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Comparative Study on the Extraction of Phytochemicals (*Saponin*) from Velvet tamarind (*Dialium guineense*) and Siam weed (*Chromolaena odorata*) in Enugu State, South Eastern Nigeria

Okoro, A.C[1]., Orakwe, L.C[1]., Nwachukwu, C.P[1*]., Ugwu, E.I[2]., and Umobi, C.O[1]

[1]Agric and Bio Resources Engineering, Nnamdi Azikiwe University, Awka
[2]Agric and Bio Resources Engineering, University of Nigeria, Nsukka

[*]Corresponding Author's E-mail: cp.nwachukwu@unizik.edu.ng

Abstract
This study tries to compare the phytochemical composition of the leaves of Velvet tamarind (*Dialium guineense*) and Siam weed (*Chromolaena odorata*). It also specifically compared the saponin contents of the leaves of these two plants. The leaf samples were collected from a forest in Emene, Enugu State and were taken to the labouratory for analytical procedure. The plant leaves were subjected to qualitative and quantitative screening for phytochemicals. The result of the qualitative analysis indicated the presence of phytochemicals such as Phenol (++), Alkaloids (+), Saponin (+), Tannin (++), Flavonoids (++) and Glycoside (+) at varying levels in Velvet tamarind (*Dialium guineense*) while the result of the quantitative analysis of Siam weed (*Chromolaena odorata*) leaves indicated the presence of phytochemicals such as Alkaloid (++), Saponin (++), Tannin (+) and Glycoside (++). The result of the quantitative analysis indicated the presence of phytochemicals in mg/g such as Phenol (0.433), Alkaloids (0.0276), Saponin (0.0378), Tannin (0.3867), Flavonoids (0.3781) and Glycoside (0.0892) at varying levels in Velvet tamarind (*Dialium guineense*) while the result of the quantitative analysis of Siam weed (*Chromolaena odorata*) leaves indicated the presence of phytochemicals such as Alkaloid (0.3234), Saponin (0.15433), Tannin (0.1278) and Glycoside (0.4234). Generally, saponin from Siam weed (*Chromolaena odorata*) leaves had higher quantity when compared to that of Velvet tamarind (*Dialium guineense*). The result of the FTIR analysis conducted showed that the functional group of the extracted saponin had close similarity with that of a standard saponin. It is recommended that Siam weed (*Chromolaena odorata*) leaves be employed during extraction of saponin for industrial uses.

Keywords: Phytochemicals, saponin, Siam weed, Velvet Tamarind, Qualitative analysis.

1. Introduction

Siam weed and Velvet tamarind leaves have been found to contain good amount of saponin and other phytochemicals and can serve as a more readily available and cheap source of saponin in South Eastern Nigeria. This study became imperative because plant-based surfactants have been found to be very useful in industries for cleaning and in agriculture for soil washing of heavy metals from contaminated soils. Chromolaena odorata (L) King and Robinson belongs to the family Asteraceae and is known locally in Nigeria through many common names as Awolowo, siam weed, Elizabeth weed, obirakara, olorohuru and independence weed (Ngozi and Theresa, 2014). It is a fast-growing perennial invasive weed native to Nigeria 50years ago. In Nigeria, the weed is found growing along road side, waste and fallow. It is an aggressive competitor that occupies different types of lands where it forms dense strands that prevents the establishment of other flora (Harini *et al.,* 2014).

Phytochemicals that can be found in plants as plant chemicals have protective and preventive properties against diseases (Breslin, 2017). The presence of numerous bioactive chemicals in plants has been revealed by many studies including alkaloids, steroids, flavonoids, phenols, glycosides, and saponins (Banothu *et al.,* 2017). Phytochemical

analysis of C. odorata leaves showed that it contained alkaloids, flavoloids, saponins, tannins, and steroids. The presence of secondary metabolites such as alkaloids, saponins, tannins, flavonoid attest to its medicinal values (Usunobun and Ewere, 2016). The saponin content of Siam weed leaves comes in different quantity as contained in different literatures and varies from plant to plant.

Velvet tamarind also known as black velvet is a common name for *Dialium guineense*, a genus of a legume belonging to the family of Fabaceae and sub-family of Caesalpinioideae. The pulp of the fruit is edible and sweet, with fairly low levels of ascorbic acid and tannin. It is a fairly good source of protein and minerals (Awotedu *et al.,* 2020). Velvet tamarind (*Dialium guineense*) can be found in West African countries such as Ghana where it is known as Yoyi, Sierra Leone, Senegal, and Nigeria where it is known as Awin in Yoruba, Icheku in Igbo and Tsamiyar kurm in Hausa (Bessong *et al.,* 2016). Abu *et al.,* (2020a) and Abu *et al.,* (2020b) found that *D. guineense* contains high levels of phenols, flavonoids, saponins, and tannins, which may explain why it is used in traditional medicine. The phytochemical analysis of ethanol leaf extract of *D. guineense* revealed the presence of alkaloids, proteins, carbohydrates, glycosides, phenols, resins, saponins, tannins and terpenoids (Eucharia *et al.,* 2022). Previous studies have shown that *D. guineense* leaves contains saponins which are presumed to add to the cleaning effect of teeth and prevent caries and plaque. (Alagbe *et al*., 2020).

Majority of available articles for saponin extraction from a plant-based source is with soapberry and shikakai plants which are not readily available in South Eastern part of Nigeria. Velvet tamarind (*Dialium guineense*) and Siam weed (*Chromolaena odorata*) are both more readily available and cheaper sources of saponin when compared to the aforementioned sources. The objective of this research is to ascertain the presence and quantity of some phytochemicals in the leaves of Velvet tamarind (*Dialium guineense*) and Siam weed (*Chromolaena odorata*) from Emene, Enugu State, South Eastern Nigeria with keen interest on saponin.

2.0 Material and methods

2.1. Collection of Plant Material.

The Plant materials Siam weed leaves and Velvet Tamarind leaves were collected around Emene, in Enugu State. The plants were identified and authenticated at the plant labouratory of the Department of Agric Technology, School of Agriculture, Enugu State Polytechnic, Iwollo. It was immediately taken to Project Development Institute's (PRODA) labouratory for qualitative and quantitative procedures.

2.2. Qualitative Screening of the Phytochemicals in the Plants Investigated

According to (Shaikh and Patil, 2020), the ethanolic extract was used to perform the phytochemical screening using standard methods, for the detection of the following:

2.2.1 Screening for Alkaloids (Mayer's Test)

1ml of the extract was measured into a watch glass and little amount of dilute hydrochloric acid and Mayer's reagents were added to the solution; the formation of a white precipitate indicated the presence of alkaloids.

2.2.2. Screening for Flavonoid (Shindo's Test)

1.3ml of the extract was mixed with 0.5g of magnesium turnings; the mixture was boiled for 5minutes; the appearance of orange to red colour indicated the presence of flavonoid.

2.2.3. Screening for Phenol

A few drops of ferric chloride solution were added to 2ml of the extract in a watch glass; the appearance of bluish green colour indicated the presence of phenol.

2.2.4. Screening for Saponin (Frothing Test)

2.5ml of the extract was mixed with a few drops of distilled water and the mixture was shaken vigorously, a cupious lather formation was noticed which indicated the presence of saponin, and the absence of the cupious lather.
meant the absence of saponin.

2.2.5. Screening for Tannin (Wohler's Test)

A few drops of basic lead acetate solution were added to 1.6ml of the extract; the appearance of a white precipitate indicated the presence of tannin in some of the plant extracts.

2.2.6. Screening for Glycoside
2.5ml of the extract was mixed with a little quantity of anthrone on a watch glass, one drop of concentrated sulphuric acid was added and made into a paste and heated gently over a water bath; a dark green colouration indicated the presence of glycoside.

2.3. Quantitative Screening of the Phytochemicals in the Plants Investigated
According to (Ajurum, 2017), the method below was adopted in the quantitative analysis of the following phytochemicals;

2.3.1. Alkaloid Determination
5g of the sample were weighed into a 250ml beaker and 200ml of 20% acetic acid in ethanol was added and covered to stand for 4 hours. This was filtered and the extract was concentrated using a water-bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the preparation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed.

2.3.2. Tannin Determination
500 mg of the sample was weighed into 100 ml plastic bottle. 50 ml of distilled water was shaken for one hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipette out into a tube and mixed with 3 ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured in a spectrophotometer at 120nm wavelengths, within 10 minutes. A blank sample was prepared and the colour also developed and read at the same wavelength. A standard was prepared using tannin acid to get 100 ppm and measured.

2.3.3. Flavonoid Determination
100g of the plant sample were extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed.

2.3.4. Saponin Determination
The samples were ground. 20g of each plant samples were dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 hours with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined nbutanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponins content was calculated in percentage.

2.3.5. Phenol Determination
For the extraction of the phenolic component, the fat free sample was boiled with 50 ml of ether for 15 minutes. 5 ml of the extract was pipette into a 50 ml flask, and then 10 ml of distilled water was added, 2 ml of ammonium hydroxide solution and 5 ml of the extract was pipette into a 50 ml flask, and then 10 ml of distilled water was added, 2 ml of ammonium hydroxide solution and 5 ml of concentration amyl alcohol were also added. The samples were left to react for 30 minutes for colour development. The absorbance of the solution was read using a spectrophotometer at 505 nm wavelengths.

2.4. FTIR Analysis
The functional groups of the extracted saponin were studied using Buck M-530 Fourier-Transform Infrared Spectroscopy (FTIR). The sample was introduced into potasium bromide (KBr) before it was placed in the FTIR spectra where it attained a wave-range of 500-4,000cm^{-1}

3.0 Result Presentation and Discussion
The result of the phytochemical screening of the leaves of Velvet tamarind (*Dialium guineense*) and Siam weed (*Chromolaena odorata*) were as summarized in tables 1 and 2. Table 1 and 2 shows the result of the qualitative and quantitative analysis of Velvet tamarind (*Dialium guineense*) and Siam weed (*Chromolaena odorata*) respectively.

3.0 Results and Discussions

The result of the phytochemical screening of the leaves of Velvet tamarind (*Dialium guineense*) and Siam weed (*Chromolaena odorata*) were as summarized in tables 1 and 2. Table 1 and 2 shows the result of the qualitative and quantitative analysis of Velvet tamarind (*Dialium guineense*) and Siam weed (*Chromolaena odorata*) respectively.

3.1 Qualitative Analysis

The phytochemical screening of the leaves of Velvet tamarind (*Dialium guineense*) and Siam weed revealed that they contain alkaloids, flavonoids, phenols, saponin, Glycosides and tannin at various degrees (table1), while steroid was found to be absent from the leaves of the two plants. The Presence of these phytochemicals had also been observed in other plants (Usunobun and Ewere, 2016). King *et al.*, (2017), also reported the presence of alkaloids, saponins, glycosides, flavonoids, tannins, phlobatannins, steroids and terpenoids in C. odorata parts. However, the present study showed absence of flavonoid, steroid, phenol, terpenoids and phlobatannins. Table 1 showed that saponin is positive $(++)$ for Siam weed leaves, this is high when compared to the result of the works of Anyadoh-Nwadike *et al.*, (2019) which showed that saponin is positive $(+)$ but, same with that of Velvet tamarind which is positive $(+)$.

With exception to steroids, the result of this work is also in tandem with work of Gloria *et al*., (2022). The result of this work on Velvet Tamarind also agrees with the result of Siti and Angelle, (2023) on Siam weed. It recorded the presence of secondary metabolites such as flavonoids, phenols, saponin and tannins. The presence of saponin in Siam weed recorded a medium presence while Velvet tamarind (*Dialium guineense*) recorded a low presence (table 1).

Table 1: **Result of Qualitative Phytochemical Analysis**

Phytochemicals	Velvet Tamarind	Siam Weed
Alkaloids	+	++
Saponin	+	++
Flavonoid	++	-
Steroids	-	-
Phenol	++	-
Tannins	++	+
Glycosides	+	++

-(not detected); + (Low presence); ++(medium presence); +++(High presence)

3.2 Quantitative Phytochemical Analysis

Quantitative phytochemical analysis as shown in table 2 revealed that the presence of Phenol was highest in the leaf of Velvet tamarind (*Dialium guineense*) (0.433 mg/g) when compared to other phytochemicals, while Glycoside had the highest value in Siam weed leaf (0.4234 mg/g) when compared to other phytochemicals. Saponin had the highest value in Siam weed leaf (0.1543mg/g) when compared to that of Velvet tamarind (*Dialium guineense*) (0.0378 mg/g), the results of saponin in this study were found to be low when compared to the result of Agaba. and Fawole, (2016) who recorded 331.7mg/g of saponin in Siam weed leaves. According to the work of John, *et al.*, (2022), Siam weed and Tarmarind velvet leaves recorded $1.74 \pm 0.03\ (mg/g)$ for saponin, which is closely related to the result of this study. The result of this work on saponin is in tandem with the result of Ugwoke et al., (2017). Bamisaye *et al.*, (2014) recorded a low value for saponin when compared to the result of this work.

Table 2: Result of Quantitative Phytochemical Analysis

	Velvet Tamarind (mg/g)	Siam weed (mg/g)
Alkaloids	0.0276	0.3234
Saponin	0.0378	0.1543
Flavonoid	0.3781	-
Phenol	0.433	-
Tannins	0.3867	0.1278
Glycosides	0.0892	0.4234

3.3. FTIR Spectra Result.

The presence of saponin was confirmed by the FTIR spectra, which showed absorptions characteristic of some functional groups at different frequencies. Absorption bands of 3,834.449cm^{-1}, 3,506.994cm^{-1} and 3,303.62cm^{-1}, indicates the presence of a hydrate (H2O), hydroxyl -OH compound while it is 3281cm^{-1} for a standard saponin (Happiness *et al.*, 2022). This also tallies with the work of Ugwu *et al.*, (2019). Bands of and 3001.123cm^{-1} indicates the presence of stretch alkyne C-H stretch. Bands of 2,870.087cm^{-1} indicates the presence of methyl C-H stretch (-CH_3) and bands of 2,731.087cm^{-1} indicates the presence of methylamino, N-CH_3, C-H stretch. Band of 2594cm^{-1} indicates the presence of thiols (S-H stretch) while bands of 2266.704 cm^{-1} and 2,164.605cm^{-1} indicates the presence of C≡C Medial alkynes. Bands of and 2,050.404cm^{-1}indicates the presence of C≡C terminal alkynes. The absorbance at 1,951.47cm^{-1} and 1,875.542cm^{-1} indicates the presence of carbonyl compound while the standard saponin gave 1727 cm^{-1} (Sharma *et al.*, 2012). Band of 1,711.722cm^{-1} indicates the presence of ketones, aldehydes and esters and 1,570.03cm^{-1} indicates the presence of aromatic rings. Bands of 1,471.794cm^{-1} and 880.4996cm^{-1} indicates the presence of carbonate ions while 1,310.836cm^{-1} indicates the presence of vinyl C-H in plane bend.

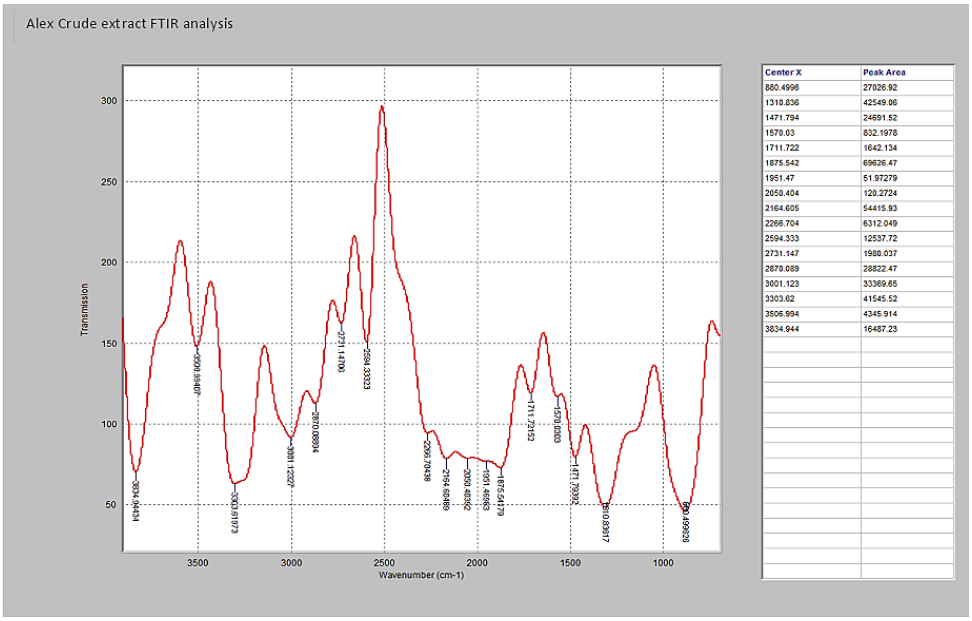


Figure 1: FTIR spectra representation crude saponin extract analysis from Siam weed.

4.0. Conclusion

This study compared the phytochemical contents of Velvet tamarind (*Dialium guineense*) and Siam weed (*Chromolaena odorata*) in which the result showed that Siam weed contains saponin (1.5433 g/100g), tannin (1.278 g/100g), alkaloids (3.234 g/100g) and glycosides (4.234 g/100g) while Velvet tamarind contains saponin (0.378 g/100g), flavonoid (3.781 g/100g), tannin (3.867 g/100g), phenol (4.33 g/100g), alkaloids (0.276 g/100g) and glycosides (0.892 g/100g). Based on the qualitative and quantitative phytochemical analysis of the leaves of the aforementioned plants. It was revealed that Siam weed (*Chromolaena odorata*) contain higher quantity of saponin when compared to Velvet tamarind (*Dialium guineense*). Siam weed from saponin was found to contain functional groups that represents standard saponin from FTIR test that was conducted from crude extract of saponin. This justifies why Siam weed is good for the extraction of saponin from a plant-based source owing to its cheap and readily available nature. It can be concluded that for a high demand for saponin extraction, Siam weed (*Chromolaena odorata*) leave is a better option.

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