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## Quantitative Determination of Some Secondary Metabolites and the Anti - Bacterial Effects of the Leave Extracts of *Duranta erecta*

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### Abstract

Analyses were carried out on methanol and n - hexane extract of the leaves of *Duranta erecta* to qualitatively and quantitatively determine the presence of some secondary metabolites. The phytochemical screening showed the presence of tannins (0.49mg/100g), flavonoids (0.85mg/100g), alkaloids (0.14mg/100g) and saponins (0.78mg/100g) in the methanolic extract while the n - hexane extract showed the presence of only alkaloid (0.18mg/100g), flavonoids (1.70mg/100g) and saponins (0.64mg/100g). The antibacterial activity of the extracts were evaluated against some human pathogenic bacteria; *Proteus mirabilis*, *Proteus mirabilis*, *Bacillus substillis*, *Salmonella typhii* and *Bacillus aereus* at 600mg/ml, 300mg/ml, 150mg/ml, and 75mg/ml by agar diffusion method. The result of the antibacterial activity showed activities against the growth of *Proteus mirabilis* (mic 129mg/ml), *Bacillus substillis* (mic 141mg/ml), *Salmonella typhii* (mic 81mg/ml) and *Bacillus aereus* (mic 100mg/ml) while the nhexane extract did not show any activity against the growth of any bacteria.

### 1. Introduction

In most developing countries of the world, access to synthetic medicine remains a serious problem till today. This has made those living in remote cities of the world dependent mainly on herbal medicine obtained from parts of plants. These parts of plants may include the seeds, barks, roots and leaves. Herbs have been prime medicinal agents in traditional and holistic therapies. About ninety percent of plants have good medicinal value. Many plants synthesize substances that are useful for the maintenance of health in human and animals. Among such substances are the secondary metabolites, of which at least 12, 000 have been isolated, a number estimated to be less than 10% of the total.

Medicinal plants have always been considered a healthy source of life in that their the rapeutical properties are very useful in healing various diseases because of their high sensitivity and versatility [1]. Though herbal medicines are very effective in the treatment of various ailments, very often these drugs are unscientifically exploited or improperly used. Therefore, these plants drugs deserve detailed studies in the light of modern science [2].

*Duranta erecta* is a bright green coloured shrub with orange fruits normally used as

ornamental plant. The pharmacological significance was noted due to the presence of various bio - active compounds such as alkaloids, flavonoids, saponins, tannins and terpenoids. The literature studies, indicated that it has been used for headache, toothache, healing of wounds and liver protection, [2] diuretics [3]. Its anti malarial properties have also been investigated [4]. The anti oxidant and anti - viral activities of its secondary metabolite have been studied by many scientists [7 - 11]. This present research is planned to identify the bioactive component of the leaves extract of the plant as well as its antibacterial activity against some human pathogenic bacteria.

## 2. Materials and Methods

### 2.1. Sample Collection

The leaves of *Duranta erecta* were collected from the surroundings of advanced research laboratory, Nnamdi Azikiwe University, Awka, Anambra state, South Eastern Nigeria. The leaves were collected between the month of April and May, 2012. They were identified and authenticated by Prof. C.V. Okeke of the Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

### 2.2. Experimental

#### *Preparation of Plant Material*

The leaves of *Duranta erecta* were air - dried and pulverized using electric blending machine. The powdered leaves were stored at room temperature in a clean sterilized air tight bottle.

### 2.3. Extraction

20g of fine powder of the leaves sample were extracted with soxhlet apparatus with solvents in increasing polarity i.e. n - hexane and methanol. The extracts obtained were concentrated under reduced pressure. The extracts were concentrated in a water bath at reduced temperature after which the percentage yield was calculated and the extracts left in desiccator until further use. The results were summarized in Table 1.

### 2.4. Qualitative Phytochemical Screening

The qualitative phytochemical screening were conducted using some analytical methods. For the analysis of tannins and alkaloid, the methods used was the method of Treas and Evans [13]. Saponins, glycoside, carbohydrates and terpenoid were studied using the method as described by Harbon, [14]. Proteins were studied using methods as described by Finar [12] while steroids and acidic compounds were analysed using the methods as described by Akpuaka [15].

### 2.5. Quantitative Phytochemical Analysis

#### 2.5.1. Quantitative Determination of Tannins

2g of the sample in a conical flask was added 10ml of 2m HCl and 29 ml was and shaken vigorously. It was then

filtered into a 50 ml volumetric flask and made up to 50 ml. 5 ml of the solution was pipetted into a test tube and 5ml of water was used as control into another test tube. In each test tube were added 3ml of FeCl<sub>3</sub>, 1ml HCl and 3ml of 0.08 [K<sub>3</sub>F<sub>6</sub> (CN)<sub>6</sub>]respectively and was allowed to stand for 30 seconds. The absorbance was read at 760nm from UV - VIZ spectrophotometer [16].

#### 2.5.2. Quantitative Determination of Flavonoids

In a 250ml beaker was added 10g of the sample and dissolved with 70ml of water at room temperature. It was allowed to stand for 15 minutes. 6g of activated charcoal was added and properly mixed which was allowed to stand for 30 minutes. It was filtered into a 400ml beaker using a vacuum, 60ml fritted glass funnel containing asbestos pad. 2 drops of HCl was added and allowed to evaporate on a steam bath to about 40ml and quantitatively transferred to a 50ml volumetric flask and made up with water. Further dilutions could be made with water. The absorbance was read at 233nm [16].

#### 2.5.3. Quantitative Determination of Saponins

0.1g of the processed sample in a test tube was added 5ml of distilled water and boiled for 5 minutes. It was filtered while hot and 10ml of the filtrate was pipette into a test tube, this is followed by addition of 10ml of distilled water. The mixture was shaken vigorously. The formation of a stable foaming froth was observed. The absorbance of the solution was read at 620nm [16].

#### 2.5.4. Quantitative Determination of Alkaloids

3ml of the extract was pipetted into 250ml beakers in duplicate, 2ml phosphate buffer was added and adjusted to pH 7.0. The water bath was incubated at 37°C, followed by the addition of 6.0ml of 5% tri chloro acetic acid and 2.0ml of casein solution previously brought to 37°C and allowed to stand for 1 hour at room temperature and filtered. The absorbance of the sample and the blank was read at 580nm [16].

### 2.6. Antimicrobial Screening of the Methanol Leaf Extract

Agar diffusion method was used in the analysis. Two fold serial dilution of the methanol leaf extract; 600, 300, 150, 50 and 75 mg/ml were prepared by mixing with sterile distilled water. The culture of the medium was prepared by weighing 17g of nutrient agar dissolved in 600ml of distilled water, heated and dispersed into bijoux bottles and sterilized by auto clarity at 121°C for 15mins. 20ml of molten nutrient agar was poured into the separate petri dishes, swirled slowly and was allowed to set and dry.

Each set of plate were streaked evenly with both culture of *Bacillus subtilis*, *Staphylococcus aureus*, *Proteus mirabilis*, *Salmonella typhi*, and *Bacillus cereus* (NRRL 14724) each using a swap stick and allowed to stand for 30mins.

Agar wells were made on the inoculated nutrient agar using cork borers. The different concentrations of the extract prepared were then poured into the well and incubated for at 37°C for 24hrs. Another agar plate containing no extract was also used (negative control). The agar plate was incubated for 24hours. The antibacterial activity of different concentrations of the extract on the bacteria were determined by measuring their inhibition zone diameter (mm), zone of observable inhibition growth of each bacteria as criteria for declaring a plant extract bioactive and is indicated by a clear zone around a well containing the plant extract.

### 3. Results and Discussion

*Table 2. Results of Qualitative Screening of the Phytochemicals in the leaves of Duranta erecta.*

Types of Phytochemical constituents	Methanol extract	Hexane extract
Alkaloids	+	++
Saponins	++	-
Tannins	+++	-
Flavonoids	+++	++
Steroids	++	-
Fat & oil	-	+
Reducing sugars	++	++
Carbohydrates	+	+
Glycosides	+	-
Acidic compounds	+	+

From Table 2, it is observed that the methanol extract contains alkaloids, saponin, tannin, flavonoid, steroid, glycoside, acidic compound, reducing sugar and carbohydrate whereas protein and fats and oil were absent. The nhexane extract on the other hand shows the presence of alkaloids, fats and oil, reducing sugar, carbohydrate and acidic compound while saponin, tannin, flavonoid, steroids

In Table 1 is shown the percentage yield of methanol and nhexane extract.

*Table 1. Results of Percentage Yield of Methanol and n Hexane extract.*

Solvent	Colour and consistency	% yield
Methanol	Dark brown and sticky	1.75
Hexane	Yellowish coloured liquid	0.10%

From Table 1, it seen that the result of percentage yield of methanol extract is higher than that of nhexane extract. This is in agreement with literature which has shown that methanol is highly potent solvent for extracting phytochemicals from plant materials [14].

The qualitative screening of the phytochemicals in the leave extracts of *Duranta erecta* was recorded in Table 2.

and glycoside were absent.

#### 3.1. Results of Quantitative Phytochemical Analysis

The result of the qualitative phytochemical analysis is shown in Table 3.

*Table 3. Results of quantitative determination of phytochemical constituents.*

Phytochemical constituents	Methanol extract (mg/100g)	Hexane extract (mg/100g)
Tannins	0.49	0.00
Flavonoids	0.85	1.70
Alkaloids	0.14	0.18
Saponins	0.78	0.64

From Table 3, it is observed that the methanol is a better extracting solvent than nhexane for all the phytochemicals studied except for flavonoids. Flavonoids were extracted the most by n - hexane at 1.70 mg/100g while by methanol gave a yield of 0.85mg/100g.

#### 3.2. Results of Qualitative Phytochemical Screening

The results of the qualitative phytochemical screening is shown in Table 4.

*Table 4. Results of qualitative Phytochemical Screening.*

Types of Phytochemical constituents	Methanol extract	Hexane extract
Alkaloids	+	++
Saponins	++	-
Tannins	+++	-
Flavonoids	+++	++
Steroids	++	-
Fat & oils	-	+
Reducing sugars	++	++
Carbohydrates	+	+
Glycosides	+	-
Acidic compounds	+	+

From Table 4, it is observed that methanol gave a better qualitative extracts for saponins, tannins, flavonoids, steroids, and glycosides than the nhexane extracts whereas for alkaloids and fats and oil phytochemicals, nhexane gave a better qualitative extracts. Both methanol and nhexane gave the same quality for reducing sugars and acidic compounds.

### 3.3. Result of Antibacterial Screening

In Table 5 is shown the result of antibacterial screening of methanol leave extract of *Duranta erecta* against some bacteria at concentrations of 600mg/ml, 300mg/ml, 150mg/ml and 75mg/ml.

**Table 5.** Results of Antibacterial Screening of methanol leave extract of *Durantaerecta* against some bacteria at various concentrations (mg/ml).

Concentration (mg/ml)	Staphylococcus aureus	Proteus mirabilis	Bacillus subtilis	Salmonella typhi	Bacillus cereus (NRRL 14724)
600	-	+	+	+	+
300	-	+	+	+	+
150	-	+	-	+	+
75	-	-	-	+	-

From Table 5 it can be seen that all the concentrations of methanol extract of *Duranta erecta* studied were biologically inactive against *Staphylococcus aureus* whereas all were active against *Salmonella typhi*. For *Proteus mirabilis* and *Bacillus cereus*, all concentrations studied were active against them except for the concentration of 75mg/ml which were inactive. On the other hand only concentrations of 600mg/ml

and 300mg/ml of the methanol extract were biological active against *Bacillus subtilis* while others were biologically inactive.

The antibacterial test result of the methanol extract of the leaves of *Duranta erecta* was expressed in terms of the Inhibition Zone Diameter (IZD) in mm and recorded in Table 6.

**Table 6.** Antibacterial test result of methanol extract of the leaves of *Durantaerecta* (mg/ml) expressed as inhibition zone diameter IZD (mm).

INHIBITION ZONE DIAMETER, IZD (mm)					
Concentration mg/ml	Staphylococcus aureus	Proteus mirabilis	Bacillus subtilis	Salmonella typhi	Bacillus cereus (NRRL 14724)
600	0	13	12	30	25
300	0	6	4	28	16
150	0	2	0	14	10
75	0	0	0	6	0

From Table 6, it can be seen that the activity increases as concentration of the extract increases and vice versa.

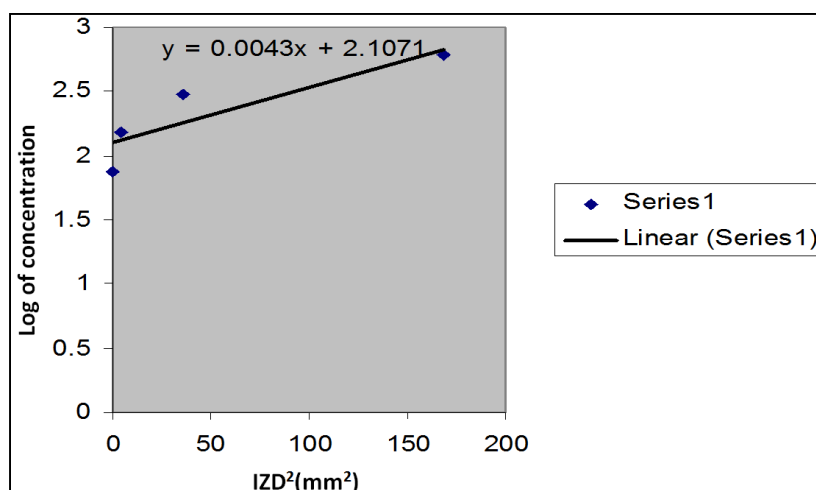
The minimum inhibition concentrations (MIC) of the test organisms were deduced by plotting graphs of logarithm of concentrations of the plant extract against square of the

inhibition zone diameters (IZD) as shown in Figures 1 to 4.

The intercept on the vertical axis gave the result of the MIC of the various micro organisms. The result of the MIC test is given in Table 7.

**Table 7.** Result of minimum inhibitory concentration (MIC) of methanol extracts of the leaves of *Duranta erecta*.

Micro Organisms	Intercept on the vertical axis	(Anti log) MIC (mg/ml)
Proteus mirabilis	2.11	129
Bacillus subtilis	2.15	141
Salmonella typhi	1.91	81
Bacillus cereus (NRRL 14724)	2.00	100



**Figure 1.** Graph of log concentration of plant extract against the square of inhibition zone diameter (IZD) in mm of *Proteus mirabilis*.

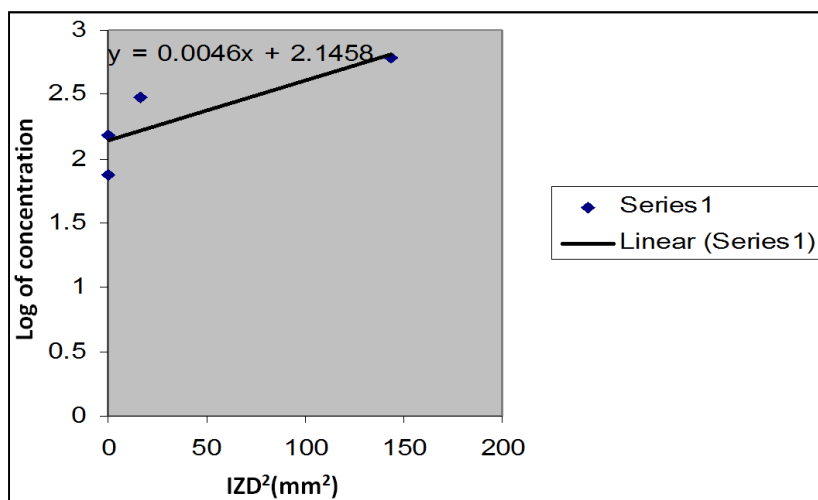


Figure 2. Graph of log concentration of plant extract against the square of inhibition zone diameter (IZD) in mm of *Bacillus subtilis*.

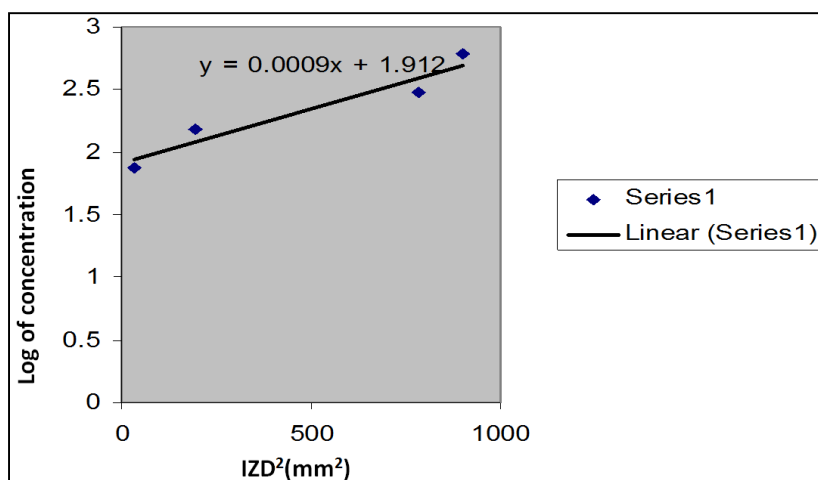


Figure 3. Graph of log concentration of plant extract against the square of inhibition zone diameter (IZD) in mm of *Salmonella typhi*.

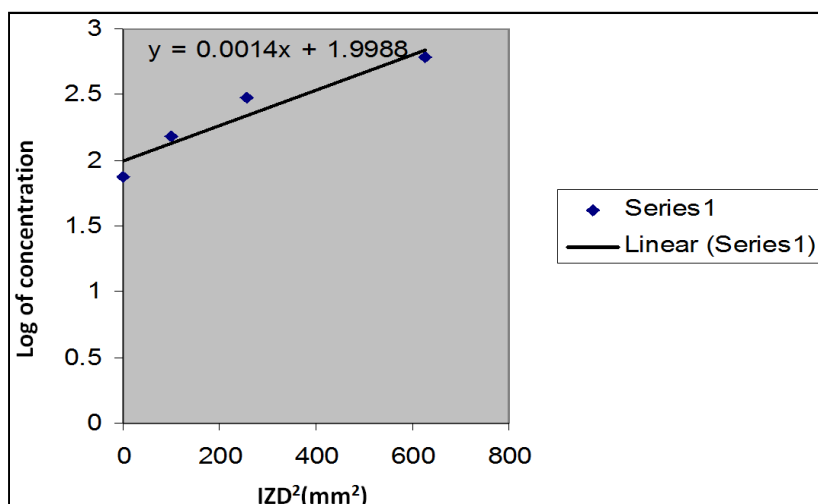


Figure 4. Graph of log concentration of plant extract against the square of inhibition zone diameter (IZD) in mm of *Bacillus cereus* (NRRL 14724).

From Table 7 it is observed that the test organism, *Salmonella typhi* has the lowest minimum inhibition concentration which shows that the plant extract was very sensitive against *Salmonella typhi* at lower concentrations.

### 3.4. Discussion

Table 1 shows the percentage yield of both methanol and n - hexane with methanol having the greater yield of 1.75%

while n hexane extracted just 0.1%. It has been shown that methanol is a better solvent than n - haxane owing to the fact it is a moderately polar solvent close to water which is a universal solvent [14].

The quantitative phytochemical analysis, it showed that flavonoid was the highest phytochemicals determined quantitatively. Flavonoids have been shown to exhibit their actions through effect on membrane permeable and by inhibitor of membrane bound enzymes and this property may explain the antioxidant property of this plant since they have been known to posses antioxidant and anti inflammatory activities hence their use in the treatment and management of cancer [3, 13]. Saponins were found in relatively large quantities in the leaves of *Duranta erecta*. Saponins are mostly used in the manufacture of insecticides, vaccines and synthesis of steroidal hormones [17].

The presence of alkaloid shows that the plant can be used as an analgesic and also in the treatment of malaria [13]. Furthermore, the presence of acidic compound shows that the leaves of *Duranta erecta* are poisonous which had been reported in the literature [11]. These findings are in line with the ethno medical uses of *Duranta erecta* from literature [9]

The antibacterial analysis carried in table 4 out showed that the methanol extract of the plant at high concentrations of 600 mg/ml and 300 mg/ml inhibited the growth of the following organisms: *Proteus mirabilis*, *Bacillus subtilis*, *Salmonellatyphi* and *Bacillus cereus* (NRRL 14724) while at lower concentration of 150 mg/ml and 75 mg/ml, the inhibition of the growth is more pronounced only in *Salmonellatyphi* and *Bacillus cereus* (NRRL 14724) while there was no inhibition at all concentrations in *Staphylococcus aureus*. This shows that the anti - bacterial activity decreased considerably with reduction in the concentration of the extract, indicating that the extract is more effective at higher concentration. The antibacterial activity of pure methanol on the micro organisms showed that there was no inhibition of the growth of micro organisms. This proved that the anti bacterial activity was as a result of the plant extract and not the pure methanol [17].

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