such as industrial processes, oil spills, and the incomplete combustion of fossil fuels, have led to the accumulation of petroleum hydrocarbons in various environments (Santos et al., 2011). Petroleum and its derivatives such as gasoline, diesel, and kerosene have a significant ecological impact, particularly in contaminated marine and terrestrial ecosystems (Santos et al., 2011). Throughout this paper, the term "petroleum" will encompass both crude oil and its derivatives. Several processes influence the environmental fate of hydrocarbons, including sorption, volatilization, abiotic transformation (chemical or photochemical), and biotransformation (Korda et al., 1997). Biodegradation of hydrocarbons whether aliphatic or aromatic can occur under both aerobic and anaerobic conditions (Hamme et al., 2003). Under aerobic conditions, oxygenase enzymes incorporate oxygen atoms into hydrocarbon molecules: monooxygenases introduce one oxygen atom, while dioxygenases introduce two. Aerobic degradation tends to be more efficient due to the availability of oxygen as

a terminal electron acceptor (Cao et al., 2009). The complete oxidation of saturated aliphatic hydrocarbons leads to the production of acetyl-CoA, which enters the citric acid cycle, generating electrons for the electron transport chain. This process continues until hydrocarbons are fully mineralized to CO_2 (Madigan et al., 2010). Aromatic compounds such as benzene, toluene, xylene, and naphthalene can also be degraded aerobically. Typically, the degradation of these compounds produces catechol or structurally similar intermediates, which are subsequently funneled into the citric acid cycle and fully oxidized to CO_2 (Madigan et al., 2010 and Cao et al., 2009). *Swietenia macrophylla*, commonly known as mahogany, is a large tropical tree of ethnomedicinal importance in countries such as India, Malaysia, China, and throughout Central and South America (Moghadamtousi et al., 2013). Traditional medicine utilizes various parts of this plant for their antimicrobial, antioxidant, and antidiabetic properties. Phytochemical analyses have identified nine phenolic acids and eighteen flavonoids in aqueous leaf extracts of *S. macrophylla*, with demonstrated antioxidant and anti-inflammatory activities in vitro (Pamplona et al., 2015). Moreover, the concentration of polyphenols in these extracts is significantly higher than in other Amazonian plant extracts obtained through similar methods (Silva et al., 2007). Polyphenols are naturally occurring, non-enzymatic antioxidant compounds that, even at low concentrations, can mitigate excessive oxidative stress (Kujawska and Jodynis-Liebert, 2018, Ciulla et al., 2019 and Singh et al., 2020).

Phytodegradation, or phytotransformation, refers to the metabolic breakdown of contaminants by plants. Organic pollutants such as PAHs and total petroleum hydrocarbons (TPHs), as well as inorganic compounds like nitrogen and sulfur oxides, can be degraded either internally via plant metabolism or externally by root-secreted enzymes (Das et al., 2010; Kavamura & Esposito, 2010). This process effectively remediates various pollutants in soil and water, including petroleum hydrocarbons, pharmaceuticals, pesticides, insecticides, and surfactants (Grzegórska et al., 2020). In addition, plant-secreted enzymes can enhance microbial activity in the rhizosphere, further supporting phytoremediation and promoting plant growth (Greipsson, 2011, Al-Baldawi et al., 2015 and Chen et al., 2016).

The present study aimed to investigate the biodegradation potential of engine oil by enzymes-rich leaves extracts of *Swietenia macrophylla, S. mahogany and Khyaya senegalensis*.

MATERIALS AND METHODS

The experimental parts of the present study were carried out at Central Laboratory for Chemical Analysis, Hort. Res. Inst., A.R.C. during 2024.

Plant materials: Leaves were collected from three mahogany trees *Swietenia macrophylla* (1), *Swietenia mahogany* (2) *and Khaya senigalensis* (3) located in the orchard of Timber Tree Res. Dept., Hort. Res. Inst., A.R.C.

Molecular characterization of trees: DNA extraction: The procedures were carried out according to manufacture instruction of QIAGEN DNase Plant Mini Kit adapted from (**www.qiagen.com/HB-1166**)

Protocol: Purification of Total DNA from plant: The tissue was grind 0.5g of sample in liquid nitrogen using a mortar and pestle until a fine powder forms. 400µl buffer AP1 and 4µl RNase A were added to the samples then Vortex and incubate for 10 min at 65°c. 130µl Buffer P3 was added to each sample then mixed and incubated for 5 min on ice. The samples were centrifuged the lysate for 5 min at 14,000 rpm. The samples were transfer the lysate into a Ql Ashredder spin

column placed in a 2 ml collection tube then centrifuged for 2min at 14,000 rpm. The supernatant of the samples were transferred into a new tube and 250µl of buffer AW1 were added and mixed by pipetting. 650µl of the mixture into a DNase Mini spin column placed in a 2 ml collection tube were transferred and centrifuged for 1 min at 8,000 rpm then the supernatant were discard. The spin column into a new 2ml collection tube was placed for each sample and 500µl buffer AW2 were added and centrifuged for 1min at 8,000 rpm then the supernatant was discarding. Another 500 µl buffer AW2 were added and centrifuged for 2 min at 14,000 rpm. In new 2 ml micro centrifuge tube the spin column was transferred and 100 µl buffer AE for elution was added then the tubes were incubated for 5 min at room temperature (15-25 °c) then followed by last Centrifuge for 1 min at 8,000 rpm

Genetic differentiation by PCR using ISSR: The samples were taken up for PCR amplification to be genetically differentiated using universal primers of ISSR Zietkiewicz et al. (1994), Genomic DNA was amplified by mixing the template DNA (50 ng), with the polymerase reaction buffer, dNTP mix, primers and Taq polymerase. Polymerase Chain Reaction was performed in a total volume of 100 µl, containing 78 µl deionized water, 10 µl 10 X Taq pol buffer, 1 µl of 1 U Taq polymerase enzyme, 6 µl 2 mM dNTPs, 1.5 µl of 100 mM reverse and forward primers and 1 µl of 50 ng template DNA. PCR was programmed for each primer condition (Hristova et al., 2011). The first program using primers were started with an initial denaturing at 94°C for 8 min. followed by 32 cycles of denaturation at 94°C for 1 min, annealing at 51 °C for 30 sec and extension at 70 °C for 2 min and the final extension at 72 °C for 7 min in a BIOER/LifeECO 96 advanced gradient Thermo cycler. PCR product (20 µl) was mixed with loading buffer (8 µl) containing 0.25% bromophenol blue, 40 % w/v sucrose in water and then loaded in 2% Agarose gel with 0.1 % ethidium bromide for examination with horizontal electrophoresis.

List of universal primers for ISSR identification

UBC- 898	CACACACACACA
UBC- 899	CATGGTGTTGGTCATTGTTCCA
UBC- 900	ACTTCCCCACAGGTTAACACA

Used oil sample:

Collection and Chromatographic analysis using GC–MS: Approximately 500 ml of oil samples collected from operating engine and stored in carefully sealed bottles. Chromatographic analysis using GC–MS was performed (Agilent Technologies 7890B GC Systems combined with 5977A Mass Selective Detector). Capillary column was used (HP-5MS Capillary; 30.0 m × 0.25 mm ID × 0.25 µm film) and the carrier gas was helium at a pressure of 8.2 psi with 1 µl injection. The sample was analyzed with the column held initially for 3 min at 60°C after injection, then the temperature was increased to 300°C with a 20°C/min heating ramp, with a 8.0 min hold. Injection was carried out in split-less mode at 300°C. MS scan range was (m/z): 50–550 atomic mass units (AMU) under electron impact (EI) ionization (70 eV) and solvent delay 4.0 minutes The constituents were determined by mass fragmentations with The NIST mass spectral search program for the NIST/EPA/NIH mass spectral library Version 2.2 (El-Naief et al., 2020).

Preparation of enzyme-rich leaf extracts: Fresh leaf samples were collected and washed from dust then stored at -20^{o}C in phosphate buffer pH 7.0 for three days and soaked using PBs (Phosphate Buffer Solution) pH 7.0 for the macerations process. Samples were filtered using filter paper Watmann No. 1 and volume was completed up to 30 ml.

Biosurfactant recovery: The biosurfactant was recovered from the plant extraction in FBs pH 7.0 supernatant by buffer

precipitation method (Pruthi and Cameotra, 1995).

Qualitative tests: Oil displacement assay: 30ml of distilled water was taken in a Petri-plate. 1ml of engine oil was added to the center of the plate containing distilled water. Then 20µl of the surfactant was poked into the oil drop. The biosurfactant producing extract displaced the oil (increase in diameter) and spread in the water. (Anandraj and Thivakaran, 2010).

Drop collapse test: A drop of used engine oil was placed on the slide and then 10µl of the surfactant was added by piercing the drop using micropipette without disturbing the dome shaped of the oil. The drop collapsed within 1min was considered to be positive for the drop collapse test. (Das and Chandran, 2010).

Biosurfactant characterization: The crude enzymatic extraction of biosurfactant recovered was characterized on the basis of structural and activity.

Structural characterization Rhamnose test: The presence of carbohydrate groups in the biosurfactant molecule was assayed by rhamnose test (Dubois et al., 1956). A volume of 0.5ml of surfactant was mixed with 0.5 ml of 5% phenol and 2.5ml of sulfuric acid and incubated for 15min before measuring absorbance at 490 nm.

Activity characterization: foaming and emulsifying properties: The foam was produced by hand shaking a 5g/l of crude biosurfactant solution for several minutes. The stability of the foam was monitored by observing it during 2h. The ability of the biosurfactant to emulsify some liquid hydrocarbons, such as sesame oil was determined. The sterile biosurfactant (2ml) was added into each test tube (in a set of three) containing the substrate (2ml). The content of the tubes was vortexed at high speed for 2min and left undisturbed for 24h (Cooper and Goldenberg, 1987).

Statistical analysis:

ANOVA ($p < 0.05$) was used to assess the significant effects of *Swietenia macrophylla, Swietenia mahogany,* and *Khaya senegalensis* drop collapse test, oil displacement assay and rhamnose ***test,*** by applying CRD design followed and using LSD test ($P \leq 0.05$) to determine significant differences among treatment means (Steel and Torrie, 1980). The data were analyzed by Co-State software Version-4 (Co-Stat Graphics, 1999)

RESULTS AND DISCUSSION

1. **Oil displacement assay and Drop collapse test:** The leaf extracts of three mahogany species and the root extract of *Swietenia mahogany* tested positive in both the drop collapse and oil displacement assays using crude engine oil. These assays were employed as preliminary screening methods to evaluate biosurfactant production mediated by oxidizing enzymes, specifically monooxygenases and alkane hydroxylases. The lysis zones, presented in **Table (1)** and **Figures (1 and 2**), were measured five minutes after enzymatic extract application without agitation. Among the samples, the leaf extract of *Swietenia mahogany* exhibited the highest lysis diameter at 17.7 mm for 100 µl, followed by 29.8 mm and 37.7 mm for 200 µl and 300 µl, respectively. In comparison, the root extract of *Swietenia mahogany* showed superior activity, with lysis diameters of 19.2 mm, 32.5 mm, and 42.6 mm for 100 µl, 200 µl, and 300 µl, respectively. These quantitative assays indicate the surface and wetting activities of the extracts (Youssef et al., 2004). The oil displacement test, in particular, provides an indirect measure of a surfactant's surface activity—larger diameters correspond to higher surfactant efficacy (Rodrigues et al., 2006)

Table (1). Effect of enzyme-rich extracts at concentrations 100, 200 and 300 µL on drop collapse of used engine oil.

Extraction volume	100 µl		200 µl		300 µl	
ODA time	Zero time	5 min	Zero time	5 min	Zero time	5 min
Swietenia macrophylla	**11.2**	**13.3**	**17.6**	**21.3**	**20.2**	**28.6**
Swietenia mahogany	**13.2**	**17.7**	**18.1**	**29.8**	**28.7**	**37.7**
Khaya senigalensis	**15.2**	**15.5**	**16.6**	**23.2**	**28.8**	**36.4**
Root of *S. mahogany*	**41.2**	**43.1**	**57.8**	**63.5**	**72.2**	**83.5**
L.S.D. 5%	**1.86**	**0.91**	**1. 41**	**2.27**	**2.32**	**1.23**

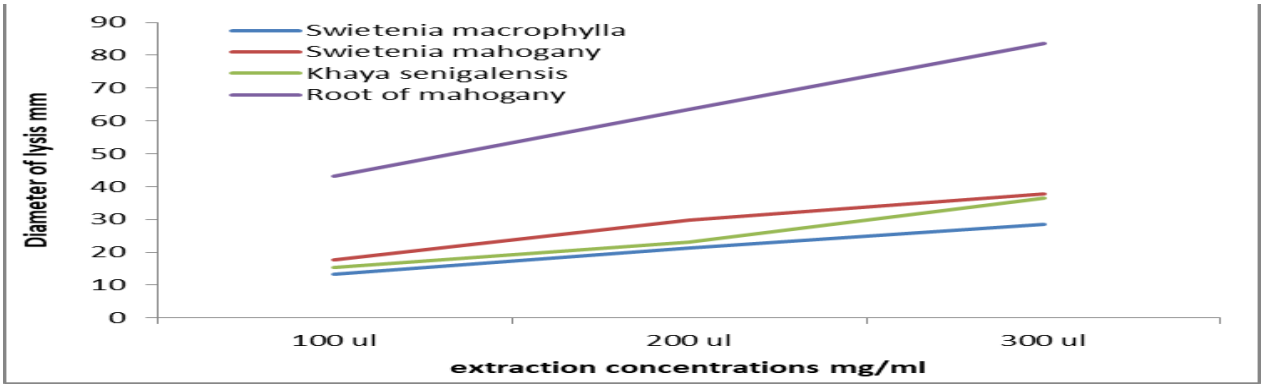


Fig. (1). Effect of enzyme-rich extracts at concentrations 100, 200 and 300 µL on drop collapse of used engine oil after exposed to extractions

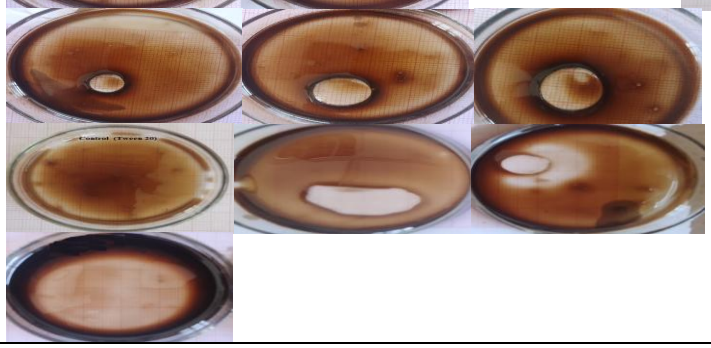
Source of extraction	Control (tween 20)	100 µL	200 µL	300 µL
Swietenia macrophylla				
Swietenia mahogany				
Khaya senigalensis				
Root of *S mahogany*				

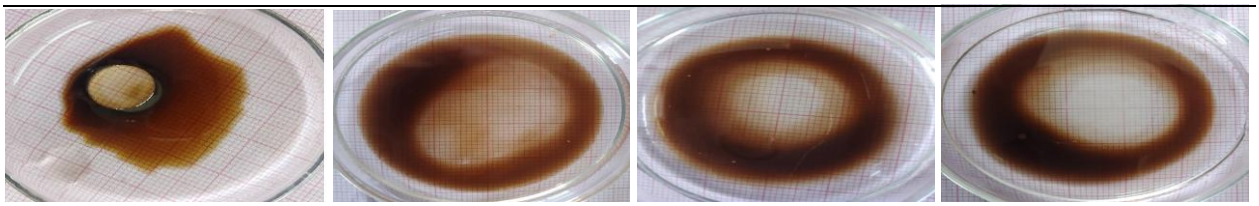
Fig. (2). Effect of enzyme-rich extracts at concentrations of 100, 200 and 300 µL on drop collapse of used engine oil.

The presence of biosurfactants led to oil displacement and the formation of a clearing zone, as shown in **Table (2) and Figure (3).** The diameter of the clearing zone on the oil surface was correlated with the activity of oxidative enzymes. A linear relationship was observed between oxidative enzyme activity and clearing zone diameter, indicating that

higher enzyme activity was associated with increased oil displacement. A positive oil displacement test served as a preliminary indicator of oil degradation, likely mediated by monooxygenases and alkane hydroxylases. This observation suggests that the plant extract facilitated biosurfactant production. According to the data in Table 2 and Figure 4, the highest oil displacement diameter was recorded for the root extract of *Swietenia mahagoni* (30.66 mm), followed by the leaf extract of *S. mahagoni* (26.77 mm). *Khaya senegalensis* also demonstrated significant activity, with a clearing zone diameter of 25.66 mm.

Table (2). Effect of enzyme-rich extracts on Oil displacement assay of used engine oil .

Extract source	Start	After 5 min
Swietenia macrophylla	**10.22**	**20.86**
S. mahogany	**11.45**	**26.77**
Khaya senigalensis	**11.21**	**25.66**
Root of *S. mahogany*	**12.92**	**30.66**
L.S.D. 5%	**0.69**	**1.28**



Swietenia macrophylla | ***Swietenia mahogany*** | ***Khaya senigalensis*** | **Root of *S. mahogany***

Fig. (3). Effect of enzyme-rich extracts on oil displacement test of used engine oil.

2. Biosurfactant (extract) characterization:

a. **Structural characterization:** The optical density varices with different extract source of supernatant which confirmed that the rhamnose test was positive and separated biosurfactant could be of glycolipid type. **(Table 3).** Gujar and Hamde (2012) also reported rhamnose test positive indicating biosurfactant could be of rhamnolipid type. Abouseoud et al. (2007) also reported rhamnose test was positive which indicates that the separated biosurfactant was of glycolipid type.

Table (3). Quantitative estimation of carbohydrate by rhamnose test.

Extract source	Rhamnose concentration mg/ml
Swietenia macrophylla	**1.02**
Swietenia mahogany	**1.52**
Khaya senigalensis	**0.98**
Root of *S. mahogany*	**0.88**
L.S.D. 5%	**0.076**

The extract obtained from *S. mahogany* gave 1.52 mg/ml of rhamnose contents that indicating degradation oil obtained by biosurfactant effect returned to glycolipid produced by plant cell as shown in GC-Mass which appeared Cholest-7-en-3-ol, 14-methyl-, (3.beta.)- in extract.

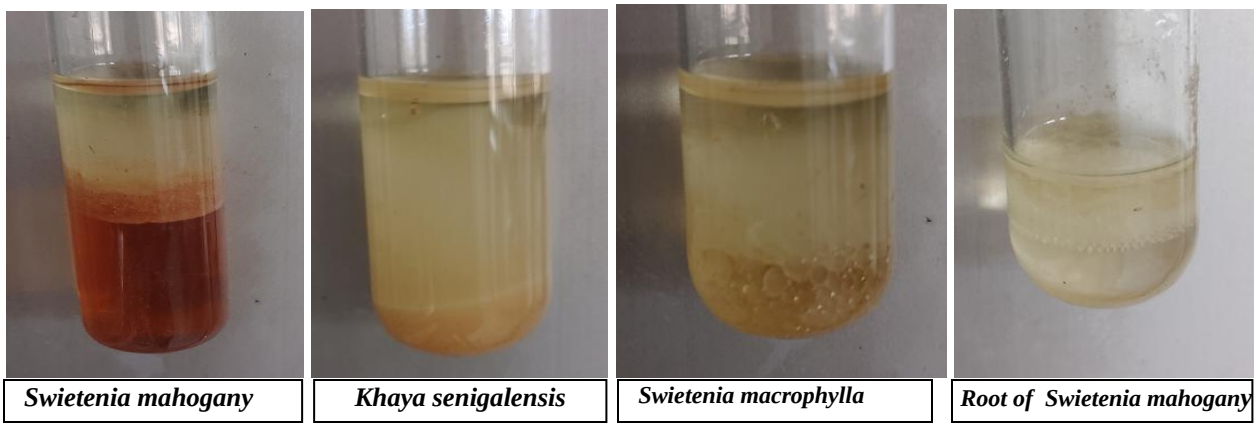
Glycolipids (Rhamnolipids) caused greater dispersion of water-insoluble n-alkanes in the aqueous phase due to their amphipathic properties and the molecules consist of hydrophilic and hydrophobic moieties reduced the interfacial tension of oil-water systems. This resulted in higher interaction of cell extractions with solubilized hydrocarbon droplets much smaller than the cells and rapid emulsifying of hydrocarbon in to the cell extractions. In this concern, Diaz et al. (2002) reported that immobilization of bacterial cells enhanced the biodegradation rate of crude oil compared to free living cells in a wide range of culture salinity. Immobilization can be done in batch mode as well as continuous mode. Packed bed reactors are commonly used in continuous mode to degrade hydrocarbons. Cunningham et al. (2004) used polyvinyl alcohol (PVA) cryogelation

as an entrapment matrix and microorganisms indigenous to the site. They constructed laboratory biopiles to compare immobilized bioaugmentation with liquid culture bioaugmentation and biostimulation. Immobilised systems were found to be the most successful in terms of percentage removal of diesel after 32 days.

b. Activity characterization:

Foaming and emulsifying properties:

Total disappearance of the foam was observed at 24 hrs. Abouseoud et al. (2007) reported total disappearance of the foam was detected after 2h. Gujar and Hamde (2012) also reported total disappearance of the foam was detected after 2h. Emulsification activity gave indication on the presence of biosurfactant. Higher emulsification index indicated a higher emulsification activity of the tested biosurfactant. Sesame oil was the best substrates for biosurfactant having higher emulsification activity 24 h. **(Fig 4).**



Swietenia mahogany | ***Khaya senigalensis*** | ***Swietenia macrophylla*** | ***Root of Swietenia mahogany***

Fig. (4): Effect of biosurfactant obtained from enzymatic leaves extract of three mahogany species on foam disappearance.

As shown in the **Fig. (4)** *S. mahogany* was more positive effect to disappear foam after adding enzymatic extract to sesame oil following by roots extraction of the same tree. In this concern, Willumsen and Kosaric (1997) and Lima et al. (2011) proposed that the three bacterial strains were grown in the appropriate media, temperatures and incubation period of each strain for the production of the bio-surfactants. After the incubation period, the cell free broth culture containing the biosurfactant (supernatants) of *P. aeruginosa* SH 29 was sterilized and applied for the cleaning of oil-contaminated vessels, while the sterilized supernatants of the two *microorganism and plantlets*. were applied for enhancing the biodegradation of oil sludge, this is in addition to a phytogenic biosurfactant as a comparison.

The biosurfactant can be used directly to recover oil remaining in the oil - contaminated vessels, oil tanks, storage oil tanks, tank cars, pipelines and other containers used to transport or store crude oil. During petroleum oil storage, large amount of oily sludge are developed at the bottom of the storage tanks, causing reduction of the tank capacity. Lima et al. (2011) reported that reduction in the capacity of storage tanks and development of corrosion makes it necessary to dispose this oily sludge through periodic cleaning of the tanks. Traditionally, methods may be used for cleaning such tanks and recovery of the sludge from the tank bottoms. These methods are expensive, time consuming and need specialized and skilled personnel. As an alternative, removal of oil sludge and cleaning the storage tanks may be carried out using biosurfactants that are able to reduce the interfacial tension and the viscosity in order to facilitate the oily sludge removal from tanks. Chamanrokh et al.

(2010) found that washing the oil - contaminated vessels with 10 to 20 mg/ml of biosurfactant solution readily forms an oil-water emulsion. Banat (1995) cleaned oil storage tanks and recovered oil from the sludge by using a biosurfactant produced by bacterial strains.

Pacwa-Plociniczak et al. (2011) reported that the effective microbiological method in bioremediation of oil-polluted polluted sites is the use of biosurfactants-producing bacteria without necessary characterization of the chemical structure of the biosurfactants. The cell free culture broth containing the biosurfactant can be applied directly or diluting it appropriately to the contaminated site. The other benefit of this approach is that bio-surfactants are very stable and effective in the culture medium that was used for their synthesis. Das and Mukherjee (2007) inoculated an oil-polluted soil sample with mineral salt medium inoculated with *Bacillus subtilis* or *P. aeruginosa* M and NM strains. Helmy et al. (2010) studied the application of a biosurfactant for the recovery of oil and enhancing the biodegradation rate. They found that addition of biosurfactant to a mixed culture (consortium) of oil-degrading bacteria increased the biodegradation rate to 70% after 70 days; this is in contrast to only 55% in the absence of biosurfactant. They then suggested that the addition of both oil-degraders and biosurfactant may increase the biodegradation rate. Cameotra and Makkar (2010) explained that biosurfactants can enhance the solubilization of the hydrocarbon contaminants from the polluted soil which in turn enhances the biodegradation process

3. Oxidizing enzymes in plant extracts:

The data presented in **Table (4)** elucidate the role of initial enzymatic extracts in the oxidative degradation of organic pollutants. This process involves the enzymatic activation and incorporation of molecular oxygen, primarily catalyzed by oxygenase and peroxidase enzymes. Among the enzymatic activities assessed, polyphenol oxidase (PPO) exhibited the highest activity in the leaves and roots of *Swietenia mahagoni*, with measured values of 0.048 and 0.036 U/mg protein, respectively. Similarly, peroxidase activity was notably high, reaching 0.129 U/mg in leaves and 0.127 U/mg in roots. Hydrocarbon biodegradation, encompassing both aliphatic and aromatic compounds, can occur under aerobic and anaerobic conditions (Hamme et al., 2003). Under aerobic conditions, oxygenase enzymes are responsible for initiating the breakdown process by incorporating oxygen atoms into hydrocarbon molecules. Monooxygenases insert a single oxygen atom, whereas dioxygenases insert two oxygen atoms, often derived from hydrogen peroxide (H_2O_2), which is oxidized through the action of peroxidases and PPOs.

Gharib et al. (2018) demonstrated peroxidase gene expression in the leaf tissues of three *S. mahagoni* genotypes using 10% native polyacrylamide gel electrophoresis (PAGE). The electrophoretic profiles revealed three distinct peroxidase bands (Px1, Px2, Px3) with variations in band intensity and density across samples, though the relative front (Rf) values were approximately consistent. These findings suggest that salt stress induces increased peroxidase activity, likely due to upregulation of the encoding gene(s). Similarly, electrophoretic analysis of polyphenol oxidase revealed two distinct bands (PPO1 and PPO2) among the examined genotypes, differing in intensity. These results align with findings by El-Sayed et al. (2007), who reported that salinity stress influences the presence and intensity of protein bands in wheat cultivars, indicating stress-induced modulation of PPO expression. **Figure (5)** illustrates the mechanistic interaction of monooxygenase enzymes with peroxidase and polyphenol oxidase systems, contributing to the oxidative breakdown of both aliphatic and aromatic hydrocarbons. The initial enzymatic attack on xenobiotic compounds

is typically mediated by oxygenases (Fritsche and Hofrichter, 2000), which require activation by oxygen insertion depending on the substrate's molecular characteristics, such as chain length. In higher eukaryotes, cytochrome P450 monooxygenases (CYPs) constitute a large family of enzymes, with multiple isoforms contributing collectively to substrate metabolism. In contrast, such diversity is limited in microorganisms (Zimmer et al., 1996). Nevertheless, microbial species such as *Candida maltosa*, *Candida tropicalis*, and *Candida apicola* have demonstrated the presence of multiple microsomal P450 enzymes capable of utilizing n-alkanes and other hydrocarbons as sole carbon and energy sources (Scheue et al., 1998).

Diverse alkane-oxidizing enzyme systems are involved in aerobic hydrocarbon degradation across prokaryotic and eukaryotic organisms. These include cytochrome P450 enzymes, integral membrane di-iron alkane hydroxylases (e.g., alkB), soluble di-iron methane monooxygenases, and membrane-bound copper-containing methane monooxygenases (Van Beilen and Funhoff, 2005). The potential of phytoremediation for the treatment of hydrocarbon-contaminated sites has also been investigated. At a site approved by the Alabama Department of Environmental Management, approximately 1,500 cubic yards of soil—70% of which initially contained >100 ppm total petroleum hydrocarbons (TPH) were subjected to vegetative cover. After one year, 83% of the soil samples had TPH concentrations below 10 ppm. Similar reductions in TPH levels, originally ranging from 1,700 to 16,000 mg/kg, have been reported in sites contaminated with crude oil, diesel fuel, and refinery waste (Hecht & Badiane, 1998; Nedunuri et al., 2000). Plant species were found to significantly influence the extent of TPH reduction, with some promoting greater hydrocarbon degradation than others or compared to unvegetated control soils.

In the Pacific Islands, several tropical plant species, including *Cordia subcordata* (kou), *Thespesia populnea* (milo), *Prosopis pallida* (kiawe), and *Scaevola sericea* (beach naupaka), have shown tolerance to contaminated field conditions and contributed to effective phytoremediation of diesel-contaminated soils (U.S. Army Corps, 2003).

Table (4). Oxidized enzymes a source of oxygen atoms used in monoxygenasis reaction

Source of enzymes	POD U/mg	PPO U/mg
Khaya senegalensis	0.061	0.0303
Swietenia mahogany	0.129	0.0485
Swietenia macrophylla	0.103	0.0303
Root of *S. mahogany*	0.127	0.0364

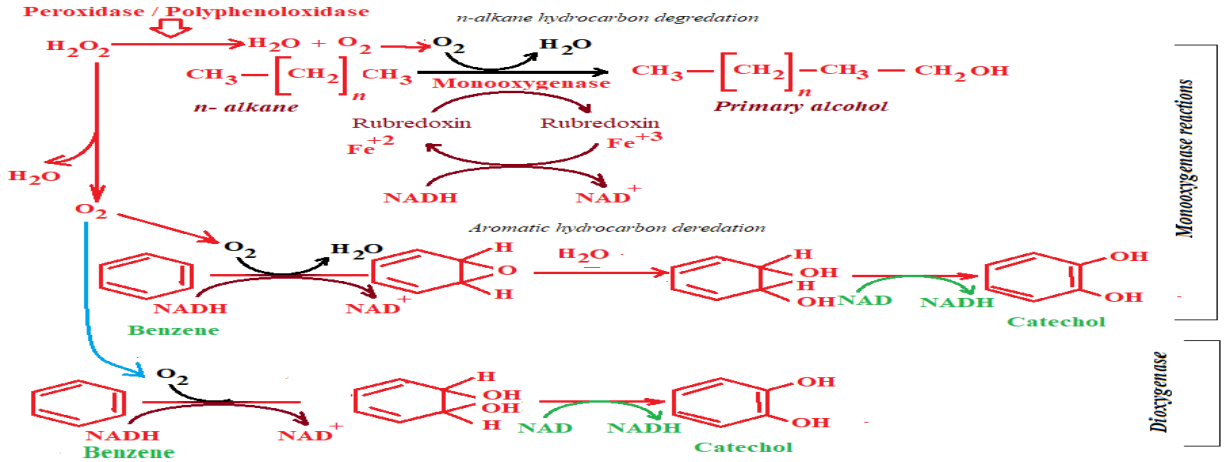


Fig. (5). Hydrocarbon biodegradation mechanism by enzymatic extraction (biosurfactant)

4. **GC –Mass of engine oil:** The crude oil was added to the buffer solution as the sole source of aliphatic-hydrocarbons and cyclo-hydrocarbons for 7 days of incubation. Generally, most of n-alkanes and cycalo-hydrocarbons were degraded by the enzymes in leaves extractions. However, the crude oil contain short chain n-alkanes, were more degraded than those with longer chains or cyclic hydrocarbons. The GC-Mass of crude oil **(Table 5)** showed the highest percent 15.35 % of (1-Hexadecanol, 2-methyl-); 15.39 % (erythro-9,10-Dibromopentacosane); 8.23 % (Octatriacontyl pentafluoropropionate); 7.35 % (2-Piperidinone, N-[4-bromo-n-butyl]-) and 7.23 % of (17-Pentatriacontene).

Table (5). Crude engine oil fractionation by GC-Mass

No	RT	Area PCT	Compound	carbon	qul
1	12.2981	**3.3052**	Octadecanal*	C18	87
2	12.4697	**0.6136**	Oxirane, tetradecyl-*	C16	89
3	12.7387	**7.3533**	2-Piperidinone, N-[4-bromo-n-butyl]-**	C9	87
4	12.9275	**0.0231**	2-Octadecyl-propane-1,3-diol*	C21	90
5	13.1564	**1.8957**	Tetradecanal**	C14	68
6	13.5855	**1.2375**	benzenesulfonyl fluoride, 4-(hexadecyloxy)-3-nitro-*	C22	81
7	13.7057	**1.3789**	Tetradecane, 1-bromo- **	C14	60
8	13.8544	**0.9257**	Heneicosane, 11-cyclopentyl-*	C26	64
9	13.9803	**3.983**	Carbonic acid, but-2-yn-1-yl eicosyl ester**	C25	64
10	14.2435	**2.208**	Cyclohexane, (1-hexadecylheptadecyl)-*	C39	53
11	14.461	**2.8822**	Octatriacontyl trifluoroacetate*	C40	60
12	14.5182	**2.3855**	Disparlure **	C19	50
13	14.5525	**1.341**	Dimethylmalonic acid, 4-chlorophenyl pentadecyl ester*	C26	64
14	14.5868	**15.395**	erythro-9,10-Dibromopentacosane*	C25	76
15	14.6269	**0.9101**	Tetratriacontyl pentafluoropropionate*	C37	81
16	14.8157	**6.1812**	Octadecane, 1-[2-(hexadecyloxy)ethoxy]-*	C36	90
17	14.9359	**6.1388**	5-Methyl-Z-5-docosene*	C23	55
18	14.9707	**8.2394**	Octatriacontyl pentafluoropropionate*	C41	90
19	15.2449	**15.3546**	1-Hexadecanol, 2-methyl-*	C17	89
20	15.7999	**3.7786**	Hexacosanal*	C26	76
21	16.3721	**2.2807**	Oxalic acid, allyl octadecyl ester*	C23	74
22	16.7268	**3.829**	Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy-*	C23	42
23	17.0931	7.7091	17-Pentatriacontene*	C35	86
24	18.289	**0.1087**	benzenesulfonyl fluoride, 4-(hexadecyloxy)-3-nitro- **	C22	58
25	18.329	**0.5421**	Cholest-7-en-3-ol, 14-methyl-, (3.beta.)- **	C28	83

*** Aliphatic hydrocarbons** **** Cyclic hydrocarbons.**

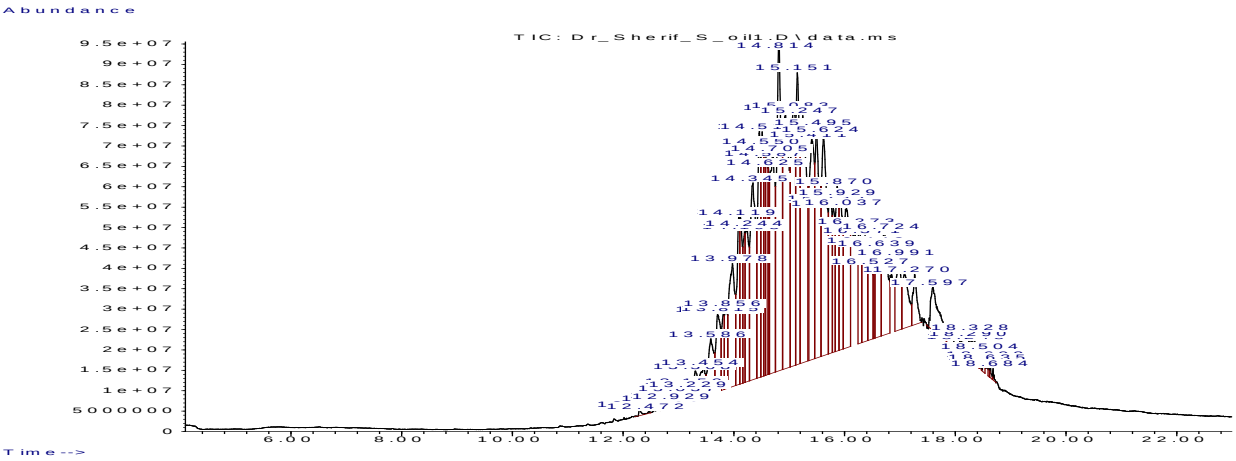


Fig. (6): GC-Mass fractionation of crude engine oil.

According to the **Table (5) and Fig. (6)** the crude oil containing aliphatic hydrocarbon and cyclic hydrocarbons like (C9) 2-Piperidinone, N-[4-bromo-n-butyl]- 7.35 % ; (C14) Tetradecanal (1.89 %); (C14) Tetradecane, 1-bromo- 1.37 %; (C25) Carbonic acid, but-2-yn-1-yl eicosyl ester 3.89 % ; (C19) Disparlure 2.38 % and (C22) benzenesulfonyl fluoride, 4-(hexadecyloxy)-3-nitro- 0.11%. Moreover, (C28) cholesterol ring was found as Cholest-7-en-3-ol, 14-methyl-, (3.beta.)- 0.54%.

Table (6). Fractionation crude engine oil by GC-Mass after incubation with enzyme-rich extract of *Swietenia macrophylla* for 7 days

PK	Area PK	RT	Library/ID	Qual	Formula	Carbon
1	35.6629	5.14	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane	56	C11H32O4Si4	c11
2	5.8193	5.203	Cyclotetrasiloxane, octamethyl-	47	C8H24O4Si4	C8
3	4.0754	6.4046	3-Hydroxybenzoic acid, 2TMS derivative	50	C13H22O3Si2	C13
4	3.9777	7.4345	Pentasiloxane, 1,1,3,3,5,5,7,7,9,9-decamethyl-	25	C10H30O4Si5	C10
5	13.1327	14.793	2-Piperidinone, N-[4-bromo-n-butyl]-**	64	C9H16BrNO	C9
6	11.0575	14.8845	Heneicosane, 11-cyclopentyl-	64	C26H52	C26
7	10.2634	14.9647	5-Methyl-Z-5-docosene	43	C23H46	C23
8	16.011	15.1077	Cholest-7-en-3-ol, 14-methyl-, (3.beta.)-	64	C28H48O	C28

According to **Table (6) and Fig. (7)** many aliphatic and cyclic hydrocarbons decreased and disappears as (C11) trimethylsiloxy) trisiloxane 35.66 %; (C8) Cyclotetrasiloxane, octamethyl- 5.8%; (C13) 3-Hydroxybenzoic acid, 6.40 % (salicylic acid); (C10) Pentasiloxane, 1,1,3,3,5,5,7,7, 9,9-decamethyl- 7.4 % and (C9) cyclic hydrocarbon [2-Piperidinone, N-[4-bromo-n-butyl] 14.79 %

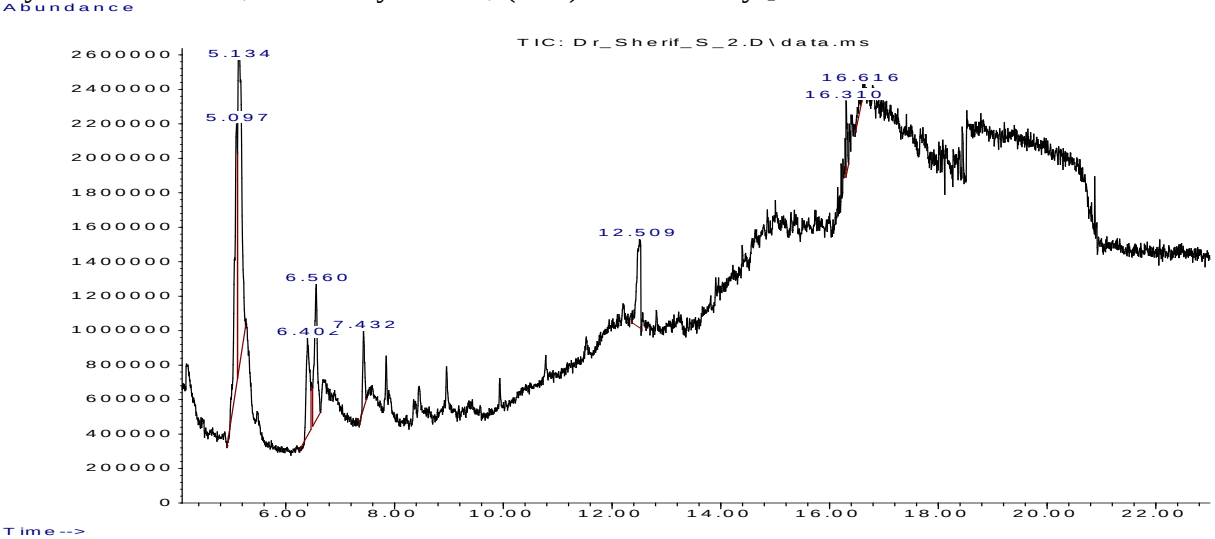


Fig. (7) GC- Mass chromatogram fractionation of crude engine oil after incubation with enzyme-rich extract of *Swietenia macrophylla* for 7 days

Four compounds were not degradation by enzymes (C9) cyclic hydrocarbon [2-Piperidinone, N-[4-bromo-n-butyl] 14.79 %; (C26) Heneicosane, 11-cyclopentyl- 14.8 %; (C23) 5-Methyl-Z-5-docosene 14.9 % and (C8) Cholest-7-en-3-ol, 14-methyl-, (3.beta.)- 15.1 %.

Table (7). Fractionation crude engine oil by GC-Mass after incubation with enzymes extraction of *Swietenia mahogany for 7 days*

PK	RT	Area Pct	Library/ID	Qual	Formula	C
1	5.0485	21.8757	Cyclotetrasiloxane, octamethyl-	64	C8H24O4Si4	C8
2	5.1114	29.4174	trisiloxane, 1,1,1,5,5,5-hexamethyl-3-[(trimethylsilyl)oxy]-	35	C9H27O3Si4	C9
3	5.1629	27.7524	Cyclotetrasiloxane, octamethyl-	40	C8H24O4Si4	C8
4	6.5648	20.9545	Cyclopentasiloxane, decamethyl-	52	C10H30O5Si5	C10

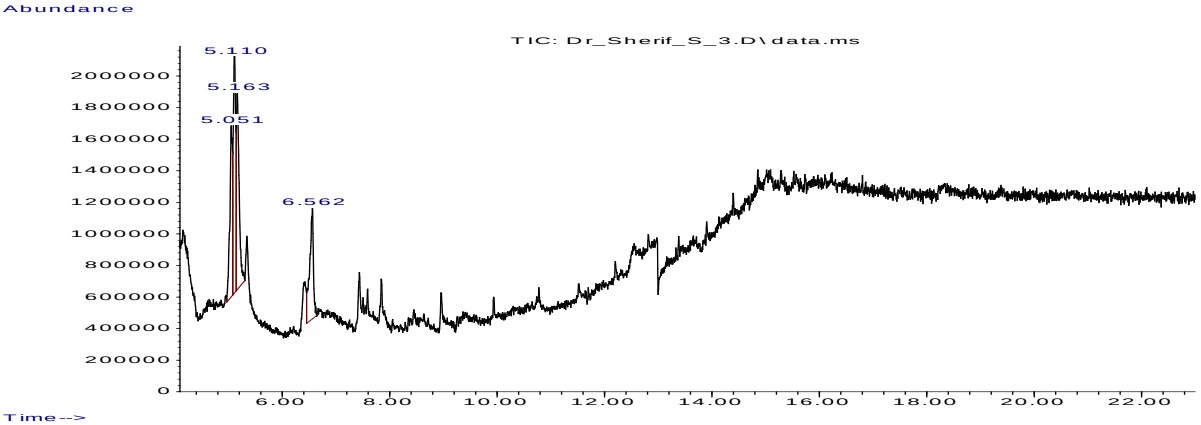


Fig. (8). GC- Mass chromatogram fractionation of crude engine oil after incubation with enzyme-rich extract of *Swietenia mahogany* for 7 days

Swietenia mahogany have a monooxygenases and alkane hydroxylase which responsible for degradation many hydrocarbon long chain and cyclic hydrocarbon. Only four compounds were observed **(Table 7 and Fig. 8)** in the sample at the end of incubation period (C8) Cyclotetrasiloxane, octamethyl- 21.87 % ; (C9) trisiloxane, 1,1,1,5,5,5-hexamethyl-3-[(trimethylsilyl)oxy]- 29.41 %; (C8) Cyclotetrasiloxane, octamethyl- 27.75 % and (C10) Cyclopentasiloxane, decamethyl- 20.95 %.

Table (8). Fractionation crude engine oil by GC-Mass after incubation with enzyme-rich extract of *Khaya senigalensis* for 7 days.

PK	RT	Area Pct	Library/ID	Qual	Formula	C
1	5.0944	20.5327	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane	37	C11H32O4Si4	C11
2	5.1344	38.2188	Cyclotetrasiloxane, octamethyl-	32	C8H24O4Si4	C8
3	6.4047	9.7827	4-Trimethylsilyl-9,9-dimethyl-9-silafluorene	35	C17H22Si2	C17
4	6.5592	12.7014	Cyclopentasiloxane, decamethyl-	43	C10H30O5Si5	C10
5	7.4347	4.2088	Phosphonoacetic Acid, 3TMS derivative	9	C11H29O5PSi3	C11
6	12.51	10.3582	2-Buten-1-ol, (E)-, TBDMS derivative	38	C10H22OSi	C10
7	16.3094	2.6337	2-Piperidinone, N-[4-bromo-n-butyl]-	52	C9H16BrNO	C9
8	16.6184	1.5636	Oxalic acid, cyclobutyl heptadecyl ester	68	C23H42O4	C23

The third species of mahogany trees was *Khaya senigalensis* which gave a few hydrocarbon compounds (C11) 3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane 20.53 %; (C8) Cyclotetrasiloxane, octamethyl- 38.21 %; (C17) 4-Trimethylsilyl-9,9-dimethyl-9-silafluorene 9.78 %; (C10) Cyclopentasiloxane, decamethyl- 12.7 % ; (C11) Phosphonoacetic Acid, 3TMS derivative 4.2 %; (C10) 2-Buten-1-ol, (E)-, TBDMS derivative 10.35 %; (C9) 2-Piperidinone, N-[4-bromo-n-butyl]- 2.63 % and (C23) Oxalic acid, cyclobutyl heptadecyl ester 1.56% **(Table 8 and Fig. 9)**

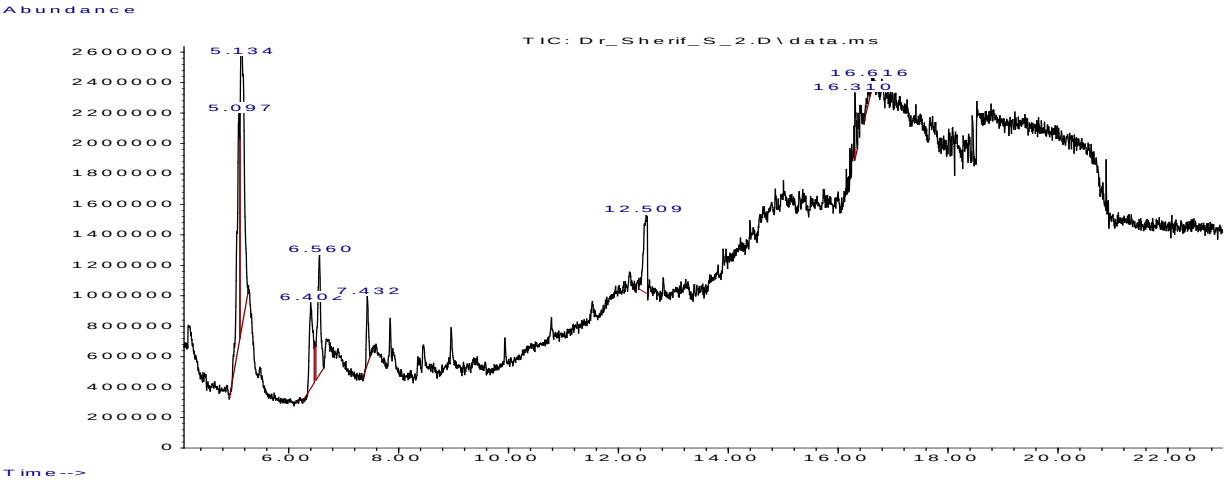


Fig. (9). GC- Mass chromatogram fractionation of crude engine oil after incubation with enzyme-rich extractof *Khaya senigalensis* for 7 days

According to GC-Mass analysis of crude oil incubated with enzymatic extraction of *S. mahogany* for 7 days, root enzymatic extraction of *S. mahogany* was analyzed by GC-Mass due to high degradation level of crude oil obtained from enzymatic leaves extraction. So, root extraction only obtained from this species was analyzed according to **Table (9) and Fig. (10)** It showed a new compound appears (C15) 4'-Nitroflavone 33.43 % which synthetic in the plants and not found in the crude engine oil. Also, the 4'-Nitroflavone work as substrate for monoxygenasis which responsible for hydrocarbon degradation. Other compound observed in the analysis was (C4) Acetic acid, chloro- 6.10 % that obtained from metabolism of indole acetic acid. Also, 3-Hydroxybenzoic acid, 26.0 % which known salicylic acid that synthetic in plant cells which gave the plant tolerance to differences in temperatures.

Table (9). Fractionation crude engine oil by GC-Mass after incubation with enzyme-rich extract of *S. mahogany* root for 7 days

PK	RT	Area Pct	Library/ID	Qual	Formula	C
1	5.1343	6.1039	Acetic acid, chloro-	16	C4H4Cl4O4	C4
2	6.416	26.0035	3-Hydroxybenzoic acid, 2TMS derivative	47	C13H22O3Si2	C13
3	6.5476	33.4334	4'-Nitroflavone	22	C15H8BrNO4	C15
4	7.4288	15.3561	Tetrasiloxane, decamethyl-	25	C10H30O3Si4	C10
5	14.8502	16.8317	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane	27	C18H52O7Si7	C18
6	15.7085	2.2713	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	18	C16H48O7Si8	C16

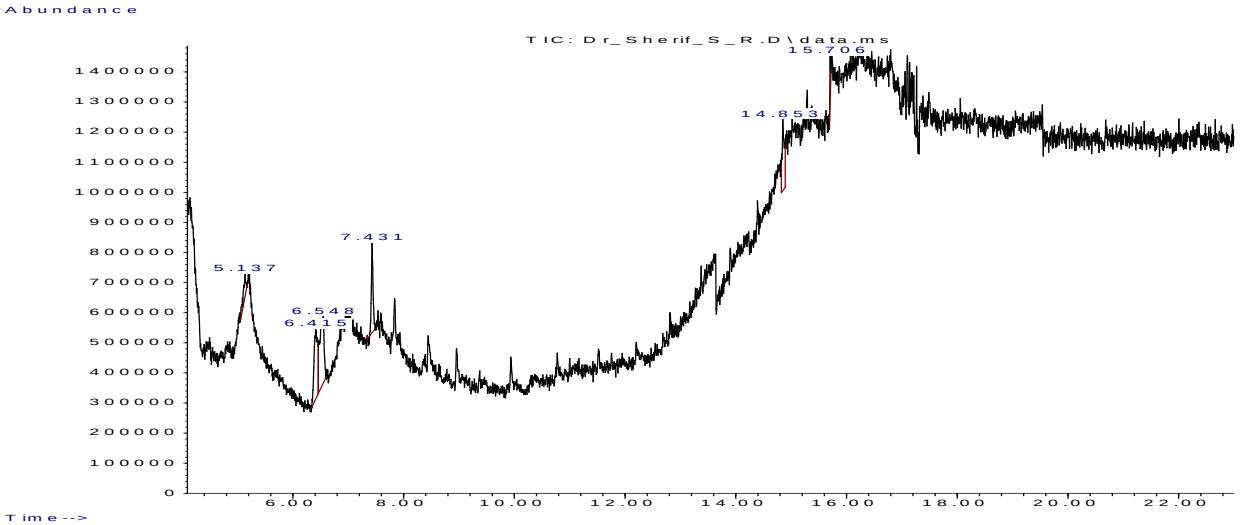


Fig (10). Fractionation crude engine oil by GC-Mass after incubation with enzyme-rich extract of *Swietenia mahogany* root for 7 days.

According to the previous data the highest amount of crude oil was 1-Hexadecanol, 2-methyl- 15.35% and erythro-9,10-Dibromopentacosane 15.39 % it was disappear after incubation with enzymatic

5. **Genetically evaluation of mahogany trees by ISSR amplification profiles:**

Genomic DNA from the three samples (*Swietenia macrophylla* (S1), *Swietenia mahogany* (S2), and Khaya *senegalensis* (S3) was amplified using three distinct Inter Simple Sequence Repeat (ISSR) primers (UBC- 898, UBC- 899and UBC- 900). A scorable bands were generated across all samples and primers. A genetic variation can be detected among the three samples in comparison of the three primers which emphasis the difference in the genetic sequences. The generation of a considerable number of polymorphic bands across all primers highlights the utility of ISSR markers in discriminating between these samples (Hristova et al., 2011). The varying number and size of amplified bands observed with each primer (UBC- 898, UBC- 899and UBC- 900).) underscore the sequence-specific nature of ISSR amplification (Guo et al., 2016). The ability of these primers to generate unique banding patterns for each sample suggests their suitability for individual identification or germplasm characterization.

The 11 ISSR primers allowed the amplification and satisfactory visualization of fragments from the PCR reactions from the genomic DNA of 3 individuals of *Swietenia macrophylla, Swietenia mahogany*, and *Khaya senegalensis* Of this total, 3 primers enabled good amplification, being visible and of high quality, and classified as suitable. The other primers showed barely visible marks and were classified with a moderate standard, but as reproducible as those considered suitable.

The primers generated 27 bands for three types of plants with an average of 9 bands per primer, the first primer (UBC 900) produced 8 bands for three types their relative mobility ranged from 0.44 to 0.8. On the first type *Swietenia macrophylla* two polymorphic bands were observed followed by five polymorphic bands for *S. mahogany* and three polymorphic bands for *K. senegalensis*. The total polymorphism percentage was 100 % for all bands. Total polymorphic bands percentage was 25 % for S1, followed by 62 % for S2 and 37.5 % for the last one.

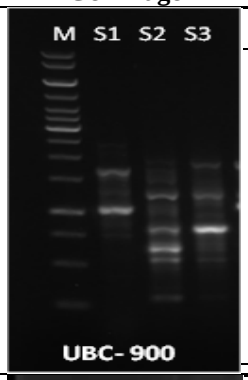
The second primer (UBC 898) produced 15 bands for three types their relative mobility ranged from 0.43 to 0.86. On the first type *Swietenia macrophylla* 10 polymorphic bands were observed followed by five polymorphic bands for *S. mahogany* and three polymorphic bands for *K. senegalensis*. The total polymorphism percentage was 95 % for all bands and 5% monomorphism.

Finally, UBC- 899 primer scored RF ranged from 0.52 to 0.66 and showed one band

for polymorphism and one band monomorphism for S1. The bands of S2 on band monomorphic pattern and 2 bands polymorphic pattern as the same of S3. 60 % similarity for *S. mahogany* and the two types *Swietenia macrophylla and K. senegalensis.*

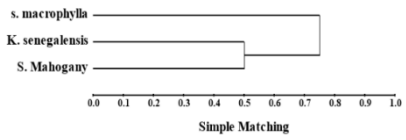
Table (10). ISSR analysis, polymorphism; monomorphism and similarity percentage for three types of mahogany trees.

Primer: ACTTCCCCACAGGTTAACACA

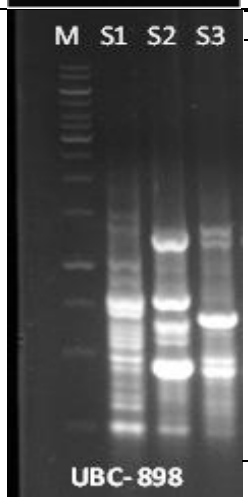


RF	MS/MW	S1	S2	S3
0.44	551.85	0	0	1
0.46	506.02	1	0	0
0.52	401.56	0	1	1
0.56	337.63	1	0	0
0.61	279.81	0	1	1
0.66	231.89	0	1	0
0.7	200.69	0	1	0
0.8	137.83	0	1	0

Source of Polymorph	S1	S2	S3
No. of M. B.	0	0	0
No. of P.B	2	5	3
Total Bands	2	5	3

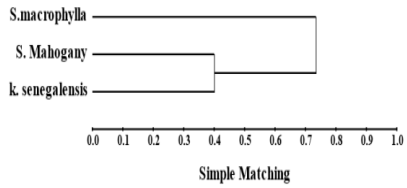


Primer: CACACACACA

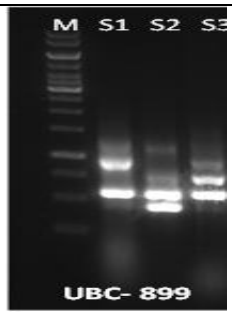


RF	MS/MW	S1	S2	S3
0.43	490.65	1	0	0
0.48	404.14	0	1	1
0.53	341.6	1	0	0
0.57	304.07	1	0	0
0.61	267.19	0	1	0
0.62	257.02	1	0	0
0.64	240.93	0	0	1
0.65	228.79	0	1	0
0.69	201.04	1	1	0
0.72	181.28	1	0	1
0.74	167.75	1	1	1
0.77	153.23	1	0	0
0.79	141.79	1	0	0
0.81	132.91	1	0	0
0.86	113.81	1	1	0

Source of Polymorph	S1	S2	S3
No. of M.B	1	1	1
No. of P. B.	10	5	3
Total Bands	11	6	4

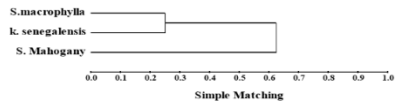


Primer: CATGGTGTTGGTCATTGTTCCA



RF	MS/MW	S1	S2	S3
0.52	327.05	1	0	1
0.56	279.41	0	1	1
0.61	228.68	1	1	1
0.66	189.86	0	1	0

Source of Polymorph	S1	S2	S3
No. of M.B	1	1	1
No. of P. B.	1	2	2
Total Bands	2	3	3



In this concern, In ISSR analysis, 3 of the ISSR primers generated variable banding patterns. A total of 27 out of 3 ISSR fragments were polymorphic. 28 amplified fragments were considered as cultivar-specific markers.

The effectiveness of enzymatic bioremediation can be significantly enhanced by molecular tools, making it especially suitable for situations requiring rapid remediation (Sutherland et al., 2004). According to Al-Calde et al. (2006), advances in protein engineering, metagenomics, and proteomics are reducing costs, minimizing chemical usage, and improving cost-benefit ratios. The application of molecular tools in

biocatalysis can also help address concerns related to the environmental release of genetically modified organisms (GMOs). For example, if a modified enzyme is produced in vitro, there is no need to introduce the genetically modified organism into the natural environment.

Numerous PCR primers have been developed to target genes encoding petroleum-degrading enzymes under both aerobic and anaerobic conditions. The use of these well-characterized primers can facilitate environmental screening of degradation capabilities and help evaluate the potential of microbial isolates. Additionally, new primers can be developed to target specific metabolic pathways or to improve the comprehensiveness of existing primers using available genomic databases.

CONCLUSION :

The present results show that extracts from mahogany leaves contain enzymes capable of degrading high molecular weight aliphatic and aromatic hydrocarbons. These extracts may also function as biosurfactants, containing glycolipids that reduce the surface tension between oil and water, facilitating emulsion formation. The mahogany leaf extract can potentially be applied directly to contaminated sites without the need for isolating and characterizing the chemical structure of the biosurfactant. This approach represents a promising, simple, and cost-effective method for treating oily sludge generated during the routine cleaning of oil storage tanks and other petroleum industry processes. Additionally, the phytogenic surfactant used in this study shows potential for detoxifying soil polluted with oily sludge.

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الملخص العربي

تقييم التحلل البيولوجي المحتمل لزيت المحركات المستعملة باستخدام مستخلص من بعض النباتات من العائلة *Meliaceae*

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تشكل الهيدروكربونات البترولية مصدر قلق بيئي كبير نظرًا لانتشارها الواسع وتأثيراتها الضارة على صحة الإنسان. ويعتبر التحلل البيولوجي استراتيجية فعالة لمعالجة تحلل الهيدروكربونات من البترول المسكوب. في هذه الدراسة، تم التحقيق في إمكانية مستخلصات أوراق الماهوجني المحلية لتحلل البترول في المختبر. تم عزل زيت المحرك الخام وتحليله بواسطة كروماتوغرافيا الغاز-مطياف الكتلة (GC-MS) دون أي معالجة مسبقة. ثم تم تحضين الزيت لمدة 7 أيام مع مستخلصات أوراق من ثلاثة أنواع من الماهوجني: *Swietenia macrophylla* و *Swietenia mahagoni* و *Khaya senegalensis*. في نهاية فترة الحضانة، أجريت اختبارات مختلفة، بما في ذلك إزاحة الزيت وانهيار القطرات وتحليل محتوى الرامنوز واختبارات خصائص الرغوة والاستحلاب. كما هدفت الدراسة الحالية إلى فحص التنوع الجيني لثلاثة أنماط جينية من *Swietenia mahagoni* باستخدام علامات تكرار التسلسل البسيط (ISSR). تم الحصول على الأنماط الجينية من بستان قسم أشجار الأخشاب، معهد بحوث البستنة، محافظة الجيزة. أظهر التوصيف الجزيئي أن مستخلص سويتينيا ماهاجوني كان الأكثر فعالية في تحليل النفط الخام. أُجري تحليل تكرار التسلسل البسيط (ISSR) لتقييم التباين الجيني بشكل أعمق. أنتجت ثلاثة بادئات ISSR أنماطًا مميزة للخطوط. من بين 62 قطعة مُضخّمة من ISSR، كان 27 منها متعدد الأشكال، وتم تحديد 28 منها كعلامات خاصة بالصنف. **بشكل عام**، أظهرت مستخلصات سويتينيا ماهاجوني - من الأوراق والجذور - أعلى كفاءة في تحليل النفط الخام.