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Phytochemical, Vitamins, Micro And Macro Elements And Antimicrobial Analysis Of The Leaves Of *Napoleona Vogelii* (Akpaesu)

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Abstract
The phytochemical, vitamins, micro and macro elements and antimicrobial analysis of the leaves of *Napoleona vogelii* were investigated. Qualitative and quantitative evaluation of the phytochemicals was made using standard methods. The antimicrobial analysis of ethanol, chloroform and butanol extract of the sample were performed on the bacterial *Eschericha coli, Staphylococcus epidermidis, Staphylococcus aureus , Proteus mirabilis* and on the fungus *Candida albicans*. The disc diffusion method was used for the screening using Muller Hinton agar for the in vitro antimicrobial activities. The vitamins were determined by using spectrophotomeric method. The micro and macro elements were determined by using the wet digestion method and an atomic absorption spectrophotomer AAS-Biotech 896, UK. The result for the quantitative analysis showed the presence of rich bioactive compounds such as alkaloid (6.50%±1.50), saponin(3.50%±0.05), Tannin(0.70%±0,09), Flavonoid(6.50%±0.04), phytate (3.10mg/100g± 0.03) and oxalate(4.25mg/100g±1.02). The vitamins presence includes vitamin A (0.72mg/100g±0.07), vitamin B1 (5.27mg/100g±0.02), B2 (0.51mg/100g±0.41), B3(0.92mg/100g±0.12), B9 (2.98mg/100g±0.32), B12(8.28mg/100g±0.06), vitamin C (3.20 mg/100g±1.03) and E(2.02mg/100g±0.05). The result of the heavy metals and minerals showed the presence calcium (2030.60mg/kg), iron (281.41 mg/kg), zinc (6.80 mg/kg), manganese (74.01 mg/kg), copper (2.56 mg/kg), chromium (0.12 mg/kg), nickel (0.57 mg/kg) and magnesium (90.85 mg/kg). The antimicrobial activities showed significant inhibition of test organisms. The result showed that the leaf of *Napoleona vogelii* have potent medicinal uses and suggests that the folkloric uses of these maybe based on these results.

Keywords: Phytochemicals, Vitamins, Micro and Macro elements, Antimicrobial, *Napoleona vogelii*

1.0 Introduction

Humans are dependent on plants directly or indirectly for their wide importance. They are becoming known more and more for their vital usage in many arenas, as food, shelter, fiber, fuel, and many other necessities of life, including medicinal purposes (Tapsell *et al*, 2014). Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against attack from predators such as insects, fungi, herbivores mammals. These Bio-active compounds in plants have shown remarkable potential and tremendous benefit in accelerating medicinal activities (Elvin, 2012; Rios *et al*, 2005). They owe their medicinal activity to the presence of the constituent secondary metabolites such as terpenoids, alkaloids, tannins, steroids, glycosides, phenolics, saponins, flavonoids etc that are contained in their leaves, stems, barks, fruits, seeds, roots and flowers (Lai, 2014; Rois *et al*, 2005). These metabolites differ among the plant community and provide tremendous reservoir of various chemical substances that are potential therapeutic agents (Talalay, 2011). These bioactive compounds are called phytochemicals. Phytochemicals are non nutritive plant chemicals that have protective or diseases preventive properties (Thomas, 2004).

Chemical compounds in plant mediate their effects on the human body through processes identical to those well understood by conventional drugs; thus herbal medicines do not differ greatly from

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conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines (Eric *et al,* 2014).

Before the modern day medicine and its pharmacopeia of synthetic drugs, there were plants, and ancient civilization knows how to use them strategically to treat common ailments and even life threatening disease. Today the world health organization estimates that 80 percent of the population of some Asian and African countries presently uses herbal medicine for some aspect of primary health care (Arun *et al,*2013; Sharma *et al,* 2013). There are three ways in which plants have been found useful in medicine. Firstly, they may be used directly as teas or in other extracted forms for their natural constituents. Second, they may be used as agent in the synthesis of drugs. Finally, the organic molecules found in plants maybe used as models for synthetic drugs. A typical example is the *Napoleona vogelli* whose leaf extract are sometimes prepared as polyherbal medicine, having shown to have curative effects for ailments like diarrhea and arthritis, aside its wound healing effect (Srinivas *et al,* 2007, Enye *et al,*2013).

The objective of this study is to evaluate the phytochemical constituent of the leaf of *Napoleona vogelii*, the micro and macro nutrient, the vitamins composition of the plant leaf and the antimicrobial properties of the leaf of *Napoleona vogelii* using selected bacteria and fungi.

MATERIALS AND METHOD

Sample Collection

Fresh leaves sample of *Napoleona vogelii* were collected from Uturu, in Isikwuato local government area of Abia State. The plants were identified by by Mr. Ibe Kalu Ndukwe of Michael Okpara University of Agriculture, Umudike, Abia State.

Micro-Organisms Collections

Pure cultures of bacterial strains, Eschaeria coli (gram negative), Proteus mirablis (gram negative), Staphylococcus Aureus (gram positive), Staphylococcus Epidermins (gram positive) and fungal strain of Candida were obtained from the department of microbiology, university of Abuja, Nigeria.

Preparation of Plant Materials

Freshly collected leaves of *Napoleona vogelii* were washed and dried under a shade at normal room temperature for 10 days. Upon drying, the leaves were blended to powder using an electric blender. The powdered sample was then stored in airtight containers and kept under normal room temperature until required.

Preparation of Extracts

The ethanolic, butanoic and chloroform extract of *Napoleona Vogelii* was prepared by immersing 80g of the dried material in 500 ml the different solvent for 24 hours at room temperature contained in a 1000 ml flask. The flask was covered with and then allowed to stand for 24 hours. After 24 hours, the suspension was shaken vigorously and filtered using a muslin cloth and filter paper. The filtrate was then concentrated by air drying. The concentrated extract was then stored in airtight sample bottle until required. For the preparations of dilutions of crude extracts for antimicrobial screening, a reconstituted ethanolic, butanolic and chloroform extract was prepared by dissolving 200 mg, 400 mg, 600mg and 800 mg of the extract in 1ml of methanol to obtain a concentration of 200 mg/ml, 400 mg/ml, 600mg/ml and 800 mg/ml respectively.

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Disc diffusion Evaluation of Antimicrobial Activity

The method used for the evaluation of the antimicrobial activity of the leaf of *Napoleona vogelii* plant is the disc diffusion method.

Determination of Heavy Elements and Minerals

1gram of the sample was weighed into a beaker and 10ml of digestion mixture (nitric acid, sulphuric acid and perchloric acid) in the ratio 2:2:1 was added and the mixture was heated on a hot plate in a fume hood at 105^0C until the production of white fumes which indicates complete digestion. 10ml of de-ionized water was added to the digestate and was allowed to cool down to room temperature and filtered using No 42 whatman 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 spectrophotomerAAS-Biotech896, UK

RESULTS AND DISCUSSION

Table 1: Qualitative phytochemical result of aqueous, ethanolic, butanolic and chloroform extract of the leaf of *Napoleona vogelii*

Parameters	Aqueous extract	Butanol extract	Chloroform	Ethanol extract
Tannis	+	+	-	+
Steroid	-	+	-	-
Triterpernoids	+	-	-	-
Glycosides	+	+	+	-
Saponnins	-	-	-	-
Phytate	+	-	-	+
Alkaloids	+	-	+	-
Terpenoid	+	+	+	+
Carbohydrate	+	+	-	+
Flavonoid	+	+	-	-
Oxalate	_	+	+	_
Phlobataninns	-	-	-	-
Resins	-	-	-	-
Balsam	-	+	+	+
Volatile oil	-	-	-	-

Key:+ = parameter present , - = parameter absent

Table 1 shows the qualitative phytochemical result of the leaves of *Napoleona vogelii*. The leaves of the ethanolic, butanolic and chloroform extract were investigated for the phytochemical constituent and it reveals the presence of Tannis,

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Triterpernoids, Glycosides, Saponnins, Phytate, Alkaloids, Terpenoid, Carbohydrate, Flavonoid, Oxalate, and Balsam. These componenents are known to be biologically active because they protect the plant against infections and predations by animals (Igidi and Edene, 2014).

According to Adiele *et al,* (2013), the phytochemical analysis of the methanol extract of the leaf of *Napoleona vogelii* revealed the presence of glycosides, alkaliods, saponins, terpeniods, steroids, flavonoids, resins, proteins and carbohydrates. The wound healing effect of *Napoleona Vogelii* leaf extract could be due to the presence of some of these phytochemicals notably tannins, alkaloids glycosides and saponins (Sabale *et al,* 2012).

According to Onyegbule *et al,* (2011). The results of phytochemical screening of *Napoleona imperialis* showed the presence of alkaloid, saponins, tannins, glycosides and proteins while Flavonoids, resins and steroid were absent.

QUATITATIVE PHYTOCHEMICAL RESULT

The quantitative phytochemical composition of *Napoleona Vogelii* leaves extracts are shown in Table 2

Table 2: Quantitative phytochemical result of the leaves of *Napoleona vogelii*

Parameters	*Napoleona vogelii* leaves (mg/100g) (%)
Saponin	3.50 ± 0.05
Tanin	0.76 ± 0.09
Flavonoid	6.50 ± 0.04
Alkaloid	6.50 ± 1.50
Terpenoid	0.00 ± 0.00
Phytate	3.00 ± 0.03
Oxalate	4.24 ± 1.02

The saponin value is 3.50% (Table 2). This is quite high compared to the value of 0.75% reported for the fruit of *Napoleona vogelii* (Igidi and Edene, 2014), 0.68 reported for the *Napoleona imperialis* seed (Martin *et al,* 2010). 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) (Shi, *et al,* 2004). The value obtained for tannin is 0.7%. This value is small compared to 333.4mg/kg (1.667mg/5g,) reported on the fruit. According to Martin *et al,* 2010, 1.35% was recorded for *Napoleona imperialis*. The presence of tannin implied that the leaf has astringent properties, quickens the healing of wound and inflamed mucous membrane (Farquar, 1996), anti-microbial properties and protecting protecting the kidney from inflammation (Adeolu and Enesi, 2013). This supports its folkoric uses.

The phytochemical result also showed that the flavonoid content of *Napoleona vogelii* leaf is 6.50%, this is higher compared with 4.65% reported for the fruit (Igidi and Edene, 2014). It inferred that the leaf have biological functions such as protection against allergies, inflammation free radical, palate aggregation, microbes, ulcer and strong anticancer activities.(Farquar 1999: Okwu 2004).

Alkaloid content of the leaf is 6.50% as against 0.08% recorded for the fruit (Igidi and Edene, 2014). According to Martins *et al,* 2010, the alkaloid content of *Napoleona imperialis* seed is 0.56%. Alkaloids are the most efficient therapeutically significant plant substances. Pure isolated and the synthetic derivatives are used as basic medicinal agent because of their analgesic, antiplasmodium and bacterial properties. Caution should be taken in the consumption of plant materials with very high

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concentration of Alkaloid because they could inhibit certain mammalian enzymes activities such as those of cyclic adenosine monophosphate (AMP) (Raymond *et al*, 2010).

The value of Oxalate is 4.25mg/100g. Oxaletes have biopesticide properties in bee keeping. The value of the phytate is 3.10mg/100g, this value is high when compared to the value of 1.56mg/100g was reported by martin *et al*, 2010. Phytates provide antioxidant effect, food additive and preservative, though it is capable of forming insoluble complexes with zinc, iron and calcium thereby interfering with their absorbtion in the body (Adeolu and Enesi, 2013).

ANTIMICROBIAL RESULT

Table 3:Antibacterial activity of the extract

		Zone of inhibition (cm)		
Test organism	Concentration	Ethanol extract	Butanol extract	Chloroform extract
E. Coli	800mg/ml	12	13	NA
(Gram negative)	600mg/ml	11	12	NA
	400mg/ml	11	11	NA
	200mg/ml	10	6	NA
CONTROL	500mg/ml	14	18	12
S.Aureus	800mg/ml	12	13	12
(Gram positive)	600mg/ml	11	10	11
	400mg/ml	10	8	11
	200mg/ml	8	8	10
CONTROL	500mg/ml	16	14	18
P. Mirabillis	800mg/ml	13	12	12
(gram negative)	600mg/ml	11	11	12
	400mg/ml	10	10	10
	200mg/ml	9	8	9
CONTROL	500mg/ml	16	16	14
S. Epidermia	800mg/ml	12	12	12
(gram positive)	600mg/ml	11	10	11
	400mg/ml	10	10	10
	200mg/ml	NA	8	10
CONTROL	500mg/ml	14	20	14
Candida	800mg/ml	10	11	NA
	600mg/ml	NA	9	NA

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	400mg/ml	NA	NA	NA
	200mg/ml	NA	NA	NA
CONTROL	500mg/ml	18	18	18

NA = No activity

TABLE 4: % Inhibition of the leaves extracts at 800mg/ml against the test organism compared to their standards

Test organism	Ethanol (%)	Butanol (%)	Chloroform (%)
E.coli	86	86	-
S. aureus	75	75	67
P.mirabilis	81	81	86
S. epidermis	86	60	86
Candida	56	61	-

The antibacterial assay was performed using the disc diffusion method which best showed the clear zones of inhibition in diameters. Table 3, 5 and 6 show the varied susceptibility of the bacterial in the crude extract on the basis of zones of inhibitions, Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). These differences are dependent on the microorganisms and extracting solvents. Lengths of zones of inhibition from different studies vary from one organism to another, and concentration difference. The organisms which are sensitive tend to move away from the region around the extract while those that are resistant show no zone of inhibition. From the result shown by the different extract of *Napoleona vogelii* using the zone of inhibition of the standard,(Amocillin for gram negative, Ampiclox for gram positive and Nyxtatin for the fungus) The organisms used for the antimicrobial screening are well known pathogens and these include *Eschericha coli* , *staphylococcus aureus*, *Staphylococcus epidermidis*, P. Mirabillis and *candida albicans* (Otto, 2009)

The ethanol, butanol and chloroform extract of the leaves *N. vogelii* displayed various activities against all test organisms at various concentration ranging from 200mg/ml to 800mg/ml, except for the chloroform extract which showed no activity against E-coli and Candida. The percentage inhibition of the test organisms at 800mg/ml of the different extract when compared to their standards ranges from 56% to 93%. The butane extract showed the highest percentage of inhibition of 93% against S. aureus when compared to the standard Ampiclox. The extract demonstrated activities against certain test organism confirming the use of the plant in ethno pharmacology. More work should be done to ascertain the active principles responsible for this action.

Table 5: **Minimum Inhibition Concentration *N. vogelii* Leaves Extract**

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Test organisms	CONCENTRATION (mg/ml)	ETHANOL	BUTANOL	CHLOROFORM
E.coli	125	+	+++	+++
	250	_	++	+++
	500	_	_	++
S. aureus	125	++	+	+
	250	_	_	_
	500	_	_	_
P.mirabilis	125	++	+	+++
	250	_	_	++
	500	_	_	_
S.epidermidis	125	+++	+	++
	250	++	_	_
	500	_	_	_
Candida	125	+++	+++	+++
	250	+++	+++	++
	500	++	+++	++

The MIC for the ethanolic extract shows inhibition for all the test organisms at 500mg/ml except for candida. At 250mg/ml, the extract inhibited the growth of *P.mirabillis*, *E.coli* and *S.aureus* at 125mg/ml, no total inhibition was observed, but minimal to high turbid extract was seen.

Table 6: Minimum Bactericidal Concentration

For the MBC, minimal to heavy growth was observed for all the test organism at 250mg/ml concentration for the whole extract except for *E.coli* and *P.mirabilis* that showed no growth for the ethanol and butanol extract respectively. There was moderate to heavy growth for the fungus, candida throughout the entire concentration. At 500mg/ml, *S.aureus, P.mirabilis,* and *S. epidermidis* showed no growth in the entire extract except for *S.epidermidis* where there was minimal growth in the ethanol extract.

Test organism	Concentration (mg/ml)	ETHANOL	BUTANOL	CHLOROFORM
E.coli	250	_	++	+++
	500	_	+	++
S. aureus	250	+	+	+
	500	_	_	_
P.mirabilis	250	+	_	+
	500	_	_	_
S. epidermidis	250	++	+	+
	500	+	_	_
Candida	250	++	++	++
	500	++	++	++

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VITAMIN COMPOSITION OF THE LEAF EXTRACTS OF *NAPOLEONA vogelii*

The vitamin composition of the leaves extracts of *Napoleona vogelii* is shown in Table 7**.**

Table 7: Vitamin Content of *Napoleona vogelii*

Vitamins	**Amount (µg/ml)**
A	0.72±0.07
B_1	5.27± 0.02
B2	0.51± 0.02
B3	0.92±0.12
B9	2.98 ± 0.32
B12	8.28± 0.06
C	3.20 ±1.03
E	2.02 ± 0.05

Table 8 shows the vitamin content of the leaves of *N. vogelii*. A total of 13 vitamins were analysed, 9 vitamins were present while 5 vitamins were absent; three water soluble vitamins (B5, B6 and B7) and two fats soluble (D and K). Vitamin B1 showed the highest concentration of 8.28mg/ml while vitamin B2 of 0.51mg/ml showed the lowest concentration.

The result revealed that *Napoleona vogelii* contained 0.72mg/ml of vitamin A, which apart from helping in growth, also promote resistance to disease, delays ageing, and preside over the health of the eyes, nails and hairs (Adeolu and Enesi, 2013). The sample contained the B complex vitamin B1(5.27mg/ml), B2 (0.51mg/ml), B3 (0.92mg/ml), B9 (2.98mg/ml) and B12(8.28mg/ml) which was required for normal growth, functioning of the heart and nervous system, eyes, formation of co-enzyme for cellular respiration (Food and Nutrition Board, 2004). The sample contains vitamin C (3.20mg/ml) which helps in the health of lungs and bronchia, teeth and gums, bones and joints and purifies the blood. It prevents the free radical damage that triggers the inflammatory cascade and associated with reduced severity of inflammatory conditions such as asthma, osteoarthritis and rheumatoid arthritis (Picciano, 2012; Traber *et al*, 2008). Therefore, it could be used in the herbal medicine for the treatment of common cold and prostate cancer (Mason, 2008). It also contains vitamin E (2.02mg/ml) which is a good antioxidant, necessary for the formation of red blood cells and the structure, recovery and maintenance of muscle and other tissue (Helzlsouer, 2009).

No literature was found to have recorded the vitamin composition of *N. vogelii.* However, works have been reported on the proximate nutritional composition. According to Afamefula *et al*, 2006 *Napoleona vogelii leaves contains* 25.55% crude protein and crude fiber 8.80% while according to (Igidi and Edene, 2014), the fruit contains, moisture content of 69%, protein 1.93%, fat 2.1%, crude fibre 16%, ash 3.5% and carbohydrate 7.47%.

Estimation of micro and macro elements Intake through the Consumption of *Napoleona Vogelii*

Table 8: micro and macro elements Concentration of *Napoleona vogelii*

Minerals	Amount in mg/kg
Calcium	2030.60
Iron	281.41
Zinc	6.80
Manganese	74.01
Copper	2.56
Lead	BDL
Chromium	0.12
Cadmium	BDL
Nickel	0.57
Magnesium	90.85

BDL = below detection point, 0.001mg/kg

The result of the present study showed that *Napoleona vogelii* is a good source of mineral elements. The result indicates the concentration of these elements reported as milligram per kilogram (mg/kg) on a dry weight basis.

Table 9 showed that the leaf of *Napoleona vogelii* contained Iron (281.41 mg/kg), Zinc (6.80 mg/g), Calcium (2030.60 mg/g), Chromium (0.21 mg/kg), Manganese (74.01 mg/kg), Magnesium (90.25 mg/kg), Copper (2.56 mg/kg), Nickel (0.57mg/kg) Lead and cadmium were found to be below detection limit .

Calcium was found to be of the highest amount (2030.60 mg/kg). High concentration of calcium in the body is very important because of its role in forming of bones and teeth, clothing of blood, muscle contraction and synaptic transmission of nerve impulse (Ghani *et al*, 2012). It participates in cell division and the regulation of cell proliferation and differentiation. (Kathleen, 2014). Thus the high concentraction of calcium contained in *Napoleona vogelii* may be of high therapeutic value.

The amount of copper recorded by the leaf of *Napoleona vogelii* is 2.26mg/kg. Copper is also an essential micronutrient which functions as a biocatalyst, required for body pigmentation, in addition of iron, maintains a healthy central nervous system, prevents anemia and interrelated with the function of zinc and iron in the body (Racheal, 2013).

The Manganese concentration of the leaves of *Napoleona vogelii* was 74.01mg/kg. Manganese acts as a catalyst and co factor in many enzymatic processes, involved in the synthesis of fatty acid and chloresterol (Kathleen, 2014). The lead and cadmium concentration was found to be below detection limit. It has been shown that potassium, calcium, and magnesium take part in neuro muscular transmission and together with zinc, manganese, copper, cadmium and chromium are involved in biochemical reactions in the body. The elements also serve as constituents of biological molecules and co-factor for various metabolic processes. Deficiency or excess of these elements may cause many metabolic disorders. Zinc, magnesium and chromium have important roles in the metabolism of cholesterol and in heart disease (Rachael, 2013).

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