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Evaluation of qualitative and quantitative phytochemical constituents of *Napoleona imperialis* stem bark

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Abstract

The aim of this study was to determine the qualitative and quantitative phytochemicals of *Napoleona imperialis* stem bark. Methods*:* Extractions and Phytochemical analysis of *Napoleona imperialis* stem bark were done using standard procedures. Result: Aqueous *Napoleona imperialis* stem bark extract had yield of 76.9g (7.69%) and methanol *Napoleona imperialis* stem bark extract also yielded 58.5g (5.85%) when 1000g pulverized was used for each. Qualitative phytochemical analysis of this extract exhibited moderate quantities of alkaloid, steroid and terpenoid in aqueous extract while methanol extract had flavonoid, saponin and tannin also in moderate quantities. Scanty quantities of flavonoid, saponin, cardiac glycoside and tannin were seen in aqueous extract so also, scanty quantities of alkaloid, steroid, terpenoid and cardiac glycoside were seen in methanol extract. The quantitative analysis of the phytochemicals was carried out twice and the average taken. Phenol had a concentration of 1.173mg/kg, Steroids had 0.623mg/kg, Terpenoid had 3.031mg/kg, Flavonoid showed 58.240mg/kg, Saponin had 13.190mg/kg, Alkaloid had 13.530mg/kg, Cardiac glycoside with 3.910mg/kg, Phytate showed 2.320mg/kg, Tannin had 52.500mg/kg and Hydrogen cyanide showed13.500mg/kg. This research proves that *Napoleona imperialis* contains some phytochemicals that are useful for mankind and also the part of this plant extracts exhibit medicinal properties.

Keywords: *Napoleona imperialis*; Aqueous extract; Methanol extract; Phytochemical analysis

1. Introduction

Napoleona imperialis, a wild plant found in South eastern Nigeria is an evergreen non timber plant which grows abundantly in bush fallows, secondary bushes and marginal lands in most of the tropical humid zones of West Africa [1]. It is commonly known as Utum in Ikwuano dialect in Igbo language in Nigeria [2].

Phytochemicals are chemicals of plant origin [3]. They have biological activity in the plant host and play a role in plant growth or defense against competitors, pathogens and predators [4]. Phytochemicals which are secondary metabolites are produced by plants and they give plants it's colour, flavor, smell and serve as part of plant's natural defense system [5]. Plants do produce primary metabolites that are essential for the biochemical pathways that control growth, photosynthesis, respiration or flowering. The plants also produce secondary metabolites that help them to adapt to environmental conditions competing with other plants and chasing off attacks by predatory insects and animals or attracting those that play importance in pollination, fruit dispersal or protection. They are naturally occurring and are

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believed to be effective in combating or preventing disease due to their antioxidant properties [6]. The compounds contribute to protection against degenerative diseases in human [8]. There are several classes of phytochemicals present in plants and they include; alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, steroids, saponins, resin, terpenes and terpenoids [7; 8]. Furthermore, this study was carried out to assess the qualitative and quantitative phytochemicals of *Napoleona imperialis* stem bark.

2. Material and methods

2.1. Plant material

(*Napoleona imperialis* stem bark, was collected from the bush and used for the study). It was identified as *Napoleona imperialis* by Mr. Felix Nwafor, a plant taxonomist at the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka, Enugu State. Instruments, Equipment, Chemicals, Kits and all consumable reagents were of analytical grade and purchased from a reputable company

2.2. Methods

2.2.1. Collection of Napoleona imperialis stem bark

The stem bark of the plant was collected from the plant from the bush, dried under shade for forty (40) days, ground first, with clean mortar and pestle and then with clean grinding machine. They were stored in brown bottles in the cupboard until ready for extraction.

2.2.2. Plant Identification

The plant was identified as *Napoleona imperialis* by Mr. Felix Nwafor, a plant taxonomist at the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka, Enugu State

2.3. Extraction

2.3.1. Preparation of the aqueous and methanol extracts of Napoleona imperialis stem bark

The dried stem bark were ground into powder using grinding machine. Exactly 1kg of the ground stem bark powder of *Napoleona inperialis* was soaked in 5 litres of distilled water for 72hrs for aqueous extraction. Another 1kg of the ground stem bark of *Napoleona inperialis* was soaked in 4.5litres of 80% methanol for 72hrs for methanol extraction. The aqueous extraction was sieved and filtered using whatman no 1 (125mm) filter paper. The filtrate was concentrated using water bath at 50 degree centrigade. The methanol extraction was also sieved and filtered using whatman no 1 (125mm) filter paper. The filtrate was concentrated using water bath at 50 degree centigrade. The aqueous and methanol extracts were separately stoppered in universal bottles and preserved in the refrigerator for use. The extracts were therefore prepared by solubilizing it with water before use.

2.4. Phytochemical analysis

2.4.1. Qualitative Analysis of Phytochemicals

Qualitative analyses were carried out using the methods of Trease and Evans [9] and Harborne [10], to ascertain the presence of the different phytochemicals in the stem bark of *Nopoleona imperialis* plant before quantitative analysis are carried out.

2.5. Quantitative analysis

2.5.1. Alkaloids Determination

Five grams (5g) of the sample was weighed into a 250ml beaker and 200ml of 20% acetic acid in ethanol was added and covered and allowed to stand for 4 hours at 25 ^{o}c. This was filtered with filter paper No. 42 and the filtrate was concentrated using a water bath (Memmert) to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH_4OH (1% ammonia solution). It was then, filtered with pre-weighed filter paper. The residue on the filter paper is the alkaloid, which is dried in the oven (precision electrothermal model BNP 9052 England) at 80 ^{o}c. The alkaloid content was calculated and expressed as a percentage of the weight of the sample analyzed [10;11].

Calculation:

$$\%\text{weight of alkaloid} = \frac{\text{weight of filter paper with residue} - \text{weight of filter paper x 100}}{\text{Weight of sample analyzed}}$$

2.6. Flavonoids Determination

The plant sample (10 g) was extracted repeatedly with 100ml 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 into dryness over a water bath and weighed to a constant weight [12].

Calculation

$$\%\text{flavonoids} = \frac{(\text{weight of crucible} + \text{residue}) - (\text{weight of crucible}) \text{ x } 100}{\text{Weight of sample analyzed}}$$

2.7. Determination of Saponin

Exactly 5g of the sample (*Napoleona imperialis* stem bark) was put into 20% acetic acid in ethanol and allowed to stand in a water bath at 50^0c for 24hours. This was filtered and the extract was concentrated using a water bath to one-quarter of the original volume. Concentrated NH_4OH was added drop-wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed. The saponin content was weighed and calculated in percentage [11].

Calculation

$$\%\text{saponin content} = \frac{(\text{weight of filter paper} + \text{residue}) - (\text{weight of filter paper}) \text{ x } 100}{\text{Weight of sample analyzed}}$$

2.8. Cardiac Glycosides Determination

Wang and Filled's method was used. To 1 ml of extract was added 1ml of 2% solution of 3,5-DNS (Dinitro Salicylic acid) in methanol and 1ml of 5% aqueous NaOH. It was boiled for 2 minutes (until brick-red precipitate was observed) and the boiled sample was filtered. The weight of the filter paper was weighed before filtration. The filter paper with the absorbed residue was dried in an oven at 50^0c till dryness and weight of the filter paper with residue was noted.

The cardiac glycoside was calculated in %.

Calculation

$$\%\text{cardiac glycoside} = \frac{(\text{weight of filter paper} + \text{residue}) - (\text{weight of filter paper}) \text{ x } 100}{\text{Weight of sample analyzed}}$$

2.9. Tannin Determination by Follins dennis Titration

The follins dennis titrating method as described by Trease and Evans [9], was used. To 20g of the crushed sample (*Napoleona imperialis* stem bark) in a conical flask was added 100ml of petroleum ether and covered for 24 hours. The sample was then filtered and allowed to stand for 15 minutes allowing petroleum ether to evaporate. It was then re-extracted by soaking in 100 ml of 10% acetic acid in ethanol for 4hrs. The sample was then filtered and the filtrate collected. NH_4OH (25 ml) was added to the filtrate to precipitate the alkaloids. The alkaloids was heated with electric hot plate to remove some of the NH_4OH still in solution. The remaining volume was measured to be 33ml. Five milliliter (5ml) of this was taken and 20 ml of ethanol was added to it. It was titrated with 0.1M NaOH using phenolphthalyne as indicator until a pink end point is reached. Tannin content was then calculated in % ($C_1V_1 = C_2v_2$) molarity.

Calculation

Data
C_1 = conc. of Tannic Acid
C_2 = conc. Of Base
V_1 = Volume of Tannic acid

V_2= Volume of Base

Therefore

$$C1 = \frac{C_2 V_2}{V_1}$$

$$\% \text{ of tannic acid content} = \frac{C_1 \text{x } 100}{\text{Weight of sample analyzed}}$$

2.10. Phytate Determination

Phytate contents were determined using the method of Young and Greaves (1940) as adopted by lucas Markakes [13]. Into 250 ml conical flask was weighed 0.2 g of the sample (*Napoleona imperialis* stem bark). The sample was soaked in 100ml of 2% concentrated HCL for 3hr, the sample was then filtered. Fifty milliliter (50ml) of the filtrate was laced in 250ml beaker and 100ml distilled water added to the sample. Ten milliliter (10ml) of 0.3% ammonium thiocynate solution was added as indicator and titrated with standard iron (111) chloride solution which contained 0.00195 g iron per 1ml.

$$\text{Phytic acid} = \frac{\text{Titre value x } 0.00195 \text{ x } 1.19 \text{ x } 100}{\text{Wt of sample}}$$

2.11. Phenol Determination

The quantity of phenol is determined using the spectrophotometer method. The sample (*Napoleona imperialis* stem bark) was boiled with 50 ml of $(CH_3CH_2)_20$ for 15min. Five milliliter (5 ml) of the boiled sample was then pipetted into 50ml flask, and 10ml of distilled water was added. After the addition of distilled water, 2ml of NH_4OH solution and 5ml of concentrated $CH_3(CH_2)_3CH_2OH$ was added to the mixture. The sample was made up to the mark and left for 30min to react for colour development and measured at 505nm wavelength using spectrophotometer.

2.12. Determination of steroid content

One gram (1.0 g) of the powdered sample (*Napoleona imperialis* stem bark) was weighed and mixed in 100ml of distilled water in a conical flask. The mixture was filtered and the filtrate eluted with 0.1N ammonium hydroxide solution. 2ml of the eluent was put in a test tube and mixed with 2ml of chloroform. Three milliliter (3ml) of ice cold acetic anhydride was added to the mixture in the flask. Two drops of (200mg/dl) standard sterol solution was prepared and treated as described for test as blank. The absorbance of standard and test was measured, zeroing the spectrophotometer with blank at 420nm.

$$\text{Calculation (mg/100ml)} = \frac{\text{Absorbance of test x Conc of std}}{\text{Absorbance of std}}$$

2.13. Cynogenic Glycoside Determination

2.13.1. Acid Titration Method

Ten to twenty grams (10 -20 g) sample, ground to pass N0.20 sieve was placed in 800 ml kjeldahl flask and 100 ml H_2O was added. It was macerated at room temperature for 2hours and 100ml of H_2O was added and steam distill, collecting distillate in 20 ml 0.02N $AgNO_3$ acidified with 1ml HNO_3. Before distillation, it was adjusted appropriately, so that tip of condenser dips below surface of liquid in receiver. When 150 ml has passed over, the distillate was filtered through gooch wash receiver and gooch with little H_2O. Excess $AgNO_3$ was titrated in combined filtrate and washings with 0.02N KCN, using Fe alum indicator (1ml 0.02N $AgNO_3$ = 0.54mg HCN).

3. Results

Aqueous *Napoleona imperialis* stem bark extract had yield of 76.9 g (7.69%) using 1000 g pulverized material while methanol *Napoleona imperialis* stem bark extract had yield of 58.5 g(5.85%) also when 1000 g pulverized is used (Table 1).

There were moderate quantities of alkaloid, steroid and terpenoid in aqueous extract while methanol extract had flavonoid, saponin and tannin in moderate quantities. Scanty quantities of flavonoid, saponin, cardiac glycoside and tannin were seen in aqueous extract while alkaloid, steroid, terpenoid and cardiac glycoside were also detected in scanty quantities in methanol extract (Table 2).

The test was carried out twice and the average taken. Phenol had a concentration of 1.173 mg/kg, Steroids had 0.623 mg/kg, Terpenoid had 3.031 mg/kg, Flavonoid showed 58.240 mg/kg, Saponin had 13.190mg/kg, Alkaloid had 13.530mg/kg, Cardiac glycoside with 3.910mg/kg, Phytate showed 2.320 mg/kg, Tannin had 52.500 mg/kg and Hydrogen cyanide showed 13.500 mg/kg (Table 3).

Table 1 Extract yield of aqueous and methanol extracts *Napoleona imperialis* stem bark

Extract	Pulverized materials (g)	Yield (g)	% yield
Aqueous	1000 g	76.9	7.69
Methanol	1000 g	58.5	5.85

Table 2 Qualitative Phytochemical constituents of aqueous and methanol extracts of *Napoleona imperialis* stem bark

Parameters	Aqueous	Methanol
Alkaloid	++	+
Flavonoid	+	++
Saponin	+	++
Steroid	++	+
Terpenoid	++	+
Cardiac Glycoside	+	+
Tannin	+	++

+: Detected; - : Not detected

Table 3 Quantitative phytochemical composition of *Napoleona imperialis* stem bark

Parameters	Concentration (mg/kg)
Phenol	1.173
Steroid	0.623
Terpenoid	3.031
Flavonoid	58.240
Saponin	13.190
Alkaloid	13.530
Cardiac glycoside	3.910
phytate	2.320
Tannin	52.500
Hydrogen cyanide	13.500

4. Discussion

From the findings in this research work, aqueous *Napoleona imperialis* extract had more yield than methanol *Napoleona imperialis* extract when 1000g pulverized materials was used for each extract. This is in line with the work done by Egbuchulem *et al.,* [14], in which the methanol extract of the seed of *Napoleona imperialis* had low yield, though the

pulverized materials were soaked for 48hrs but for this research work, they were soaked for 72hrs. This could probably mean that aqueous extraction could produce more yield that methanol extract. This is in contrast to previous work done by Chukwu *et al.,* [15], that showed lesser yield in the fruits of *Napoleona imperialis* using cold extraction. The qualitative phytochemical analysis of the stem bark in this research work showed the presence of flavonoids, saponins, alkaloids, which is in line with the work of Etim *et al*., [16] and Egbuchulem *et al.,* [14]. Egbuchulem *et al.,* [14] also reported the qualitative presence of phenols, carotenoids, phytates and oxalates which were not reported in this study and in the research conducted by of Etim *et al*.,[16]. Uchegbu *et al*., [17], recorded the presence of phytate, tannins, alkaloids, saponins in the seed extract which is also in line with this work. Etim *et al.,* [16], detected the qualitative presence of tannins, steroids, glycoside, and resin in their work. This is also in line with this research work but presence of resin was not checked for qualitatively while presence of cardiac glycoside was assessed. This study, and that of Etim *et al*., [16], checked for steroid which was not done in the work of Egbuchulem *et al.,* [14]. Egbuchulem *et al.,* 2018 and Uchegbu *et al*., [17] researched on *Napoleona imperialis* seed, Etim *et al.,* [16], worked on *Napoleona imperialis* leaves and this study was on *Napoleona imperialis* stem bark. Ogbonanya, [18] showed that the leaves of *Napoleona imperialis* contain cyanide. Dalzie, [19], and Irvine, [20] also showed that the leaves also contain glycosides, tannins and saponins which are also contained in the stem bark according to this research. Comparing the root extract with the stem bark extract, Etim *et al.,* [16] reported that *Napoleona imperialis* root contains alkaloid, flavonoids, tannins, steroids, glycosides, saponons, carbohydrates, resin and protein. This is in accordance with Harborne, [10], that plants have different classes of phytochemicals which include alkaloids, flavonoids, coumarins, glycosides, polysaccharides, phenols, tannins, steroids, resin, terpenes and terpenoids. The phytochemicals of *Napoleona imperialis* stem bark reported in this research is the same with the seeds in the work of Uchegbu *et al.,* [21], using raw *Napoleona imperialis* seeds; saponin, alkaloids, hydrogen cyanide, phytates and tannin. Terpenoids can exhibit medicinal properties such as anticarcinogenic (examples are, Taxol and perilla alcohol), antimalarial (example is the artemisinin), anti-ulcer, antimicrobial or diuretic (example is glycyrrhizin) activity [22].

5. Conclusion

This research proves that *Napoleona imperialis* contains some phytochemicals that are useful for mankind and also the part of this plant extracts exhibit medicinal properties

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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