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EXTRACTION AND PHYTOCHEMICAL SCREENING OF *Elaeis guineensis* SHELL WASTE

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Abstract: **Phytochemicals from *Elaeis guineensis* shell waste can be a source of starting materials in pharmaceutical and food processing industries. Since time immemorial, truckload of *Elaeis guineensis* shell are being treated as solid waste and the wasted shells are sometimes used by traditional herbalist who believe in the superstition working power of the shell in the treatment of some illness which is attributed to god of Satan (esu) in Southwest geopolitical zone in Nigeria. Therefore, the aim of this study was to evaluate the phytochemical composition of the methanolic extract of *Elaeis guineensis* shell waste for the presence of active phytochemicals and to scientifically validate its medicinal actions which was misunderstood by traditional herbalist based on superstition. The result of the phytochemical analysis in this present study revealed the presence of phenolics (11.4 ± 0.2g/100g), flavonoids (5.67 ± 0.23 g/100g), tannins (6.67 ± 0.12 g/100g), terpenoids (4.53 ± 0.12 g/100g), saponins (1.99 ± 0.01 g/100g) and alkaloids (1.81 ± 0.03 g/100g). Quantitatively, the phenolics content was found to be the highest (11.4 ± 0.2g/100g) in the sample while the alkaloids content was the least (1.81 ± 0.03 g/100g). Thus, our finding nullifies the traditional herbalist superstitious believe about *Elaeis guineensis* shell waste and suggests a baseline information that ethanolic shell extract of *Elaeis guineensis* contain phytochemicals that possess antioxidant, anti-inflammatory and astringent properties and the extract could possibly be used in the management of wound in traditional medicine because of the key functions of those phytochemicals reported in the past and present documented literatures. The ultimate utilization of this shell waste should be encouraged in order to enhance solid waste management as well as pollution control and thus converting waste to estimable material.**

Keywords: **Alkaloids, *Elaeis guineensis*, methanolic extract, phenolic, phytochemical analysis, waste.**

1. INTRODUCTION

The screening of plants for medicinal use has received long standing interest in the science community because plants and humans have co-existed since time immemorial and man has been able to exploit plants in several ways including his primary healthcare delivery to preserve life (Edewor *et al.*, 2015), and this has led to the utilization of large number of medicinal plants with therapeutic properties to combat various diseases such as cough, malaria, colds, wounds among others (Praveen and Ashalatha, 2014). Phytochemistry mean herbal-chemistry and it is the study of chemical composition of medicinal vegetation. Phytochemicals are compounds found in plants that are not prerequisite for normal working of the body, but have a valuable influence on health or play a dynamic role in amelioration of diseases (Ayoola *et al.*, 2008). These bioactive compounds occur naturally in plants and plants use them to defend themselves. Research has established its protective special effects against countless diseases, and examples of this phytochemicals include flavonoids, coumarins, alkaloid, terpenoid, saponin, tannin, glycoside and so on (Altiok, 2010).

Elaeis guineensis of the genus *Elaeis* is single-stemmed multiuse evergreen plants in the family *arecaceae* reaching a height of 20 m and above at maturity. These plant is native to West and Africa, East India's and Central America geographically and it is locally called "ope" in the western part of Nigeria, while the fruit and the shell of the fruit is

called “ekuro or ekuo”and “eesan” respectively (Orwa *et al*., 2009). The plant is used in the traditional medicine in west Africa for the treatment of various ailments and all parts of this plant are useful (Sreenivassan *et al.*, 2013; Olasunkamin *et al*., 2018). The local people used the oil from the plant fruit as hair cream, cure headache, stomach upset, poison antidote for goats and other ruminant animals, plant for fire wood, for roofing and for generating heat by the local goldsmith. It has been reported that the Oil obtained from its fruit mesocarp and palm kernel oil of *E. guineensis* are administered as poison antidote and can as well be used externally with several other herbs as lotion for skin diseases. More so, the leaf extract and juice from young petioles are applied to fresh wounds (Sreenivassan *et al.*,2013) and the pulverised roots of this plant are added to drinks to treat gonorrhoea, menorrhagia and as a cure for bronchitis (Olasunkamin *et al*., 2018). In addition to the above, Nigerians used the root decoction for headaches (Sreenivassan *et al.*,2012). Thus far, despite previous documented literatures and vast ethnomedicinal uses of various parts of *Elaeis guineensis*, there is no report on detailed phytochemical studies on the waste shell of *Elaeis guineensis*. This is what prompted the investigation of the phytochemical composition of the shell extract of *Elaeis guineensis*, which to the best of our knowledge has not been studied previously. Therefore, the present research work aimed at screening for the presence of active phytochemicals in *Elaeis guineensis* shell waste and scientifically validate its medicinal properties which was misunderstood by traditional herbalist based on superstition.

2. MATERIAL AND METHODS

Sample collection and Identification

Fresh shell of *Elaeis guineensis* was collected from palm oil processing factory in Ilorin, Kwara State, Nigeria. The plant shell was then identified at the Department of Plant Biology Herbarium of the University of Ilorin, Ilorin, Nigeria and given voucher number, UILH/001/2019/880.

Sample preparation and Extraction

The *Elaeis guineensis* shell was thoroughly washed with domestic water from the water works, follow by distilled water and then air-dried in the laboratory. The dried sample was then pulverized with the aid of a mechanical grinder and was stored in a clean air-tight container prior to analysis.

A specific weight (500g) of the powdered dried shell was be subjected to cold extraction (Maceration) using methanol (MeOH) as the organic solvent for 72 hours. The shell extract was filtered through Whatman No.1 filter paper into a bottle and was concentrated using a rotary evaporator to obtain the crude methanolic shell extract.

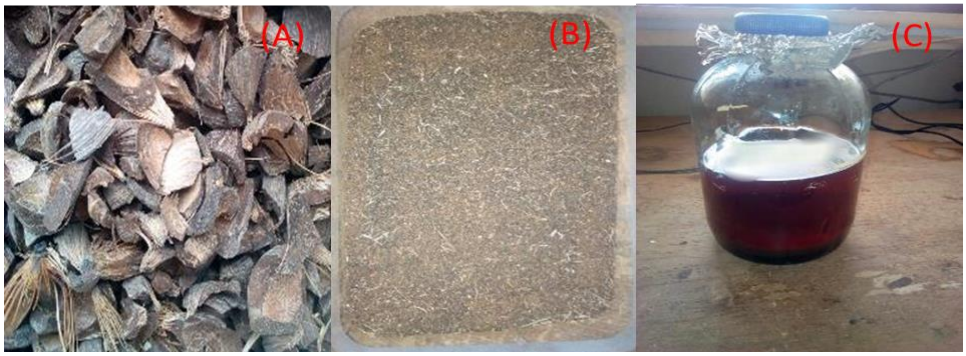

Figure 1: (A) Freshly collected *Elaeis guineensis* shell waste sample, (B) Pulverized sample, (C) Macerated sample

Qualitative Phytochemical Screening

A part of the crude extract was subjected to qualitative phytochemical screening to test for the presence of plant secondary metabolites using standard procedures described by Ayooal *et al.*, 2008; Oluwaniyi and Bazambo, 2014; Alaje *et al.*, 2014; Oluwaniyi and Oladipo, 2017; Nilugal *et al*., 2017 with slight modification.

Test for Alkaloids

To about 0.3g of the extract, 2% H_2SO_4 was be added and then filtered. Mayer’s reagent was then added to the filtrate. Formation of orange red colour shows the presence of alkaloids (Noorshilawati *et al.*, 2015).

Test for Anthraquinones

1g of the extract was dissolved in 2ml of distilled water, followed by addition few drops of 2 % hydrochloric acid. Appearance of red colour specifies the presence of anthraquinones (Praveen and Ashalatha, 2014).

Test for Anthocyanins

This was done by adding 3ml of 2M HCl and ammonia solution to a quota of the extract. The presence of pink-red colouration which turns blue-violet indicates the presence of anthocyanins (Savithramma *et al.*, 2011).

Test for Glycosides (Keller-Killiani test)

0.3g of the extract was treated with 3ml of glacial acetic acid containing one drop of ferric chloride solution. This was then under-layered with 1ml of concentrated sulphuric acid (H_2SO_4). Formation brown ring at the interface which indicates a positive test for glycosides (AzhaguRaj *et al.*, 2017).

Test for Coumarins

This was done by adding 5ml of 10% NaOH to a portion of the extract follow by addition of few drop of chloroform. The formation of yellow colouration indicates the presence of coumarins (AzhaguRaj *et al.*, 2017).

Test for Chalcones

To 2g of the extract, 3ml of Ammonium hydroxide was added to it. The formation of reddish colour shows the presence of chalcones (Khandekar *et al.*, 2013).

Test for Emodins

To 2g of the extract, 3 ml of NHOH and 5ml of Benzene was added. Appearance of red colour indicates the presence of emodins. (Savithramma *et al.*, 2011; AzhaguRaj *et al.*, 2017).

Test for Flavonoids

4 ml of dilute ammonia solution was added to a portion of the extract followed by addition of concentrated sulphuric acid (H_2SO_4). A yellow colouration indicates the presence of flavonoids (Khandekar *et al.*, 2013; Alaje *et al.*, 2014).

Test for Phenolic Compounds

This was done by dissolving 2 g of the extract in 10ml of distilled water, follow by addition few drops of neutral 5 % ferric chloride solution. Appearance of a dark green colour shows the presence of phenolic compounds (Khandekar *et al.*, 2013).

Test for Tannins

Few drops of 1 % lead acetate was added to 0.2 g of the extract and was observed for the formation of yellow precipitate (Savithramma *et al.*, 2011).

Test for Terpenoids (Salkowski test)

This was done by mixing 0.3 g extracts with 3 ml of acetic anhydride and concentration of H_2SO_4. Formation of blue-green rings indicates the presence of terpenoids (Savithramma *et al.*, 2011).

Test for Saponins

About 2g of the extract was boiled in 10ml of distilled water in a water bath and filtered. 5ml of the filtrate was further mixed with 3ml of distilled water and shaken vigorously for a stable resistant froth. The frothing was then mixed with 3 drops of olive oil, shake vigorously. The formation of emulsion shows the presence of saponins (Alaje *et al.*, 2014; Noorshilawati *et al.*, 2015; Tsado *et al.*, 2015).

Test for Steroids

To 2 ml of the extract, 2 ml chloroform was added, and then follow by addition few drops of concentrated sulphuric acid. A brown ring colouration indicates the presence of steroids (Praveen and Ashalatha, 2014).

Quantitative Phytochemical Analysis

A part of the crude sample was subjected to quantitative phytochemical analysis to estimate the quantities of the phytochemicals present using standard procedures with slight modification.

Determination of Alkaloids

5g of the sample will be weighed into a 250 ml beaker, and 200 ml of 10 % acetic acid in ethanol was added and then covered and allowed to stand for 4 hours. The solution was filtered, and the filtrate will be concentrated on a water bath to about three-quarter of the original volume. Concentrated ammonia solution will be added dropwise to the extract to precipitate the alkaloids. There resulting solution was allowed to settle, and the precipitate will be filtered and weighed (Omeh *et al.*, 2014).

Determination of Glycosides

For glycosides determination, 8ml of plant extract to a 100ml volumetric flask and 60ml of water and 8ml of 12.5% lead acetate was added, mixed and filtered. 50ml of the filtrate was transferred into another 100ml flask and 8ml of 47% Na_2HPO_4 was added to precipitate excess Pb^{2+} ion. This was mixed and make up to mark with water. The mixture was filtered twice through same filter paper to remove excess lead phosphate. 10ml of purified filtrate was transferred into clean Erlyn-Meyer flask and treated with 10ml distilled water and 10ml Baljet reagent. A blank titration was carried out using 10ml distilled water and 10ml Baljet reagent. This was allowed to stand for 1 hour for complete colour development. The colour intensity will be measured colorimetrically at 495nm and the totala glycoside was calculated using the equation below (El-Olemy *et al*., 1994).

% Total glycosides = $\frac{A \times 100}{77}$

Where A is the Absorbance.

Determination of Flavonoids

5g of the sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The solution was filtered. The filtrate was transferred into a beaker and evaporated to dryness over a water bath and weighed to a constant weight (Omeh *et al.*, 2014; Oluwaniyi and Bazambo, 2014; Oluwaniyi and Oladipo, 2017).

Determination of Tannins

The powdered sample (5g) was weighed into a conical flask and 100ml of 2M HCl was added. The content was boiled on a water bath for 30 minutes. The resulting solution was cooled and then filtered. The filtrate was then extracted twice with 40ml of diethylether. The ether extract was then concentrated to dryness and weighed and flavonoid content was calculated using the equation below (Okwu and Iroabuchi, 2004; Oluwaniyi and Oladipo, 2017).

Flavonoid content = $\frac{weight\ of\ dry\ residue}{weight\ of\ sample}$ x 100

Determination of Total Terpenoids

About 3g of the sample was weighed and soaked in 50ml of 95% aqueous ethanol for 1day. The extract was filtered and the filtrate was extracted with petroleum ether and concentrated to dryness. The dried ether extract was weighed and treated as total terpenoids (Oluwaniyi and Oladipo, 2017).

Determination of Total Phenolics

The sample (3g) was soaked in n-hexane in 200ml n-hexane for about 4 hours. The mixture will then be filtered and the procedure was repeated on the residue. This is performed to remove all the fat in the sample. The defatted sample was then extracted with diethyl ether. 10% NaOH solution and distilled water was then added to the diethyl ether extract in a separating funnel and the aqueous layer separated was acidified to pH of 4 by adding 10% HCl solution. 50ml of dichloromethane was used to finally extract the sample. The organic layer was collected, dried and weighed and the total phenolics content was calculated using the equation below (Oluwaniyi and Bazambo, 2014; Oluwaniyi and Oladipo, 2017).

Total phenolics content = $\frac{weight\ of\ dry\ residue}{weight\ of\ sample}$x100

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Determination of Saponins

5g of the sample was dispersed in 100ml of 20% ethanol. The suspension was heated and stirred continuously on a water bath for 3hours at about 55°C. The mixture was then filtered using Whatman No.1 filter paper and the residue re-extracted with another 100ml of 20% ethanol. The combined extract was concentrated to a volume of about 40ml over a water bath at about 90°C. The concentrate was washed with diethyl ether and extracted with n-butanol and the n-butanol extract was washed with 5%aqueous sodium chloride. The residual solution was firstly heated in a water bath and then dried in the oven to constant weight. The saponin content was calculated in percentage using the equation below (Omeh *et al.*, 2014; Oluwaniyi and Bazambo, 2014).

Saponin content = $\frac{weight\ of\ dry\ residue}{weight\ of\ sample}$ x 100

Statistical analysis

All the results were expressed as Mean ± S.D. Data analysis was done using SPSS software version 16.0.

3. RESULTS AND DISCUSSION

Phytochemical Screening

The phytochemical results in **Table 1,** revealed the presence of tannins, alkaloids, terpenoids, saponins, phenolics and flavonoids with anthraquinones, chalcones, emodins, coumarins and anthocyanins not being detected. **Table 2** shows that the amount of the phenolics content was found to be the highest (11.4 ± 0.2%) in *Elaeis guineensis* methanolic shell extract, followed by flavonoids (5.67 ± 0.23%), tannins (6.67 ± 0.12%), terpenoids (4.53 ± 0.12%), saponins (1.99 ± 0.01%) and alkaloids content (1.81 ± 0.03%) was found to be the least. The result obtained here was almost similar to that reported by Adeniyi and his coworkers in 2018 with phenolics content of 11.4 ± 0.1% and saponins of 1.99 ± 0.2%. Also our quantitative phytochemical result is in contrary to the statement made by Tsado research group in 2015 which accentuated that flavonoids are the most diversified groups of compounds present in plants, whereas phenolics was found to be the highest in this present studies.

Table 1: Qualitative phytochemical analysis of ethanolic shell extract *Elaeis guineensis*

Phytochemicals	
Tannins	++
Alkaloids	+
Terpenoids	+
Anthraquinones	-
Coumarins	-
Emodins	-
Chalcones	-
Saponins	+
Flavonoids	++
Glycosides	+
Steroids	-
Phenolic Compounds	++
Anthocyanins	-

Key: ++: strongly present, +: moderately present, -: absent

Table 2: Quantitative phytochemical analysis of *Elaeis guineensis*

Phytochemicals	% Composition (g/100g)
Tannins	6.67 ± 0.12
Alkaloids	1.81 ± 0.03
Terpenoids	4.53 ± 0.12
Phenolics	11.4 ± 0.2
Flavonoids	5.67 ± 0.23
Saponins	1.99 ± 0.01

Values are means ± standard deviations of triplicate determinations.

Most of these compounds are well known for their large spectrum of pharmacological properties with alkaloid having the most significant therapeutic efficiency (Tsado *et al.*, 2015). These pharmacological properties include antimicrobial (alkaloids and saponins) (Chong *et al.*2008; Ngbolua *et al*., 2014), anti-inflammatory, antifungal and antioxidant (tannins and flavonoids) (Subhanashini and Kantha, 2011; Barku *et al.*, 2013) activities. Saponins have natural tendency to ward off microbes which makes them good candidates of anti-inflammatory. These compounds serve as natural antibiotics, helping the body to fight infections and microbial invasions (Tsado *et al*., 2015).

Previously, triterpenoids and flavonoids have been known to promote wound healing processes mainly due to their astringent and antimicrobial properties, which seem to be responsible for wound contraction and increased rate of epithelialization (Subhanashini and Kantha, 2011, Sreenivasan *et al.*, 2010, Sreenivasan *et al.*, 2012). Hence, the identified constituents like triterpenoids, tannins, alkaloids and flavonoids from the *Elaeis guineensis* could possibly play a major role in the process of wound healing. Phenols are known to protect plants from oxidative damage and perform the same functions for humans (Okwu, 2005). The outstanding feature of phenols is their ability to block specific enzymes that cause inflammations (Okwu, 2005). Therefore, the presence of phenols and flavonoids that have been known for their good antioxidant activities in this plant could probably contribute to the wound healing potential of the shell extract of *Elaeis guineensis*. In addition, tannins act as free radical scavengers (Kar, 2007), giving credence to the fact that free radical scavenging action of plants as well as their antioxidant properties enhances wound healing (Kar, 2007). The tannins (6.67 ± 0.09%) (**Table 2**) present in *Elaeis guineensis* could also be in part responsible for the wound healing potential of the plant which agrees with the report of Nilugal *et al.*, 2017.

4. CONCLUSION

The findings in this research work showed that the methanolic shell extracts of *Elaeis guineensis* shell contain important phytochemicals that can combat various kinds of infection in human and can serve as antioxidant, anti-inflammatory, antifungal and antimicrobial agent. Thus, our findings suggest a baseline information that the shell of *Elaeis guineensis* is not a waste but can be very useful in traditional medicine. Also, our finding nullifies the traditional herbalist superstitious believe about *Elaeis guineensis* shell waste, and we can infer that the superstition was due to lack of insight about the medicinal properties of *Elaeis guineensis* shell. In addendum, this shell waste could possibly gain ground in wound management as well due to the antimicrobial, astringent, anti-inflammatory and antioxidant properties of various phytochemicals present as suggested in past and present documented literatures. Also, the ultimate utilization of this shell waste should be encouraged in order to enhance solid waste management as well as pollution control and thus converting waste to estimable material.

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