



PHYTOCHEMICALS, VITAMINS, MACRO AND MICRO ELEMENTS AND ANTIMICROBIAL ANALYSIS OF THE STEM BARK OF NAPOLEONA VOGELII(AKPAESU)

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ABSTRACT

Sequel to the increasing application of plant materials in ethnomedicine and for nutritive purposes the phytochemicals, vitamins, macro and micro elements and antimicrobial analysis of the stem bark of *Napoleonavogelii* were undertaken using standard methods. The result shows that *Napoleonavogelii* contains bioactive phytochemicals such as tannin ($0.45 \pm 0.25\%$), phytosterols ($12.53 \pm 0.25\%$), alkaloids ($4.42 \pm 0.23\%$), flavonoids ($9.27 \pm 0.18\%$), saponins ($4.27 \pm 0.25\%$), hydrogen cyanide ($0.37 \pm 0.20\%$). The vitamin analysis shows that the stem bark of *Napoleonavogelii* contains vitamin A (1.20 ± 0.03 Mg/g), vitamin E (1.31 ± 0.04 Mg/g), vitamin C (2.78 ± 0.04 Mg/g), vitamin B1 (7.20 ± 0.05 Mg/g), vitamin B2 (0.90 ± 0.31 Mg/g), vitamin B9 (4.09 ± 0.05 Mg/g), vitamin B3 (1.27 ± 0.25 Mg/g), vitamin B6 (12.50 ± 0.04 Mg/g) and vitamin B12 (8.25 ± 0.04 Mg/g) in varying amounts while vitamin B5 and vitamin B7 were absent. Sodium (1247 mg/kg), Calcium (2006 mg/kg), Copper (1.23 mg/kg), Phosphorous (410.22 mg/kg), Iron (304.660 mg/kg), Manganese (56.99 mg/kg) and Zinc (2.69 mg/kg) were the detectable mineral elements, Potassium (4996 mg/kg) had the highest value while Arsenic and Nickel were below detection limit (0.001 mg/kg). The result of the antimicrobial screening of the ethanol, butanol and chloroform extracts of the stem bark of *Napoleonavogelii* against five pathogenic microbes, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus sp*, *Staphylococcus aureus*, and *Candida albicans* shows that the butanol extract showed higher antimicrobial activities compared to the other extracts. Results from this study have shown that the stem bark of *Napoleonavogelii* contains medicinal properties.

Council for Innovative Research

Peer Review Research Publishing System

Journal: Journal of Advances in Chemistry

Vol. 11, No. 9

www.cirjac.com

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INTRODUCTION

Plants are composed of vast array of phytochemicals and vitamins that characterize their pharmacologic properties and nutritional values [1]. Phytochemicals are naturally synthesized in all parts of the plant and may contain active components[2].Phytochemicals have been recognized as the basis for traditional herbal medicine practiced in the past and currently in parts of the world [3]. Plants and phytochemical compounds are used in folk medicine for the treatment of different types of diseases [4]. A typical example is the *Napoleonavogelli* whose methanol leave extract of is used in the treatment of peptic ulcer diseases [5] and the bark is used for treatment of skin diseases by the local folks [6]. *Napoleonavogelli* is an evergreen and widely distributed plant with alternate leaves, usually crowded towards the apex of the branches. About 20 genera and 450 species have been identified in the tropical regions of Africa, Asia and Australia. Its region of distribution is mostly in African countries of Nigeria, Ghana, Guinea, Togo, Benin and Ivory-coast [7]. *Napoleonavogelli*, (Lecythidaceae) is known locally as akpuruke, mkpodu, or odure by the Ibos of South-East Nigeria. Its common names are Akpako, Boribons, Burukwa. The plant is found mostly in rain forest, along the sea shores extending from Sierra Leone to Nigeria. It is an ornamental shrub or tree 2.5-5cm tall. The plant grows best in full sun but tolerates light shade. It prefers warm and humid climate and requires medium water/regular moderate drought tolerance. Its fruits are sweet and edible and the fruits ripe in dry season[8].

MATERIALS AND METHODS

Sample Collection

Fresh sample of *Napoleonavogelli* stems were collected from Uturu, in Isuikwuato Local Government Area of Abia State. The plant was identified by Mr. IbeKaluNduke of Michael Okpara University of Agriculture, Umudike, Abia State.

Micro-Organisms Collections

Pure cultures of bacterial strains, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus* sp, *Staphylococcus aureus*, and fungal strain of *Candida albicans* were obtained from veterinary diagnosing laboratory veterinary research institute out station, Umudike, Abia state.

Preparation of Plant Materials

Freshly collected *Napoleonavogelli* stem bark was washed and dried under the shade at normal room temperature for 14 days. Upon drying, the stem bark was blended to powder. The powdered sample was then stored in air tight containers and kept under normal room temperature until required.

Determination of Minerals

The method described by [9] was adopted. 1g of the sample was weighed into a beaker and 100ml of digestion mixture(nitric acid: sulphuric acid: perchloric acid) in the ratio 2:2:1 was added and the mixture was heated on a hot plate in a fume hood at 105°C until the production of white fumes which indicates complete digestion. 10ml of the de-ionized water was added to the digestate and was allowed to cool down to room temperature and filtered using No 42 watman filter paper into 50ml volumetric flask. The digestate was made to mark with de-ionized water and analysis of heavy metals was performed using Atomic Absorption Spectrophotometer AAS-Biotech 896,UK.

Determination of Phytochemicals

The plant extracts were screened for the presence of some phytochemicals using standard methods as described by[10] and [11].

Antibacterial Activity

Agar well diffusion method as described by [9] was employed to assay for the antibacterial activity.

Determination of Vitamins

The method of analysis employed were those described by [12]; [13] and[14].



RESULTS AND DISCUSSION

4.1 QUALITATIVE PHYTOCHEMICAL SCREENING

The result of the qualitative phytochemical screening of the stem bark of *Napoleonavogelii* is shown in table 1.

TABLE 1: The Result of the Qualitative Phytochemical Screening of the Stem Bark of *Napoleonavogelii*

PHYTOCHEMICALS	ETHANOL	BUTANOL	CHLOROFORM	WATER
Tannin	+	-	-	+
Alkaloid	+	-	+	+
Saponin	+	-	-	+
Flavonoid	+	-	+	-
Oxalate	-	-	-	-
Steroid	-	-	+	-
Phytosterol	-	-	+	-
Carbohydrate	-	-	+	+
Cardiac glycoside	+	+	-	+
Phenol	-	-	+	+
Hydrogen cyanide	+	-	+	-

KEY : + Present - Absent

The result for the qualitative phytochemical analysis is presented on Table 1. The result revealed the presence of different phytochemicals that slightly varied with the solvent used for the extraction [15]. The presence of alkaloids, saponins, and tannins in the stem bark extracts of *Napoleonavogelii* has medicinal implication. These phytochemicals are known to be biologically active. They possess some properties such as antibacterial properties (tannins and flavonoids) antiseptic properties (tannins), anti-inflammatory properties (flavonoids, saponins), analgesic properties (steroids) and anaesthetic properties (alkaloids) [16]. The presence of tannins was found to play a role in the antifungal, antibacterial, astringent and antibiotic activities [16]. Phenolic acids and flavonoids have been the object of a great number of studies of their anti-oxidative activity which is mainly because of their capacity to act as free radical scavengers and/or metal chelators [17][18]. Both compounds have attracted considerable interest in the past few years due to their many potential health benefits. As polyphenols, phenolic acids and flavonoids are powerful antioxidants and have been reported to demonstrate antibacterial, antiviral, anticarcinogenic, anti-inflammatory and vasodilatory actions [19][20].

The above result is similar to the findings of Christainet al., [6] who in his report of methanol and petroleum extracts of the stem bark of *Napoleonavogelii* stated the presence of tannins, alkaloids and saponin. Previous phytochemical studies indicated the presence of alkaloids, tannins and saponins on *Napoleonavogelii* stem bark [16]. Though few works have been done on the phytochemicals of the stem bark, it was found out that the leaves of the plant contained tannin, alkaloid, saponin, flavonoid carbohydrate and cardiac glycoside [21] and the fruit plant contained tannin, alkaloid, saponin, flavonoid carbohydrate and cardiac glycoside [7].

QUANTITATIVE PHYTOCHEMICAL SCREENING

The result of the quantitative phytochemical screening of the stem bark of *Napoleonavogelii* is shown in table 2

The phytochemical result indicates that the flavonoid content of *Napoleonavogelii* stem bark is 9.27%. This value is relatively high compared to the value of 4.65 reported of *Napoleonavogelii* Hook fruit [22]. However, [23] reported that high amounts of flavonoid help protect blood vessels from rupture or leakage, enhance the power of vitamin C; protect cells from oxygen damage and prevent excessive inflammation.

TABLE 2: The Result of the Quantitative Phytochemical Screening of the Stem Bark of *Napoleonavogelii*

PHYTOCHEMICALS	QUANTITY PRESENT (%)
Tannin	0.45±0.25
Phytosterol	12.53±0.25
Alkaloid	4.27±0.23
Flavonoid	9.27±0.18
Saponin	4.27±0.25
Hydrogen cyanide	0.37±0.20



The value obtained for tannin in the stem bark of this plant was (0.45%). This value is lower than that reported for *Napoleonavogelii* Hook fruit [22] and the stem bark of *Napoleonavogelii* in methanol (9.5%) and in petroleum ether (1.8%) [6]. High tannin content in this sample implies severe nutritional challenge to animals or humans due to its affinity for certain digestive enzymes. The saponin value is 4.27%. This is quite high compared to the value of 0.75% reported for *Napoleonavogelii* Hook fruit [22] and lower than the value of (1.8%) in petroleum ether reported by [6]. A high Saponin diet can inhibit dental and platelet aggregation in treatment of Hypercalciuria in human (excessive urinary calcium excretion, an antidote against acute lead poisoning [24]. Saponin also decreases blood lipids, lower cancer risks and blood glucose response as well as possesses antioxidant activity. Toxicology studies of saponin using relevant experimental models have established that even at an upper concentration of 3.5%, saponin was safe and failed to cause systemic side effect [25]. The value obtained for hydrogen cyanide in *Napoleonavogelii* stem bark is 0.37% and is lower than the 36mg/100g considered lethal dose for man [26]. The alkaloid content (4.27%) is low compared to that reported by [6]. Caution should be taken in the consumption of plant materials with very high concentration of Alkaloid because they could inhibit certain mammalian enzymes activities such as those of cyclic adenosine monophosphate (AMP) [27]. European Food Safety Authority [28] stated that since cooking only lowers alkaloid content of foods by 40 – 50%, highly sensitive individuals should avoid this category of food entirely.

VITAMINS ANALYSIS

The result of the vitamins analysis of the stem bark of *Napoleonavogelii* is shown in table 3.

TABLE 3: The Result of the Vitamins Analysis of the Stem Bark of *Napoleonavogelii*

VITAMINS	QUANTITY PRESENT (Mg/ g)
Vitamin A	1.20±0.03
Vitamin E	1.31±0.04
Vitamin C	2.780.04
Vitamin B1	7.20±0.05
Vitamin B2	0.90±0.31
Vitamin B9	4.09±0.05
Vitamin B3	1.27±0.25
Vitamin B6	12.50±0.04
Vitamin B12	8.25±0.04
Vitamin B7	NIL
Vitamin B5	NIL

Vitamins have diverse biochemical functions. Some, such as vitamin D, have hormone-like functions as regulators of mineral metabolism, or regulators of cell and tissue growth and differentiation. The largest number of vitamins, the B complex vitamins, functions as precursors for enzyme cofactors that help enzymes in their work as catalysts in metabolism. Others function as antioxidants such as vitamin E and vitamin C [29].

The presence of vitamin C (ascorbic acid) in the diet enhances iron absorption, and iron to ascorbic acid ratio provides an index of iron availability in food sample in such a way that the lower ratio, the greater the relative bioavailability of iron [21]. Vitamin C prevents many debilitating diseases, increases the body's immunity and is a powerful antioxidant [30]. It also helps in recycling other antioxidants and it also aids in the formation of collagen. Collagen, tendons and ligaments depend upon Vitamin C to stay strong and healthy [31].

Vitamin E provides protection which might include its function as an antioxidant and its roles in anti-inflammatory processes, inhibition of platelet aggregation, and immune enhancement [23]. Vitamin E also may block the formation of nitrosamines, which are carcinogens formed in the stomach from nitrites consumed in the diet [32]. It also may protect against the development of cancers by enhancing immune functions. Some evidence links higher intake of vitamin E to a decreased incidence of prostate and breast cancers [23]. The vitamin A content of the plant is 1.20mg/g. Vitamin A serves as an antioxidant and is essential for normal growth and for the formation of strong bones and teeth in children, for normal vision and cell structure, for protecting the lining of the respiratory, digestive, and urinary tracts against infection, and for healthy skin [33]. It also functions as a hormone, visual pigment of the vertebrate eye and regulates gene expression in the development of epithelial tissue, including skin [34].

MACRO AND MICRO ELEMENTS ANALYSIS

The result of the macro and micro elements analysis of the stem bark of *Napoleonavogelii* is shown in table 4

TABLE 4: The Result of the Macro and Micro Elements Analysis of the Stem Bark of *Napoleonavogelii*

PARAMETERS	<i>NAPOLEONA VOGELII</i> (mg/kg)
Arsenic	BDL
Sodium	1247
Calcium	2006
Copper	1.23
Nickel	BDL
Phosphorous	410.22
Iron	304.66
Potassium	4996
Manganese	56.99
Zinc	2.69

NOTE; BDL – Below Detection Limit (0.001mg/kg)

Minerals are absolutely necessary for most metabolic processes. They serve as cofactors, help in transmission of nerve impulses and water balance [35]. The level of Calcium is relatively high compared to the level reported for the African yam bean varieties (70-128 ppm) by [36]. Calcium (2006mg/kg), is needed for the formation of bones and it supports the synthesis and function of blood cells. Calcium in conjunction with magnesium, chlorine and proteins are involved in the formation of bones [37]. The availability of calcium in the body depends on calcium to phosphorus ratio and presence of antinutritional factors such as oxalate and phytate [38]. Sodium and Potassium are necessary to maintain osmotic balance in the body as well as the pH.. Iron is present in considerable amount (304.66mg/kg) and its necessary in formation of haemoglobin and normal functioning of the central nervous system. Phosphorus, a mineral in bone formation is also present (410.22mg/kg). Phosphorus is required for normal function of the body since it is a major structural component of bone and teeth and helps to maintain normal pH [39]. It is crucial for production of ATP and also plays important role in the growth, maintenance and repair of cells and tissues [21]. The other Micro minerals like Manganese, Copper and Zinc are also present and they play important roles in metabolic activities. These elements support human biochemical processes by serving structural and functional roles as electrolytes [34]

ANTIMICROBIAL ANALYSIS

The result of the antimicrobial activity screening of the stem bark of *Napoleonavogelii* is shown in table 5

TABLE 5: The Result of the Antimicrobial Activity of the Stem Bark of *Napoleonavogelii*

	<i>STAPPHYLOCOCCUS</i>	<i>E.COLI</i>	<i>PSEUDOMONAS</i>	<i>STREPTOCOCCUS</i>	<i>CANDIDA</i>
BUTANOL	14.3 ±1.20	17.7 ±1.50	12.3 ±1.20	14.7 ±1.20	20.3 ±1.20
ETHANOL	11.7 ±1.20	9.7 ±0.60	16.7 ±1.20	13.7 ±0.60	10.3 ±1.20
CHLOROFORM	12.3 ±1.20	12.3 ±0.60	10.7 ±0.60	14.7 ±0.60	17.3 ±1.20
STD	24.3 ±1.20	24.7 ±1.20	12.3 ±1.20	18.7 ±0.60	12.3 ±1.20

Values show means of triplicate analysis ±standard deviation.

The antimicrobial activity of the extracts of *Napoleonavogelii* were studied in different concentrations (50,100,150,200) against four bacterial staphylococcus aureus, streptococcussp, Escherichia coli, pseudomonas aeruginosa and a fungal strain candida albicans. Antibacterial and antifungal potential of extracts were assessed in terms of zone of inhibition of bacterial growth. The results of the antibacterial and antifungal activities are presented in the table. The antibacterial and antifungal activities increased linearly with increase in the concentration of extracts. The result of this study agrees closely with the work of [6] on antimicrobial activity of *Napoleonavogelii*. In both works, Pseudomonas was significantly inhibited. However, the result could possibly be due to the different extraction solvents used in the different works [15].



DIAMETER OF INHIBITION ZONE

The result of the diameter of inhibition zone of the stem bark of *Napoleonavogelii* is shown in table 6

TABLE 6: The Result of the Diameter of Inhibition Zone (mm) of the Stem Bark of *Napoleonavogelii*

ORGANISM	CONC. (mg/ml)	Diameter Of Inhibition Zone (mm)		
		BUTANOL EXTRACT(mm)	ETHANOL EXTRACT(mm)	CHLOROFORM EXTRACT(mm)
<i>STAPPHYLOCOCCUS AUREUS</i>	50	-	-	-
	100	-	-	-
	150	10	8	8
	200	14	12	12
	Chloramphenicol (25mg/ml)	25	25	23
<i>ESCHERICHIA COLI</i>	50	-	-	-
	100	10	-	-
	150	13	-	9
	200	18	10	12
	Chloramphenicol (25mg/ml)	26	24	24
<i>PSEUDOMONAS AERUGINOSA</i>	50	-	-	-
	100	-	13	-
	150	8	14	-
	200	12	17	11
	Chloramphenicol (25mg/ml)	13	11	11
<i>STREPTOCOCCUS SP</i>	50	-	-	-
	100	-	-	-
	150	9	10	10
	200	15	14	14
	Chloramphenicol (25mg/ml)	18	19	19
<i>CANDIDA ALBICANS</i>	50	-	-	-
	100	9	-	10
	150	13	-	13
	200	20	10	17
	Ciprofloxacin (50mg/ml)	33	31	31
ORGANISM	CONCENTRATIN(mg/ml)	Diameter Of Inhibition Zone (%)		
		BUTANOL EXTRACT (%)	ETHANOL EXTRACT (%)	CHLOROFORM EXTRACT (%)



<i>STAPHYLOCOCCUS AUREUS</i>	50	-	-	-
	100	-	-	-
	150	40	32	32
	200	56	48	48
<i>ESCHERICHIA COLI</i>	50	-	-	-
	100	40	-	-
	150	52	-	36
	200	72	40	48
<i>PSEUDOMONAS AERUGINOSA</i>	50	-	-	-
	100	-	52	-
	150	32	56	-
	200	48	68	44
<i>STREPTOCOCCUS SP</i>	50	-	-	-
	100	-	-	-
	150	36	40	40
	200	60	56	56
<i>CANDIDA ALBICANS</i>	50	-	-	-
	100	18	-	20
	150	26	-	26
	200	40	20	34

The antimicrobial activity of the extracts were studied in different concentration (50,100,150,200) against four pathogenic bacterial strains, two Gram-positive (*Staphylococcus aureus*, *Streptococcus sp.*) and two Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*), and a fungus strain *Candida albicans*). Antibacterial and antifungal potential of extracts were assessed in terms of zone of inhibition of bacterial growth. The growth inhibition zone measured ranged from 7 to 19 mm for all the sensitive bacteria, and ranged from 9 to 21 mm for fungal strain. In this study, the results showed that the three extracts of *Napoleonavogelii* possess antimicrobial activities against the tested organisms at different concentrations. With the butanol extract, the highest antimicrobial activity of 18mm in *E.coli* and least activity of 8mm in *streptococcus* were recorded. With the ethanol extract, the highest antimicrobial activity of 17mm in *pseudomonas* and least activity of 8mm in *staphylococcus* was recorded. With the chloroform extract, the highest antimicrobial activity of 15mm in *streptococcus* and least activity of 8mm in *staphylococcus* were recorded. Butanol extract demonstrated a better antifungal activity than the other extracts. The results of the present investigation clearly indicates that the antibacterial and antifungal activity vary with the different solvent used in extraction. Thus the study ascertains the value of solvents used for extraction which could be of considerable interest to the development of new drugs.



4.5.2 MINIMUM INHIBITION CONCENTRATION (MIC)

The minimum inhibition concentration of the stem bark of *Napoleonavogelii* is shown in table 7

TABLE 7: The Minimum Inhibition Concentration (MIC) in mg/ml of the Stem Bark of *Napoleonavogelii*

EXTRACTS	<i>STAPHYLOCOCCUS</i>	<i>E.COLI</i>	<i>PSEUDOMONAS</i>	<i>STREPTOCOCCUS</i>	<i>CANDIDA</i>
BUTANOL	150	100	150	150	100
ETHANOL	150	200	100	150	200
CHLOROFORM	150	150	200	150	100

The minimum inhibition concentration (MIC) presented in the table above shows that the values of the different extracts ranged between 100mg/ml – 200mg/ml. With the butanol extract, the MIC ranged from 100mg/ml on *E.coli* and *Candida* to 150mg/ml on *staphylococcus*, *pseudomonas* and *streptococcus*. Ethanol extract however had MIC range between 100mg/ml for *pseudomonas*, 150mg/ml for *staphylococcus aureus* and *streptococcus* and then 200mg/ml for *E.coli* and *Candida*. Chloroform extract had MIC range between 100mg/ml for *Candida*, 150mg/ml for *staphylococcus*, *E.coli* and *streptococcus*, and 200mg/ml for *pseudomonas*.

CONCLUSION

The result of this study revealed the presence of most of the phytochemicals with various biological activities. This might be responsible for the observed antimicrobial activities against test organisms. The important minerals found in the stem bark may also be major contributors to the medicinal use of the plant. This data indicates that *Napoleonavogelii* stem bark contains potent bioactive compounds and justifies the claimed use of the stem bark in the traditional system of medicine to treat infectious diseases such as skin diseases caused by microbes

ACKNOWLEDGEMENT

The authors are grateful to the National Root Crop Research Institute Umudike for assisting their equipment for the anti microbial analysis and the Golden Year Ltd Port Harcourt for the use of their AAS equipment.

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