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Phytochemical Analysis and Antioxidant Activities of Dry and Fresh Leaves of *Petivera alliacea* and *Ocimum gratissimum*.

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Abstract

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. It is well-known that plants produce these chemicals to protect them but recent researches demonstrate that they can also protect humans against diseases. The aim of the study was to screen and determine the phytochemicals content (Saponins, Alkaloid, Flavonoids, Phenolics, Terpenoid) and the anti-oxidant activities in dried and wet samples of *Ocimum gratissimum* and *Petiveria alliacea*. The samples of *Ocimum gratissimum* and *Petiveria alliacea* were obtained from market in Ibadan, Oyo state, Nigeria. They were then taken to the herbarium of Obafemi Awolowo University, Ile-Ife, Nigeria for Identification. Analysis was done on both dry and fresh samples of the leaves using standard procedures of the Association of Analytical Chemists (AOAC). The results showed that the dry leaves of *Ocimum gratissimum* and *Petiveria alliacea* had significantly higher values in all phytochemical contents worked on than the fresh leaves. Moreover, *Petiveria alliacea* had significantly higher values than *Ocimum gratissimum* in Saponins (955.00 ± 13.33 vs 720 ± 10.00); Alkaloid (1935.00 ± 10.00 vs 1225 ± 13.33), Flavonoids (1531.67 ± 18.89 vs 1278 ± 8.89) and Anti-oxidants (59.23 ± 0.16 vs 55.13 ± 0.16) $p < .05$. However, *O. gratissimum* had significantly higher values in Phenolics (57.43 ± 0.11 vs 47.40 ± 0.20) and Terpenoids (1240 ± 10.00 vs 860.00 ± 10.00), $p < .05$.

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From the result, it was concluded that both leaves have disease preventive properties and are better used in dry forms. Based on this, necessary recommendations were made.

Keywords: Ocimum gratissimum; Petiveria alliacea; Phytonutrients, Phytochemicals, Disease preventive properties; Medicinal purpose.

1. Introduction

Phytochemicals are naturally occurring plant chemicals found in fruits, vegetables, legumes, grains, plant leaves, and so on. They give plants its colour, flavor, smell and are part of a plants defence system [1]. Eating lots of plant foods rich in phytochemicals may help to prevent at least one in every five cases of cancer, as well as other serious ailments such as heart disease. Plants produced these chemicals to protect themselves, but recent researches demonstrate that they can also protect humans against diseases [2, 3]. There are about 6000 known phytochemicals in natural products and they have been isolated and characterized from fruits, vegetables, spices, beverages and many other sources [4, 5].

1.1 Phytochemicals

There are different classes of phytochemicals however this study focused on alkaloids, flavonoids, phenolics and saponins.

1.1.1 Alkaloids

Alkaloids are the largest group of secondary metabolite made largely from ammonia compounds comprising of nitrogen bases synthesized from amino acid building block. The compounds have basic properties and are alkaline in nature [6]. Alkaloids have diverse and important physiological effects on humans and other animals. Well-known alkaloids include morphine, strychnine, quinine, ephedrine, and nicotine. They are widely exploited as stimulants, narcotics and poisons due to their potent biological activities. Moreover, alkaloids have pharmacological applications as anaesthetics and Central Nervous System stimulants [7]. Alkaloids are found primarily in plants and are especially common in certain families of flowering plants. More than 3,000 different types of alkaloids have been identified in a total of more than 4,000 plant species.

Many alkaloids possess local anesthetic properties, though clinically they are seldom used for this purpose.

1.1.2 Flavonoids

Flavonoids are important group of polyphenols widely distributed among the plant flora. They are the most diverse group of phytochemicals. Flavonoids are a class of non-nitrogenous biological pigments (biochromes) that includes the anthocyanins and the anthoxanthins. Though extensively represented in plants, the flavonoids are of relatively minor and limited occurrence in animals, which derive the pigments from plants [8].

Flavonoids are a group of plant metabolites thought to provide health benefits through cell signalling pathways and

antioxidant effects [9]. They have also been shown to possess anti-inflammatory, anticarcinogenic, antithrombotic, antiallergic and hepatoprotective capabilities [10].

1.1.3 Saponins

The term saponin is derived from *Saponaria vaccaria*, a plant, which abounds in saponins and was once used as soap. Saponins therefore possess 'soaplike' behaviour in water, i.e. they produce foam. On hydrolysis, an aglycone is produced, which is called sapogenin. There are two types of sapogenin: steroidal and triterpenoidal. Saponins are extremely poisonous as they cause haemolysis of blood [9]. However, they are shown to have hypolipidemic and anticancer activity [6].

1.1.4 Phenolic

Phenolics are chemical components that occur ubiquitously as natural color pigments responsible for the color of fruits of plants. Phenolics in plants are mostly synthesized from phenylalanine via the action of phenylalanine ammonia lyase (PAL). They are very important to plants and have multiple functions. The most important role may be in plant defence against pathogens and herbivore predators, and thus are applied in the control of human pathogenic infections [11]. They are classified into (i) phenolic acids and (ii) flavonoid polyphenolics (flavonones, flavones, xanthenes and catechins) and (iii) non-flavonoid polyphenolics. Phenolics essentially represent a host of natural antioxidants, used as nutraceuticals, and found in apples, green-tea, and red-wine for their enormous ability to combat cancer and are also thought to prevent heart ailments to an appreciable degree and sometimes are anti-inflammatory agents. [12].

1.1.5 Terpenoids

Terpenoids are flammable unsaturated hydrocarbon of plant origin of general formula $(C_5H_8)_n$ existing in liquid form commonly found in essential oils, resins or oleoresins [13]. Terpenoids are classified according to the number of isoprene units in their structures. The diterpenes, C₂₀ (4 isoprene units) are used as anti-cancer agents, the triterpenes C₃₀, (6 isoprene units) show anti-inflammatory, sedative and insecticidal activities [14].

1.2 Functions of Phytochemicals in the Body

- To stimulate the immune system in the body and body's defense against bacteria, viruses and other disease-causing agents.
- Phytochemical block the potential carcinogens i.e cancer causing substances to be formed in the body from substances we eat, drink and absorb from the environment.
- It slows the growth rate of cancer cells.
- It helps to reduce inflammation that provides a setting favorable for cancer growth.
- To prevent DNA damage and help with DNA repair and mechanisms.
- Phytochemical reduce oxidation, the damage to cells that occurs with aging and exposure to pollution.
- It regulates the hormones such as estrogen and insulin. [15]

1.3 Antioxidant Activity

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction involving the loss of electrons or an increase in oxidation state. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid (vitamin C), or polyphenols [16]. Natural antioxidants play a key role in health maintenance and prevention of chronic and degenerative diseases such as cancer, coronary heart disease and even altitude sickness [17, 18, 19]. Antioxidants also have many industrial uses, such as preservatives in food and cosmetics and to prevent the degradation of rubber and gasoline.

1.4 benefits of antioxidant

Antioxidant is helpful in different parts of the body system like the following:

- It improves the memory of the body
 - It enhances the immune function
 - It helps the oral health
 - Decreased risk of kidney stones
 - Reduces obesity
 - Mostly epidemiological research and research on tea consumption, concentrated tea extracts may not be safe
 - Prevent food containing fat or oil from going rancid due to oxidation, i.e. developing an unpleasant odour or flavour.
 - Prevent the browning of cut fruit, vegetables and fruit juices (and so increase shelf life and appearance).
- [20]

1.5 *Ocimum gratissimum*

Ocimum gratissimum is commonly called alfavaca and also known as African Basil. It belongs to the Kingdom: Plantae, Order: Lamiales, Family: Lamiaceae, Genus. Its local names include: Ncho-achrou, Ahuji (Igbo), Efinrin (Yoruba), Aramogbo (Edo) and Daidoya (Hausa). *Ocimum gratissimum* is known as a food spice and traditional herb used for the treatment of various diseases. It is commonly grown in domestic gardens and is widely used both in cooking for flavouring, as well as for its therapeutic properties as an antiseptic and for the treatment of digestive problems [21]. Several species and varieties of the genus *Ocimum* have been reported to yield oils of diverse nature; these are commonly called basilica oils. According to the literature, the oils produced from *O. gratissimum* are active against several bacteria (including *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, etc.) and fungi (including *Trichophyton rubrum*, *T. mentagrophytes*, etc [22]. The oils are used in the treatment of many ailments, including upper respiratory tract infections, diarrhea, headache, fever, eye problems, skin diseases, and pneumonia. The oil is also a potent anti-diabetic agent [23].



Figure 1: *Ocimum gratissimum*

Ocimum gratissimum is easy to cultivate and provides an inexpensive means of combating vitamin and mineral deficiency in less developed regions of the world. It is found throughout the tropics and sub-tropics, both wild and cultivated. Its greatest variability occurs in tropical Africa [24].

1.6 *Petiveria alliacea*

Petiveria alliacea belongs to the Phytolaceae family which is regarded as the most archaic family of the Caryophyllales known as singawalang in traditional uses in Indonesia. It grows in the Caribbean, Latin America, West Africa and other regions. For hundreds of years it has been used for pain relief, and as an anti-influenza, anti-inflammatory, anti-tumor, anti-bacterial, anti-fungal, anti-hyperlipidemia, and anti-diabetic drug [25]. *Petiveria alliacea* is commonly known in the South-West of Nigeria as ‘*Anamu*’ or ‘*Ewe Aja*’, In Jamaica as the “Guinea Hen Weed”. It is used in folk medicine to enhance memory and in the treatment of the common cold, flu, other viral or bacterial infections, inflammation, diabetes, and cancer [23, 26].

The leaves and also the roots are used for medicinal purposes. This plant has been used to reduce inflammation and pain, to eliminate bacteria, fungi, candida, and viruses. It is also used to enhance the immune system and increase urination [27]. Recent studies report beneficial results in the use of this plant to lower the blood sugar levels, to treat arthritis, allergies, as therapy for fever, malaria. and in the elimination of cancer cells [28,29].

1.7 Aim and Objectives of the Study

The study aimed at providing empirical evidence to give basis for or against the use of these leaves for medicinal purposes. The objective of the study is to investigate the phytochemical components of the dry and wet (fresh) samples *O. grstissimum* and *P. alliacea* and also to determine the antioxidant activities in these samples.



Figure 2: *Petiveria alliacea*

2. Materials and Methods

2.1 Samples Collection

The samples of *Ocimum gratissimum* and *Petiveria alliacea* were obtained in Boode market, Ibadan, Oyo state, Nigeria. They were then taken to the herbarium of Obafemi Awolowo University for Identification.

2.2 Extract Preparation

The leaves were washed to remove the dirty, sun dry for seven days and blended to give a powdery form and stored in air-tight container in a cool and dry place. Another set of the leaves were then collected also rinsed, air-dry and blended into a paste form packaged and taken immediately for analysis. The organoleptic, phytochemical and the extraction of the active components were determined by the methods outlined by [30]. The extracts were characterized using Unicam Ultra Violet visible spectrometry vision 32 software Vi 21.

2.3 Quantitative Analysis for Phytochemical

2.3.1 Terpenoids determination

1g of sample was weighed into 10ml Petroleum Ether, allowed to be extracted for 15min. Filtered and read the Absorbance at a wavelength of 420nm.

2.3.2 Flavonoids determination

1g was extracted with 10ml of 80% Methanol and left to stand for 2 hours which was filtered into a weighed Petri dish. Left to dry in the oven at 40⁰c, the Petril dish was later weighed when it dries to constant weight.

2.3.3 Alkaloids determination

A gram of the sample (W) was weighed and 20ml of 10% Acetic Acid in Ethanol was added. The sample was shook and allowed to stand for 4 hours. Filtered, the filtrate was evaporated to about a quarter of its original volume. One drop of concentrated Ammonia was added. The precipitate formed was filtered through a weighed paper to dry in the oven at 60⁰ c. The filter paper was weighed after it dries to constant weight (W2).

$$\% \text{Alkaloids} = \frac{W2 - W1}{W} \times 100$$

Where: W1 =initial weight of sample, W2 =weight of the ext(W1) filter paper. Leave the filter ract, W3 = final weight of the residue

2.3.4 Phenols/Phenolics and Antioxidants – (Extraction)

2 grams were extracted with 20ml of 80:20 acetone: 0.5% formic Acid for 2min and filter.

For Phenols/Phenolics/Phenolic Acids

2ml of the extract was mixed with 0.5ml of Folin-Ciocalteau Reagent and 1.5ml Sodium Carbonate (20%). It was mixed for 15 sec and allowed to stand at 40⁰ c for 30min to develop colour. Measure A₇₆₅. Express as mg GAE/g .

2.3.5 Saponins determination

1g of sample was weighed, 5ml of 20% Ethanol was added and the sample was put in a water bath at 55⁰c for 4hours. It was filtered, the residue was washed with 20% Ethanol twice and extract was reduced to about 5ml in the oven. 5ml of Petroleum Ether was added to the concentrated extract inside a separating funnel. The pet Ether layer was discarded and 3ml of butanol was added to it. The sample was washed with 5ml of 5% Sodium Chloride. The butanol was later poured into a weighed petri dish, and was put in the oven to evaporate to dryness and weigh the residue.

2.4 Determination of Antioxidant Capacity

The DPPH scavenging was calculated according to the following equation:

$$\text{RSC \%} = (1 - A_{\text{sample}}/A_{\text{blank}}) \times 100\%$$

2.5 Statistical Analysis

All results are expressed as mean ±standard deviation. All results are means of triplicates and the level of statistical significance is expressed at p< 0.05.

3. Result and Discussion

3.1 Result

The results of the phytochemical screening of both the fresh leaves and dry leaves of *Ocimum gratissimum* and *Petiveria alliacea* are shown below:

Table 1: Results of the Phytochemical contents of fresh and dry leaves of *Ocimum* and *Petivera*

	OCIMUM		PETIVERA	
	DRY	FRESH	DRY	FRESH
SAPONINS (mg/g)	7.20 ± 0.10 ^a	5.28 ± 0.08 ^b	9.55 ± 0.13 ^c	6.71 ± 0.08 ^d
ALKALOIDS (mg/g)	12.25 ± 0.13 ^a	4.71 ± 0.12 ^b	19.35 ± 0.10 ^c	8.78 ± 0.08 ^d
FLAVONOID (mg/g)	12.78 ± 0.09 ^a	6.88 ± 0.06 ^b	15.31 ± 0.19 ^c	9.56 ± 0.14 ^d
PHENOLICS (GAE/g)	57.43 ± 0.11 ^a	28.47 ± 0.18 ^b	47.40 ± 0.20 ^c	22.47 ± 0.24 ^d
TERPENOID(mg/g)	12.40 ± 0.10 ^a	5.52 ± 0.14 ^b	8.60 ± 0.10 ^c	3.27 ± 0.12 ^d
ANTIOXIDANTS(%)	55.13 ± 0.16 ^a	23.73 ± 0.16 ^b	59.23 ± 0.16 ^c	28.57 ± 0.18 ^d

Means on same row with different superscripts are significantly different (P < 0.05)

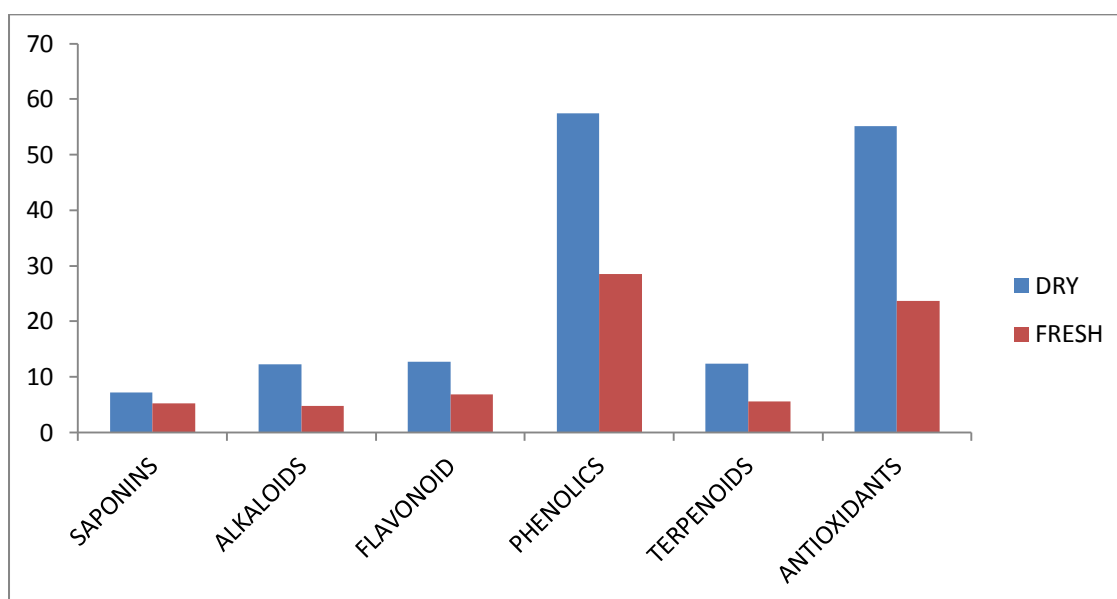


Figure 3: Phytochemical Contents of Fresh and Dry Leaves of *Ocimum* spp.

3.2 Discussion

The results presented in Table 1 showed that the phytochemical concentration and antioxidant activity in the dry leaves of *Ocimum gratissimum* and *Petivera alliacea* were significantly higher than those obtained in their fresh leaves at p < 0.05. (Figure3). This could be attributed to the fact that fresh samples have relatively higher moisture content than the dry leaf-extract making the dry leaf-extract to be more concentrated per gram of

sample than fresh leaf-extract.

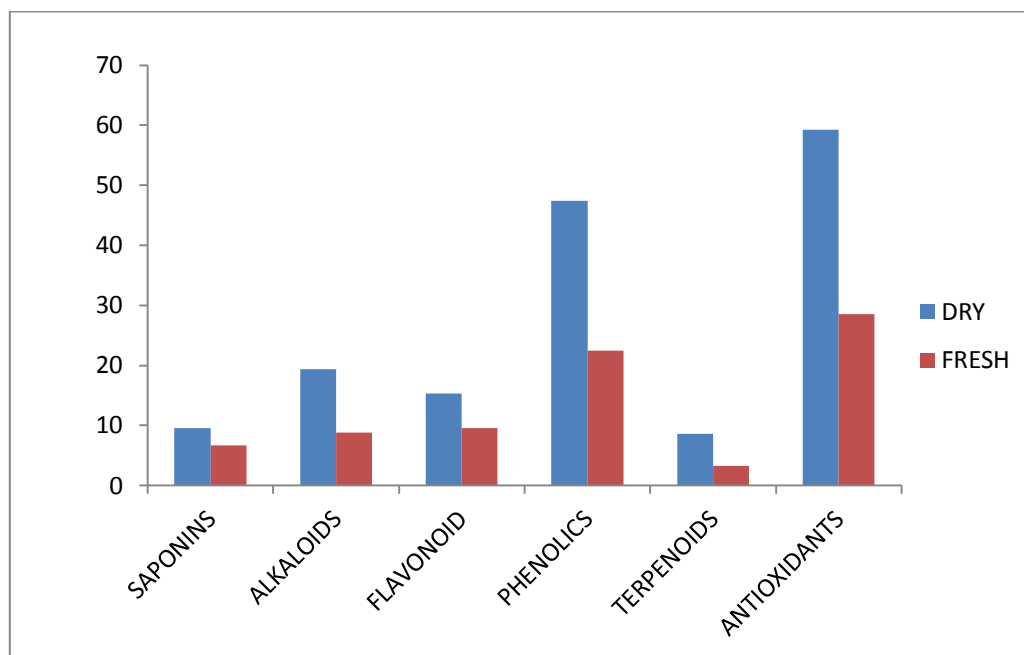


Figure 4: Phytochemical contents of fresh and dry leaves of *Petivera spp*

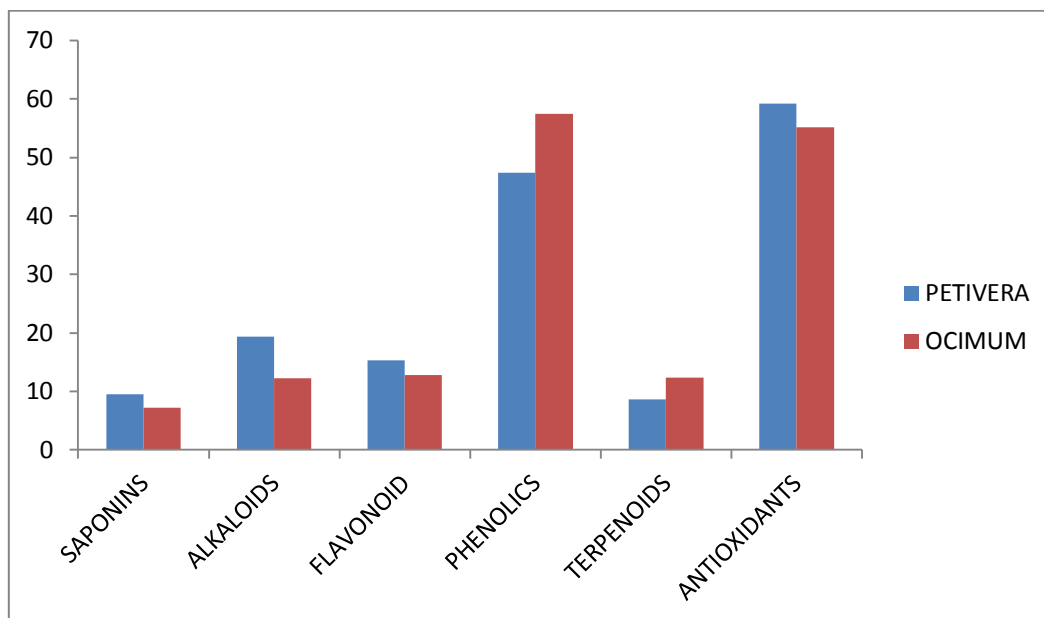


Figure 5: Quantitative comparison of phytochemical contents of *Ocimum* and *Petivera spp*. (Dry leaves) Except in phenolics and terpenoid contents, *Petivera spp* had more phytochemical contents than *Ocimum spp*.

Also, the phytochemical contents in the dry and fresh leaf-extracts of *Petivera. alliacea* in terms of Flavonoid, Saponins, Alkaloids are significantly higher than those obtained in the dry and fresh leaf-extracts of *Ocimum*

gratissimum. However, *O. gratissimum* had significantly higher values in Phenolics and Terpenoids content. Moreover, *P. alliacea* show higher antioxidant activity than *O. gratissimum*.

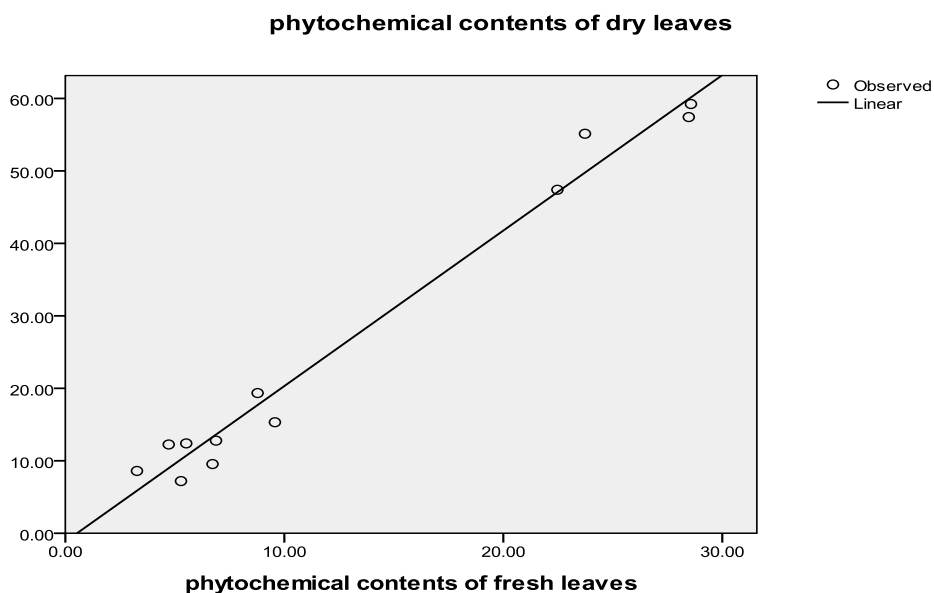


Figure 5: Linear Regression line showing the relationship between phytochemical content of wet and dry leaves of *Petivera* and *Ocimum*. There is significant correlation at 0.01 level between the Phytochemical contents of the wet and dry leaves ($R = 0.99$)

In Figure 5, Linear Regression line showing the relationship between phytochemical content of wet and dry leaves of *Petivera* and *Ocimum*. There was a strong positive correlation between them.

4. Conclusion and recommendation

4.1 Conclusions

The study has revealed that leaves of *O. gratissimum* and *P. alliacea* are rich in phytochemicals (Saponins, Alkaloids, Flavonoids, Phenolics and Terpenoids among others) which could be the basis for their being used for medicinal purposes. However, caution should be taken in the intake and administration of the leaf extracts because of the saponin and alkaloid content which may induce some side effects.

4.2 Recommendations

Toxicology studies should be carried out on both the fresh and dry samples of these plants leaves to ascertain the extent or degree of side effects attributable to them. Moreover, further studies could be carried out on other plant parts of these two plant species.

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