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Research Article

Investigation of the Phytochemical Parameters in Ethyl-Ester of Rubber Seed Oil from Imo Rubber Estate, Obiti-Ohaji, Nigeria

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Abstract

The present study focuses on the determination of the phytochemical parameters in the extract (ethyl ester) of Rubber Seed obtained from the Imo Rubber Estate, Obiti-Ohaji. The oil of the rubber seed was extracted by means of solvent (Petroleum ether), and subsequently subjected to qualitative and quantitative analysis of the phytochemical characteristics. The percent alkaloid was found to be 21%, while that of flavonoid is 43.5%. Also, the cardiac glycocides content of the oil extract is 28%, while that of saponins is 11%. The study sample also exhibited a low percentage tannic acid and that of phytate content, in the tune of 2% and 0.2088% respectively. The phenol content was discovered to be 1.605mg of the extract/100g of the seed. Phytochemical Properties, exhibited by plants, show Biochemical significances by playing essential roles in the plants to defend them against various pathogenic microbes and dangerous toxins. The secretion of these compounds varies from plant to plant. Evidence (from laboratory studies) shows that phytochemical parameters in fruits and vegetables can reduce and/or inhibit the corrosive effect inflicted on flowpipes by acid media. However, the present study sample was likened to possessing high ability to inhibit metallic corrosion owning to its high flavonoid and cardiac glycocides contents.

Keyword: *Investigation, Phytochemical Parameters, Ethyl-ester, Rubber Seed*

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Introduction

Phytochemicals are naturally present in plants, and they show biochemical significance by playing key roles in several biological and chemical applications. These properties can be found either in the leaves barks and/or seeds of the plant (Praveen *et al*, 2010). In recent years, useful impacts have been observed, at varying dimensions, in trado-medicals, nutraceuticals, food supplements, pharmaceuticals, intermediate bioactive applications and enhancement of the integrity of industrial materials (Falodun and Agbakwuru, 2004). In line with this development, production of rubber seed oil has shown a huge increase in both quantity and quality in response to its increased demand for industrial applications. Aigbodion *et al* (2000) reviewed that there is a compelling reason to explore the commercial and pharmacological benefits of low priced and unconventional sources of rubber seed oil, such as exploration of inexpensive sources of the oil, to better the economy of the world. But the rubber tree (*Hevea brasiliensis*) is exploited in Nigeria mainly for the latex, in view of its economic importance, while the auxiliary products (such as the wood and the seeds) are mostly neglected. Of these two auxiliary products, the seeds have the greatest potential and are in abundance in the country, as they are usually seen scattered all over the rubber plantations in our localities.

The yield of rubber seeds per annum in the plantations is estimated to be from 100 to 150kg/ha, even though this estimate could be influenced by factors such as abnormal leaf and phytophora diseases, species and weather (Aigbodion *et al*, 2003; George *et al*, 2006). Varying laboratory investigations have shown that rubber seed is reach in oil, whose content (in dried kernel) varies from 35 to 45%. In this documentations, rubber seed has been found to have potential applications in many areas amongst which are in the production of biodiesel, production of foaming agent in latex foam, synthesis of alkyd resin for coatings, corrosion inhibition applications, partial substitutes to mineral oil as carrier cupper fungicide in management of abnormal leaf fall disease of rubber, and so on. (Asuquo *et al*, 2012; Aigbodion and Pillai, 2000; Ramadhas *et al*, 2005; Ikwuagwu *et al*, 2000).

However, the extraction, characterization and different analysis of oils from rubber seed have been carried out extensively from samples obtained from different geographical origin. It is, thus, necessary to obtain specific data at any given moment for sample of oil from a given area, because there is always a range of variation in the phytochemical properties of oils due to environmental factors such as rain-fall, soil composition, agronomic practices, maturation period and genetic dispositions. In line with this, the

present investigation for phytochemical properties of oil from rubber seed is centered on the seed obtained from the Imo Rubber Estate, Obiti-Ohaji, in order to obtain specific composition of the sample from this origin.

Materials and Method

Sample Collection

The rubber seeds used for this study were collected (picked) from the Imo Rubber Estate, Obiti-Ohaji in the Ohaji/Egbema L.G.A. of Imo State, Nigeria. The seeds were de-shelled and sun-dried for forty-eight (48) hours, after which they were collated and ground to finer forms by means of electric blender.

Oil Extraction

The ground rubber seed was impregnated in a solution of petroleum ether for 18hours, and the mixture was subjected to mechanical pressure. The solvent was sequentially evaporated from the mixture (of oil and petroleum ether) at the temperature of 60^{o}C, using a soxhlet extractor, and the oil was then collected as a reflux.

Degumming

Hot water method of degumming was employed in this regard, as this stands to be the best way of dealing with any possible traces of the used solvent present in the mixture. 1ml of the hot water was added to 50ml of the oil sample, and the mixture is placed in a water bath at 60^{o}C for 10minutes. At the elapse of the time, the mixture was then placed under the action of a centrifuge at 80rpm for 5minutes. The process tactically separates the gums (formed) from the pure sample of the oil, which was isolated by simple decantation.

Qualitative Analysis of the Phytochemical Characteristics

Qualitative Analysis involves the determination of the simple presence of the properties in question, without knowing their actual degree of existence; it is based on the principle of simple observation of the sample behavior for a given logical inference. The methods employed in the present study are as contained in Onyeike and Acheru (2002).

Test for Alkaloid: 0.5g of each of the test samples (CSO and RSO) was treated with four (4) drops of potassium mercuric iodide solution (Dragendroff's reagent), and a cream colouration was observed, which indicates the presence of Alkaloid.

Test for Tannins: 0.5g of the sample was boiled in 20ml of distilled water in a test tube (at the boiling point of water), after which it was removed and filtered into a conical flask by means of filter paper. 0.1% $FeCl_3$ was then added to the filtrate and reddish colour change was observed, which indicates the presence of Tannins.

Test for Saponins: 0.5g of the sample was boiled with 20ml of distilled water (in a water bath), and is removed and then filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously, to obtain stable persistent froth. The frothing was then mixed with 3drops of olive oil and observed for the formation of emulsion; the formation of emulsion indicated the presence of saponins.

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Test for Flavonoid: 3drops of 1%-ammonia (NH_3) solution was added to the sample (oil extract) in a test tube, and a pay-yellow precipitate was observed, which indicated the presence of Flavonoid.

Test for Phenol: 5g of the oil extract was boiled with 2ml of phenol in a water bath, and then filtered. 2ml of the filtrate was pipetted into a conical flask, and 10ml of distilled water was added. A brownish colour was observed in each case, and this indicates the presence of Phenol.

Test for Cardiac Glycocides: 1ml of concentrated H_2SO_4 was prepared in a test tube. Then 5ml of the oil extract was mixed with 2ml of glacial CH_3CO_2H, containing 1drop of $FeCl_3$. The mixture was carefully added to the 1ml of concentrated H_2SO_4 so that the concentrated H_2SO_4 is underneath the mixture; observation for a brick-red precipitate was observed, which indicates the presence of cardiac glycocides.

Test for Phytate: 5ml of the test sample was soaked in 100ml of 2% concentrated HCl for 2hrs, after which the sample was filtered. 5ml of the filtrate was then put into a 250ml beaker, and 100ml of distilled water was added to it. A brownish colour was observed, and this indicates the presence of Phytate.

Test for Oxalate: Four drops of methyl red indicator were added to 125ml of the oil, and Calcium Chloride solution was added to the mixture drop-wise until a clear change in the salmon-pink colour was observed and noted accordingly; the colour change indicates the presence of oxalate.

Quantitative Analysis of the Phytochemical Characteristics

The quantitative analysis involves the analysis for the actual amount of the variables present in the study sample. It refers to the analysis in which the analyte may be determined (estimated) and expressed as a numerical value in appropriate unit. Quantitative analysis requires the identification (quantification) of the analyte for which numerical estimates are given.

Determination of Alkaloid Content: 5g of the sample (rubber seed oil) is weighed into a beaker, and 200ml of 10% acetic acid (in ethanol) is added to the beaker. The mixture is covered and allowed to stay for 4hrs, after which it is filtered and the filtrate was concentrated by means of water bath to one-quarter of the original volume. Then concentrated ammonium hydroxide solution (NH_4OH) is added drop-wise until precipitation is completed. The whole solution is allowed to settle and the precipitate is collected, washed with dilute NH_4OH and then filtered. The residue is the alkaloid, which is dried in an oven, weighed and recorded.

Determination of Tannins Content: The quantity of tannin was determined using Follins Denn's titration method as prescribed by Pearson (1974). 20g of the rubber seed oil was inoculated in a conical flask, and 100ml of petroleum ether was added and is covered for 24hrs. The mixture was then filtered and exposed for 15minutes to allow traces of the petroleum ether to evaporate. It was then soaked in 100ml of 10% acetic acid in ethanol for 4hrs, after which it was filtered. 25ml of NH_4OH solution was added to the filtrate to precipitate the alkaloids, which were heated in electric hot plate to remove any traces of NH_4OH still in the solution. 20ml of ethanol was added to the mixture and it was titrated with 0.1M solution of sodium hydroxide (NaOH), using phenolphthalein as indicator. Appearance of pink colour indicates the end point.

$$\text{The tannin content, } C_1 = \frac{C_2 V_2}{V_1} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \qquad (1)$$

Where, C_2 = Molar Conc. of NaOH = 0.1M
V_1 = Vol. of Tannic Acid = 5ml
V_2 = Vol. of NaOH = Titre Value; and

$$\%\text{Tannic Acid Content} = \frac{C_1 \text{ X } 100}{m} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \qquad (2)$$

Where m = Mass of sample analyzed.

Determination of Saponins Content: This described by Harbone (1975) was employed here. 5g of the rubber seed oil is weighed into a beaker containing 20% acetic acid (in ethanol) and allowed to stand in a water bath at 50^{o}C for 24hrs. This was filtered, and the filtrate was concentrated using a water bath to one-quarter of its original volume. Concentrated NH_4OH was then added drop-wise to the mixture until precipitation is completed. The whole solution was allowed to settle, and the precipitate is collected (through filtration) and weighed. The saponins content (expressed in percentage) is given by:

$$\%\text{Saponin Content} = \frac{m_2 \text{ X } 100}{m_1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

Where m_1 = mass of Sample analyzed
m_2 = mass of residue (after filtration)

Determination of Flavonoid Content: 10g of the rubber seed oil was inoculated into a beaker containing 100ml of 80% aqueous methane at room temperature. The whole solution was filtered (by means of filter paper), and the filtrate was transferred to a water bath and evaporated to dryness. The residue was then weighed and noted accordingly. The flavonoid content (expressed in percentage) is given by:

$$\%\text{Flavonoid Content} = \frac{m_2 \text{ X } 100}{m_1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4)$$

Where m_1 = mass of Sample analyzed
m_2 = mass of residue (after distillation)

Determination of Phenol Content: The quantity of phenol in the sample was determined using the spectrophotometer method. 2g of the rubber seed oil sample was combined with 50ml of ethanoic acid and allowed to boil for 15minutes. Then 5ml of the boiled sample was pipetted (after cooling) into a 50ml conical flask, and 10ml of distilled water is added to it. Also 2ml of NH_4OH and 5ml of concentrated pentanol were added to the mixture. After insuring thorough mixture of the reagents, the sample was made up to the reference mark of the flask and allowed for 30minutes for possible colour change; it was then collected and viewed in a U.V. spectrophotometer (at 505nm wavelength). The reading represents the phenol content, and was noted accordingly (in mg of solution per 100g of sample).

Determination of Cardiac Glycocides Content: Osagie (1998) was adopted in this determination.1ml of the rubber seed extract is added to 1ml of 2% solution 3,5-Dinitro Salicylic Acid (DNS) in methanol and 1ml of 5% aqueous NaOH. It was boiled for about 2minutes (until a brick-red colour is observed), and the boiled sample is filtered (haven taken note of the weight of the filter paper used). The absorbed residue was then dried, to dryness, in an oven at 50^{o}C. The weight of the dried residue is evaluated and noted accordingly, and the percentage cardiac glycocides content is given by:

$$\% \text{ Cardiac Glycocide Content } = \frac{m_2 \text{ X } 100}{m_1} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (5)$$

Where m_1 = mass of Sample analyzed
m_2 = mass of residue (after drying to dryness)

Determination of Phytate Content: The procedure adopted in determination is as prescribed by Young and Greaves (1990). 2g of the rubber seed oil is introduced into a flask, and was soaked in 100ml of 2% hydrochloric acid (HCl) for 3hrs, after which the mixture was filtered. 50ml of the filtrate was placed in a 250ml beaker and 100ml of distilled water was added. Also 10ml of 0.3% ammonium thiocyanate solution was added as indicator, and the mixture was titrated with Iron(III) Chloride solution which contains 0.00195g of Iron per ml. The percentage phytic acid content is given by:

$$\%\text{Phytic Acid Content } = \frac{V\ C\ (0.00195)\ X\ 100}{m} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (6)$$

Where V = Titre Value
C = Molar Conc. of Fe_3Cl = 1.19M
m = mass of sample analyzed

Determination of Oxalate Content: This was determined according to Osagie (1998). The procedure involved three major steps namely: Digestion, Precipitation and Permanganate titration. For the digestion, 2g of the sample was suspended in 190ml of distilled water in a 250ml volumetric flask. 10ml of 6% HCl is added, and the suspension digested at 100^{o}C for 1hr. At the elapse of the time, the mixture is cooled, and then made up to the 250ml mark before filtration.

Oxalate precipitation involves the measurement of duplicates of 125ml of the filtrate into beakers, and four drops of methyl red indicator are added. This is followed by the addition of NH_4OH solution (drop-wise) until the test solution changes from salmon-pink colour to a faint-yellow colour. Each portion is then heated to 90°C, cooled and filtered (to remove precipitate containing ferrous ion). The filtrate is, again, heated to 90°C, and 10ml of 5% $CaCl_2$ solution is then added while being stirred constantly. After heating for about 5minutes, it is allowed to cool overnight. By the next day, the solution is then centrifuged at 250rpm for 5minutes, after which the supernatant is decanted while the precipitate is completely dissolved in 10ml of 20% H_2SO_4 solution.

Finally, in permanganate Titration, the total filtration resulting from digestion of 2g of the rubber seed oil sample is made up to 300ml. Aliquots of 125ml of the filtrate is heated until it is near boiling, before it is removed and titrated against 0.05M of standardized Potassium permanganate ($KMnO_4$) solution to faint pink colour, which persist for 30sec. The oxalate content is given (in mg of solution per 100g of sample) as:

$$\text{Oxalate Content} = \frac{(T)(V_{me})(D_f)\ X\ 10^5}{M_E\ X\ M_F} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (7)$$

Where T = Titre Value
V_{me} = Molar mass/Equivalent of $KMnO_4$ *(0.00225g)*
M_E = Molarity/Equivalent of $KMnO_4$ *(0.5)*
M_F = Mass of sample analyzed *(2g)*
D_f = Oxalate factor *(125)*

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Results and Discussion

The results of the qualitative analysis are contained in Table 1, while those of the quantitative analysis are contained in Table 2 as presented below.

Table 1: Results of Qualitative Screening of the Study Sample

S/N	PARAMETERS	OBSERVATION	DEGREE OF PRESENCE
1	Alkaloid	Reddish-brown colouration	+ +
2	Tannins	Brownish-green colouration	+
3	Saponins	Formation of Emulsion	+
4	Flavonoid	Yellowish colouration	+ + +
5	Cardiac Glycocides	Appearance of brown ring	+ + +
6	Phenol	Milk colouration	+ + +
7	Phytate	Wine colour appearance	+ +
8	Oxalate	Faint-yellow colouration	+ +

Table 2: Results of Quantitative Screening of the Study Sample

S/N	PARAMETERS	RESULTS
1	% Alkaloid Content	21.00
2	% Tannic Acid Content	2.00
3	% Saponins Content	11.00
4	% Flavonoid Content	43.50
5	% Cardiac Glycocides Content	28.00
6	Phenol Content (mg/100g)	1.61
7	% Phytate Content	0.21
8	Oxalate Content (mg/100g)	59062.50

It could be observed, from Table 1, that there is little presence of tannin and saponins contents (+), when compared with other parameters assessed. The study, categorically, clarifies the fact that qualitative assessment data are not enough to deduce for the degree of presence of a given phytochemical property of a study sample. This could be demonstrated on the heavy presence of phenol content (+ + +) of the rubber seed oil (under study), in contrast to the low quantitative value (1.61%). However, the rubber seed oil indicated the presence of all the variables investigated at varying degrees, representing an embodiment of carbonyl attachment to an ether linkage. Undiandeye *et al* (2014) reviewed that the ethyl esters of a given oil sample demonstrates its

characteristics of corrosion inhibition. Also, Undiandeye *et al* (2011) reported that ethyl esters of non-edible oils usually possess high percentage contents of Flavonoid and Cardiac Glycocides than their edible counterparts. The report went further to review that these features increase the chances of edible oils for greater applications in corrosion inhibition. In line with this, the results of the quantitative/qualitative variables of the rubber seed oil sampled in the present study indicates that it would make a good corrosion inhibitor for protection of our materials.

Conclusion

Analysis of the phytochemical parameters of plant extracts is necessary to identify their inherent properties for useful biological and chemical applications. The present study shows that quantitative screening of samples gives more reliable information than the qualitative analysis, which is, more or less, mere observations. The study also demonstrated that seed oils obtained from rubber trees at Obiti-Ohaji Imo State geographical location are rich in Flavonoids and Cardiac Glycocides, and as such could serve as good inhibitors for metallic materials.

References

Asuquo J.E., Anusiem A.C.I. and Etim E.E. (2012). Extraction and Characterization of Rubber Seed Oil. *International Journal of Modern Chemistry*, 1(3): 109-115.

Aigbodion A.I., Okiemen F.E., Obazee E.O. and Bakare I.O. (2003). Utilization of Malenilized Rubber Seed Oil and its alkyd Resin as Binders in Waterborne Coatings; *Prog. Org. Coat.* (43): 28-31.

Aigbodion A.I., Menon A.R.R. and Pillai C.S.T. (2000). Processibility Characteristics and Physio-mechanical Properties of Natural Rubber Modified with Rubber Seed Oil and Epoxidized Rubber Seed Oil; *Journal of Applied Polymer Science;* 7(7): 1413-1418.

Aigbodion A.I. and Pillai C.S.T. (2000). Preparation, Analysis and Applications of Rubber Seed Oil and its Derivatives in Surface Coatings; *Prog. Org. Coat.* (38): 187-192.

Falodun A. and Agbakwuru E.O.P. (2004). Phytochemical Analysis and Laxative Activity of the Leaf Extracts of *Euphobia-heterophylla Linn*; *Pak. Journal of Sci. Ind. Res.*; 47(5): 345-348.

George K.T., Reghu C.P. and Nehru C.R. (2006). By-Products and Auxiliary Source; In: Natural Rubber Agro-Management and Crop Processing; P.J. George and C.F. Jacob (eds.); *Rubber Research Institute of India*; Kottayam: pp 504-520.

Harbon J.B. (1995). Phytochemical Methods: a Guide to Modern Techniques of Plant Analysis, 3rd Edition; *Chapman and Hall*, New York.

Harbon J.B. (1973). Phytochemical Methods; *Chapman and Hall*, New York.

Ikwuagwu O.E., Ononogbo I.C. and Njoku O.U. (2000). Production of Biodiesel using Rubber Seed Oil; *Ind. Crop Prod.* (12): 57-62.

Onyeike E.N. and Acheru G.N. (2002). Chemical Composition of Selected Nigerian Oil Seeds and Phytochemical Properties of the Oil Extracts; *Journal of Food Chemistry*; 7(6): 431-437.

Osiagie A.U. (1998). Anti-nutritional Factors; In: Nutritional Quality of Plant Foods; *Ambik Press*, Benin City-Nigeria.

Praveen N., Nayak S., Kar D.M. and Das P. (2010). Pharmacological Evaluation of Ethanoic Extracts of the *Alternan-therasessilis* Plant against Temperature Regulation; *Journal of Pharmacological Research*; 3(6): 1381-1383.

Ramadhas A.S., Jayaraj S. and Muraleedharan C. (2005). Characterization and Effect of using Rubber Seed Oil as Fuel in the Compression Engines; *Renewable Energy*; (30): 795-803.

Undiandeye J.A., Usman H.T, Abubakar M. and Offurum J.C. (2014). Kinetics of Corrosion of Mild Steel in Petroleum-Water Mixture using Ethyl Esters of Lard as Inhibitor. *International Journal of Engineering and Science*; 3(4): 18-26

Undiandeye J.A., Okewale A.O., Etuk B.R. and Igbokwe P.K. (2011). Investigation of the use of Ethyl Esters of Castor Seed Oil and Rubber Seed Oil as Corrosion Inhibitors. *International Journal of Basic and Applied Sciences*; 11(6): 48-54.