

Evaluation of proximate compositions, vitamins, micro and macro elements of *Napoleona imperialis* stem bark

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Abstract

Numerous studies done on some parts of *Napoleona imperialis* plant extracts but there is still paucity of information on the proximate composition and nutritive values of *Napoleona imperialis* stem bark.

Objective: This is to evaluate the proximate composition, vitamins, micro and macro elements of *Napoleona imperialis* stem bark. **Methods:** Determination of proximate, composition, vitamins, micro and macro elements of *Napoleona imperialis* stem bark was done using standard methods. **Results:** This study showed that Carbohydrate had the highest concentration of 59.540% followed by the ash content which was 12.768%. Potassium was found in the highest concentration of 8.246mg/kg followed by Sodium which was 5.853mg/kg. Also, magnesium had 3.497mg/kg followed by selenium of 2.701mg/kg and Calcium of 1.373mg/kg respectively. The least concentration of the mineral contents were found in Iron which had 0.076mg/kg, followed by Aluminium and Copper which were 0.075 mg/kg and 0.072 mg/kg respectively. The stem bark showed the absence of concentration of Tin, Cobalt, Lead, Manganese, Chromium and Vanadium. Vitamin A had a concentration of 5.315mg/kg, Vitamin B1 showed a concentration of 10.920mg/kg, Vitamin B2 showed 0.315mg/kg, Vitamin B3 with 5.430mg/kg, Vitamin B6 with 4012.500mg/kg, Vitamin B12 with 0.355mg/kg, Vitamin C with 1.358mg/kg and Vitamin E with 4.213mg/kg. **Conclusion:** The present study showed the presence of antioxidant properties and vitamin B complex with other nutritive values. This data indicates that *Napoleona imperialis* stem bark constitute nutritive ingredients, minerals, vitamins and antioxidants. This justifies the claimed use of the stem bark in the management of certain ailments.

Keywords: Vitamin; Microelement; Macroelement; Proximate composition; *Napoleona imperialis* stem

1. Introduction

Plants are made up of different phytochemicals properties, minerals and vitamins that provide their pharmacologic properties and nutritional values [1].

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Minerals are the most essential nutrients for the maintenance of body homeostasis and act as cofactors, aid in the distribution of nerve impulses and water balance [2]. Major minerals present in *Napoleona imperialis* stem bark extract include selenium, potassium, sodium, calcium, zinc, and others. Potassium and sodium are required to regulate the osmotic balance in the body and also maintained the pH level [2].

Selenium is an essential element that play vital role in the body and it requires to take 55 microgram daily in order to maintain the body function [3]. The antioxidants glutathione peroxidase and thioredoxin reductase, together with deiodinase enzymes, constitute selenium that is incorporated into many multivitamins and other nutritional supplements [3].

Calcium (2006mg/kg), is required for the development of bones formation and it aids in the synthesis and function of blood cells. Calcium in conjunction with magnesium, chlorine and proteins are needed in the formation of bones [4].

Vitamins play major biochemical functions in the body such as; maintenance of mineral balance and metabolism and act as antioxidant [5]. Vitamin C helps to boost immune system and fight against infection [6]. Vitamin E also can inhibit the formation of nitrosamines that may develop colon cancer as a result of consumption of diet rich nitrite [7]. Vitamin A provide antioxidant and also is needed for normal growth, bones and teeth formations especially in children [8].

The previous studies conducted on some parts of this plant still showed paucity of information on the proximate composition, vitamins, micro and macro elements of *Napoleona imperialis* stem bark.

2. Materials and Methods

Napoleona imperialis stem bark, was collected from the forest 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

2.1. Collection of *Napoleona imperialis* stem bark

The stem bark of the plant was collected from the forest, dried under shade for forty (40) days, ground 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. Proximate analysis.

2.2.1. Moisture content

Methods

A petri-dish was washed and dried in the oven. Approximately 2g of the sample (*Napoleona imperialis* stem bark) was weighed into petri dish. The weight of the petri dish and sample was noted before drying. The petri dish and sample were transferred in the oven and heated at 105°C for 3hrs cooled in a desiccator and the weight was noted. The drying procedure was continued until a constant weight was obtained [9].

$$\% \text{ moisture content} = \frac{w_1 - w_2}{\text{Weight of sample}} \times 100$$

Where w_1 = weight of petri dish and sample before drying

w_2 weight of petri dish and sample after drying.

2.3. Carbohydrate Determination

Differential method $100 - (\% \text{Protein} + \% \text{Moisture} + \% \text{Ash} + \% \text{Fat} + \% \text{Fibre})$

2.4. Ash content Determination

Principle: The ash of foodstuff is the inorganic residue remaining after the organic matter has been burnt away. It should be noted, however, that the ash obtained is not necessarily of the composition as there may be some from volatilization.

2.4.1. Methods

Empty platinum crucible was washed, dried and the weight was noted and approximately 1- 2g of dried sample was weighed into the platinum crucible and placed in a muffle furnace at 550°C for 3 hours. The sample was cooled in a desiccator after burning and weighed [10].

Calculations

$$\% \text{ Ash content} = \frac{W_3 - W_1}{W_2 - W_1} \times \frac{100}{1}$$

Where

W1 = weight of empty platinum crucible

W2 = weight of platinum crucible and sample before burning

W3 = weight of platinum and ash.

2.5. Crude Fibre Determination

2.5.1. Methods

About 2g of material (*Napoleona imperialis* stem bark) was defat with petroleum ether (if the fat content is more than 10%). It was boiled under reflux for 30 minutes with 200ml of a solution containing 1.25g of H₂SO₄ per 100ml of solution. The solution was filtered through linen and it was washed with boiling water until the washings are no longer acid. The residue was then transferred to a beaker and boiled for 30 minutes with 200ml of a solution containing 1.25g of carbonate free NaOH per 100ml. The final residue was filtered through a thin but close pad of washed and ignited asbestos in a Gooch crucible. It was dried in an electric oven and weighed. It was then incinerated, cooled and weighed.

The loss in weight after incineration x 100 is the percentage of crude fibre.

$$\% \text{ crude fibre} = \frac{\text{weight of fibre}}{\text{Weight of sample}} \times 100$$

2.6. Crude fat Determination

2.6.1. Soxhlet Fat Extraction Method

This method is carried out by continuously extracting a food using non- polar organic solvent such as petroleum ether for about 1 hour or more.

2.6.2. Procedure

Two hundred and fifty milliliter (250ml) clean boiling flask was dried in oven at 105 - 110°C for about 30 minutes. It was then transferred into a desiccator and allowed to cool. The correspondingly labeled, cooled boiling flasks was weighed. The boiling flask was filled with about 300ml of petroleum ether (boiling point 40 - 60°C) and the extraction thimble was lightly plugged with cotton wool. The soxhlet apparatus was assembled and allowed to reflux for about 6 hours. The thimble was removed with care and collect petroleum ether in the top container of the set - up was collected and drained into a container for re - use. When flask was almost free of petroleum ether, it was removed and dried at 105°C - 110°C for 1hour. It was transferred from the oven into a desiccator and allowed to cool; then it was weighed.

$$\% \text{ fat} = \frac{\text{weight of flask + oil} - \text{weight of flask}}{\text{Weight of sample}} \times 100$$

2.7. Crude Proteins

2.7.1. Methods

Exactly 0.5g of sample was weighed into a 30ml kjeldahl flask (gently to prevent the sample from touching each side of walls) and then the flask was stoppered and shaken. Then 20ml of sulphuric acid and 0.5g of the Kjeldahl catalyst was added. The mixture was heated cautiously in a digestion rack under fire until a clear solution appeared. The clear solution was then allowed to stand for 30 minutes and allowed to cool. After cooling, it was made up to 100ml with

distilled water to avoid caking and then 5ml was transferred to the kjeldahl distillation apparatus, followed by 5ml of 40% sodium hydroxide. A 100ml receiver flask containing 5ml of 2% boric acid and indicator mixture containing 5 drops of bromocresol blue and 1 drop of methylene blue was added and placed under a condenser of the distillation apparatus so that the tap was about 20cm inside the solution and distillation commenced immediately until 50 drops gets into the receiver flask, after which it was titrated to pink colour using 0.01N hydrochloric acid. (AOAC, 1992)

Calculations

% Nitrogen = Titre value x 0.01 x 14 x 4

% Protein = % Nitrogen x 6.25

2.8. Detection of vitamins

2.8.1. Estimation of vitamin A

Vitamin A was estimated by the method of Bayfield and Cole [11].

2.8.2. Methods

All procedures were carried out in the dark to avoid the interference of light. Liver homogenate (1.0ml) was mixed with 1.0ml of saponification mixture and refluxed for 20 minutes at 60^o C in the dark. The tubes were cooled and 20ml of water was added and mixed well. Vitamin A was sampled twice with 10ml of (40^o -60^o C) petroleum ether. The two samples were pooled and washed thoroughly with water. Anhydrous sodium sulphate was added to remove excess moisture. An aliquot of the sample (1.0ml) was taken and evaporated to dryness at 60^o C. The residue was dissolved in 1.0ml chloroform. Standards (vitamin A palmitate) of concentrations ranging from 0-7.5g were pipetted out into a series of test tubes.

The volume in all the tubes was made up to 1.0ml with chloroform. TCA reagent (2.0ml) was added rapidly, mixed and the absorbance was read immediately at 620nm in a spectrophotometer (Genesys 10UV). The same procedure was repeated for the sample tubes also. Vitamin A content was expressed as g/g tissue.

Calculation:

$$\text{Conc of vitamin A in sample} = \frac{\text{Abs of sample}}{\text{Abs of std}} \times \text{conc of standard}$$

2.9. Determination of vitamin E

2.9.1. Methods

This was determined by the futter – mayercolometric method with association of vitamin chemist's (kirk and sawyer 1991). One gram (1g) of the sample was mixed with 10ml of ethanolic sulphuric acid and boiled gently under reflux for 30mins. It was transferred to a separating funnel and treated with 3x, 30ml diethyl ether and recovering ether layer each time, the ether extract was transferred to a desiccator and dried under for 30mins and later evaporated to dryness at room temperature. The dried extract was dissolved in 10ml of pure ethanol. 1ml of the dissolved extract (1ml) and equal volume of standard vitamin E were transferred to separate tubes. After continuous addition of 5ml of absolute alcohol and 1ml of concentrated nitric acid solution, the mixtures were allowed to stand for 5mins and the respective absorbance measured in a spectrophotometer meter at 410nm with blank reagent at zero.

2.10. Determination of Vitamin C

2.10.1. Method

This was determined by the titrimetric method reported by (Kirk and Sawyer). A weighted sample was homogenized in 6% EDTA/TCA solution. The homogenate was filtered and used for analysis. Twenty milliliter (20ml) of 30% KI solution was added to it and it was titrated against 0.1M CUSO₄ solution. The end point was marked by a black colouration. A reagent blank was also titrated.

Vitamin C content was calculated based on the relationship below.

1ml of 0.1 mole CuSO₄ = 88mg vitamin C.

$$\text{Vitamin C mg/100} = \frac{1 \times .88 \times \text{titre} - \text{blank}}{W}$$

2.11. Determination of vitamin B₁ and B₂

2.11.1. Methods

One gram (1g) of sample (*Napoleona imperialis* stem bark) was weighed into a conical flask and was dissolved with 100ml of deionized water. This was shaken thoroughly and heated for 5 minutes and allowed to cool and filtered. The filtrate was poured into cuvette and their respective wavelength for the vitamins set to read the absorbance using spectrophotometer [12].

Vitamin B₁ = 261nm

Vitamin B₂ = 242nm

Calculations

$$\text{Concentration (mg\%)} = \frac{A \times D.F \times \text{volume of cuvette (5)}}{E}$$

Where A = absorbance

E = extinction coefficient = 25 for B₁ and B₂

DF = dilution factor

2.12. Determination of vitamin B₃ (Nicotinamide)

2.12.1. Methods

Five grams (5g) of sample was dissolved in 20ml of anhydrous glacial acetic acid and warmed slightly. Five milliliter (5ml) of acetic anhydride was added and mixed and 2 -3 drops of crystal violet solution was added as indicator. It was then titrated with 0.1M perchloric acid to a greenish blue colour [12].

Calculation

$$\text{VitaminB}_3 = \frac{\text{titre value} \times 0.0122}{0.1}$$

2.13. Determination of vitamin B₆

2.13.1. Methods

Five gram (5g) of sample was dissolved in a mixture of 5ml of anhydrous glacial acetic acid and 6ml of 0.1m mercury II acetate solution then, 2 drops of crystal violet was added as indicator. It was then titrated with 0.1m perchloric acid to a green colour end point. (Pearson, 1978).

Calculation: each meal of 0.1M perchloric acid is equivalent to 0.02056g of C₈H₁₁NO₃HCL

2.14. Determination of vitamin B₁₂

2.14.1. Methods

Spectrophotometric determination of cyanocobalamin in *Napoleona imperialis* stem bark preparations by coupling reactions with pyridine

Sample preparation: *Napoleona imperialis* stem bark (0.1g) was weighed and taken into separator. In separator, 5 ml of water was added, mixed well and extracted with 5 ml chloroform. The water layer was discarded then chloroform was taken into dry 50 ml volumetric flask by passing through anhydrous sodium sulphate and made up to 50 ml with chloroform.

Procedure: Two milliliter (2ml) of sample and blank solution was taken into test tube. In each test tube, 2 ml of 0.2% solution of phenyl hydrazine (in hydrochloric acid and alcohol in ratio of 1:5 v/v) and was added and mixed well. After that, they were heated on water bath to almost dryness and cool at room temperature. Two milliliter (2ml) solution mixture (ammonia and alcohol in ratio of 1:1) was then added in each test tube and 1ml pyridine. The absorbance was recorded at 635 nm against blank. Standard cobalamin was also analyzed and treated same as sample [12].

Calibration curve was plotted and the concentration of sample extrapolated.

2.15. Detection of minerals

2.15.1. Methods for the Elemental Analysis of samples

Two grams (2g) of the dried sample was weighed out in to a digestion flask and 20ml of the acetic acid mixture (650ml conc HNO₃; 80ml perchloric acid; 20ml conc H₂SO₄) The flask was heated until a clear digest was obtained. The digest was diluted with distilled water to the 100ml mark. Appropriate dilutions were then made for each. The sample was thoroughly mixed by shaking, and 100ml of it was transferred into a glass beaker of 250ml volume, to which 5ml of conc. nitric acid was added and heated to boil till the volume is reduced to about 15-20ml, by adding conc. nitric acid in increments of 5ml till all the residue was completely dissolved. The mixture was cooled, transferred and made up to 100ml using metal free distilled water. The sample was aspirated into the oxidising air-acetylene flame. When the aqueous sample was aspirated, the sensitivity for 1% absorption is observed [13].

3. Results

This study showed that Carbohydrate had the highest concentration of 59.540% followed by the ash content which was 12.768%. The next was moisture which was 9.506% followed by protein which was 7.350%. Fibre was 6.409% and the least was fat having the value 4.427% (figure 1).

The mineral contents was estimated twice in each of the *Napoleona imperialis* stem bark extracts and average was taken. Potassium was found in the highest concentration of 8.246mg/kg followed by Sodium which was 5.853mg/kg. Also, magnesium had 3.497mg/kg followed by selenium had 2.701mg/kg and Calcium had 1.373mg/kg respectively. The least concentration of the mineral contents were found in the Iron which had 0.076mg/kg, followed by Aluminium and Copper had 0.075 mg/kg and 0.072 mg/kg respectively. The stem bark showed the absence of concentration of Tin, Cobalt, Lead, Manganese, Chromium and Vanadium (figure 2).

The Vitamin concentration of *Napoleona imperialis* stem bark was carried out twice and the average taken. Vitamin A had a concentration of 5.315mg/kg, Vitamin B1 showed a concentration of 10.920mg/kg, Vitamin B2 showed 0.315mg/kg, Vitamin B3 with 5.430mg/kg, Vitamin B6 with 4012.500mg/kg, Vitamin B12 with 0.355mg/kg, Vitamin C with 1.358mg/kg and Vitamin E with 4.213mg/kg (Figure 3).

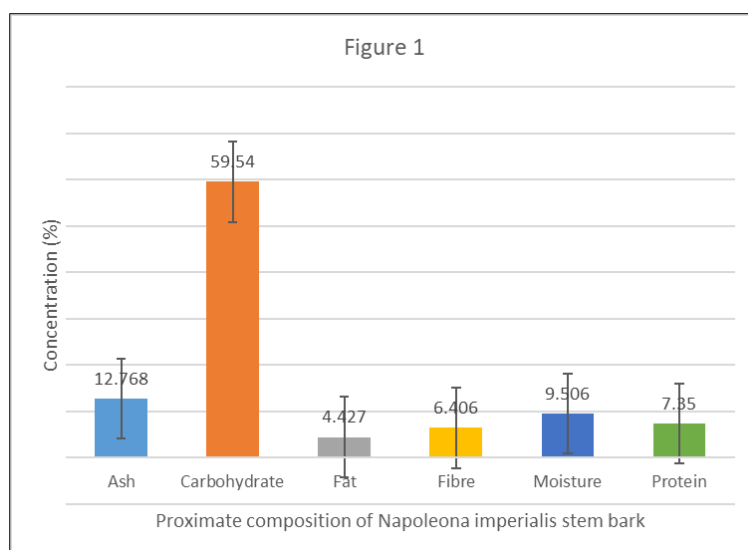


Figure 1 Proximate composition of *Napoleona imperialis* stem bark

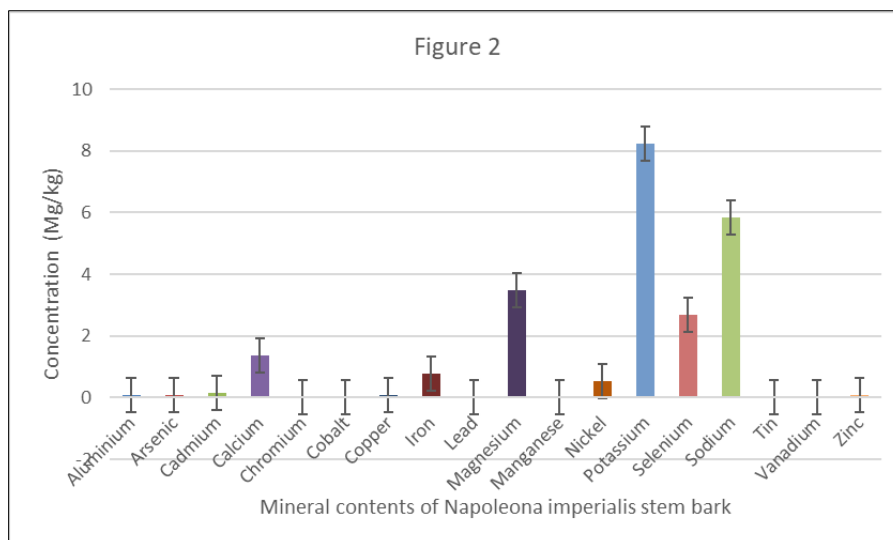


Figure 2 Mineral contents of Napoleona imperialis stem bark

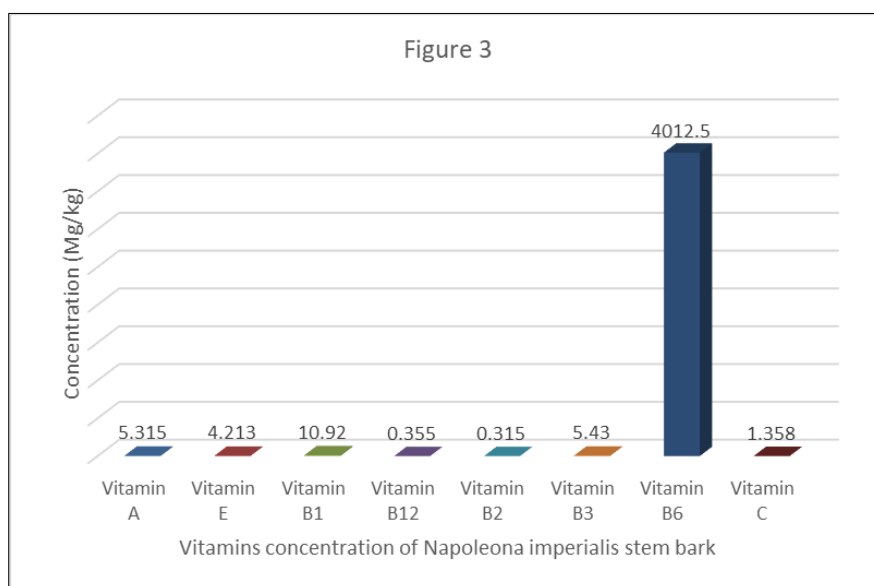


Figure 3 Vitamins concentration of Napoleona imperialis stem bark

4. Discussion

Carbohydrate is a good source of energy which is necessary for normal functioning of the brain, heart, nervous, digestive and immune systems[14]. The present study demonstrated that Carbohydrate had the highest concentration of proximate compositions followed by the ash content, the moisture, the protein, and the fibre respectively. The least of the proximal composition was found in the fat. The previous work done by Etim *et al.*, [14], reported that the proximate analysis of the leave of *Napoleona imperialis* shows the most concentration of the Carbohydrate followed by crude protein, crude fibre, crude fat and ash, this contradicts the findings in this research probably because the researchers evaluated the leaves of *Napoleona imperialis* extract thus, the part of plant extract differs from the stem bark extract evaluated in this study. Similarly, Ukpabi and Ukpabi, [15] in their dry seed meal recorded that the highest proximate contents was found in crude protein while the least was found in ether extract. However, it is important to note that the methods of plant extracts, different parts of plant extraction and concentration of extracts could affect the potency and efficacy of plant extracts.

Previous findings done by Etim *et al.*, [14], show that calcium, iron, copper and zinc in leaves of *Napoleona imperialis* had the higher concentration when compared with stem bark in this research, which were drastically reduced. Other mineral contents evaluated by Etim *et al.*, [14], demonstrated variation of concentration values in the leaves extract when compare to the present study. Copper is a co-enzyme which plays in biochemical roles in the body.

Magnesium, Selenium, Nickel, Cadmium, Arsenic, Aluminium, Tin, Cobalt, Lead, Manganese, Chromium and Vanadium were not evaluated in the leaves in the work done by Etim *et al.*, [14], but were accessed for in the stem bark of *Napoleona imperialis* in this research work. Tin, Cobalt, Lead, Manganese, Chromium and Vanadium were not detected in the stem bark of *Napoleona imperialis* in this work (0.000ppm), but Magnesium, Aluminium, Selenium, Nickel, Cadmium and Arsenic were present in low concentrations. Selenium which is described as an antioxidant, is also involved in hormone biosynthesis.

From this study, *Napoleona imperialis* stem bark had high concentration of vitamin B6 followed by vitamin B1 which are water soluble and important for protein metabolism. This Vitamin when taken at high level over a long period of time has been shown to cause irreversible nerve damage [16]. Vitamin B3 had a concentration of 5.430mg/kg. This is also water soluble and important for nervous system, digestive system and skin health. Vitamin A (fat soluble) is needed for vision, healthy skin and mucous membrane, bone and tooth growth and also in healthy immune system. Vitamin E is a fat soluble antioxidant that also protects the cell wall. Vitamin C (water soluble) which is also an antioxidant, helps in iron absorption, needed for protein metabolism and important for immune system health. Hussain *et al.*, [17], stated that sufficient amount of vitamin C in the diet is important for the body as its deficiency causes scurvy. Vitamin B2 is water soluble that is also needed for energy metabolism and important for normal vision and skin health. Vitamin B12 (0.355 mg/kg) is also present and it is important in nerve function. *Napoleona imperialis* has a lot of Vitamins that helps in the normal functioning of the body and this also justify it's medicinal use. The leaves of some plants like *Acanthus montanus*, *Pterocarpus mildbreadi* and *Datura stramonium* that are also used in south eastern Nigeria were researched by Nwaoguikpe *et al.*, [18] to contain vitamin C, vitamin B₂ and vitamin B₁.

5. Conclusions

The present study showed the abundance of antioxidant properties and vitamin B complex with others nutritive values. This could aid in the wound healing and other infectious diseases due to the great proportion of selenium content and other antioxidant properties present in this extract. The vitamin B complex especially Vitamin B6 found in the stem bark of the plant can be served as blood supplement for the management of anemia. This data indicates that *Napoleona imperialis* stem bark constitute nutritive ingredients, minerals, vitamins and antioxidants. This justifies the claimed use of the stem bark in the management of certain ailments.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No conflict of interest.

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