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Phytochemical Characterization, Acute Toxicity Studies of the Methanol Extract of *Napoleonae imperialis* Leaves

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ABSTRACT

The present study investigates the phytochemical and acute toxicity studies of the methanol extract of *Napoleonae imperialis* leaves in albino rats. The lorke's method was used for the acute toxicity study of which eighteen (18) male albino rats were used. In the first phase, the rats were divided into three groups of three rats each and received the extract at a dose of 10 mg/kg, 100 mg/kg and 1000 mg/kg. In the second phase, the rats were divided into three groups of three rats each and received the extract at a dose of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg. The animals were observed for general signs and symptoms of toxicity including mortality over a period of 48 h. The acute toxicity study (LD50) showed no adverse effect in their general behavior and mortality at the dose level given, which suggest that the leaf might be generally regarded as safe with no remote risk of acute intoxication. The phytochemical analysis showed the presence of phenol (5807.79 ± 210.87), tannins (71.49 ± 4.79), glycosides (56.97 ± 1.59), alkaloids (607.22 ± 14.42), reducing sugar (1853.26 ± 178.63), saponin (6.06 ± 0.03), carbohydrates (15547.83 ± 729.84), flavonoids (2104.38 ± 39.29), steroids (19.44 ± 1.57) and terpenoids (101.69 ± 3.88). Lately, the usages of phytochemicals are of particular interests in several disease managements, as they present natural means to suppressing of genetic transcription via some epigenetics mechanisms. The presences of some of these biological molecules in this plant justify to a large extent, some of the ethno- medicinal applications of *Napoleonae imperialis*.

Keywords: Acute toxicity, Phytochemical analysis, Phenol, Flavonoids, Steroids, Epigenetics

INTRODUCTION

Napoleonae imperialis is a small, evergreen tropical West African tree and it belongs to the family of lecythidaceae, native to Africa [1]. It grows averagely to 6 m height with a dense, and low branching crown. The showy flowers have two inner rows of petal and vary in color, usually creamy yellow, along the circumference, with the center varying from red to apricot to purple. They develop either as tender trunks or from the ancient wood of the branch. The fruit is a berry, dark orange or reddish brown containing kink a kidney shaped seed. The specie is popularly cultivated as an ornamental tree [2].

Using *Napoleonae imperialis* as a medicinal plant in an earlier study [3], it demonstrates antibacterial and wound healing properties in albino rats. In another experiment [4], prepared an herbal ointment of the methanol solution of *Napoleonae imperialis* and examined its wound healing effect by the excision wound model on guinea pigs. The result of the experiment indicates that *Napoleonae imperialis* extract possess a better wound healing property as compared to the antibiotic used as control. Other plants that had shown wound healing effect includes the following: *Kaempferia galangal*, *Radix paeoniae*, *Prosopis cineraria*, *Trigonella foenum*, *Lawsonia alba (Lynthraceae)*, *Pterocarpus santalinus*, *Clerodendum seratum*, *Ginkgo biloba*, *Euphorbia hirta*, *Cecropia pellata*, *Cathranthus roseus*, *Sesamum indicum*, *Lycopodium serratum*, *Morinda clitrifolia*, *Arternanthera sessilis*, etc.

This experiment was designed to analyze the phytochemicals properties present in the fresh leaf of the plant *Napoleonae imperialis*, in an attempt to establish scientifically its usage

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in traditional medicine as an ethanolic extract for some disease management.

MATERIALS & METHODS

Plant material collection

A fresh leaf of the plant *Napoleonae imperialis* were locally sourced in Abia State, Nigeria and was identified at the Plant Science and Biotechnology Department, Michael Okpara Uiversity of Agriculture, Umudike, Nigeria. The fresh leaves were washed and dried under shade at room temperature, using a blender; the leaves were blended into powder.

Extraction

The powdered leaves of *Napoleonae imperialis* (100 g) were soaked in methanol for 48 h and the extract filtered using a Whatman no. 1 filter paper, the filtrate was allowed to evaporate to dryness, under a water bath with a temperature set at 40°C.

Animals

Male albino rats of mean weight of 130 g were used for the study. The animals were kept in the animal house (Michael Okpara University of Agriculture, Umudike, Nigeria), allowed to acclimatized for two (2) weeks, and were used, following the approval of institutional animal ethical committee. Commercial pellet diet (Vital growers mash; Grand Cereals and Oil Mills, Nigeria) and water were given to the animals *ad libitum*.

Phytochemical analysis of the methanol extract of *Napoleonae imperialis* leaves

The preliminary phytochemical screening of the methanol extract of *Napoleonae imperialis* leaves were carried out, to ascertain the presence of phytochemicals, those detected were quantified. Both qualitative and quantitative analyses were done using standard methods previously described [5].

Acute Toxicity Studies

Lethal dose (LD_{50}) determination was conducted using the Lorke's method. Nine mice, divided into 3 groups. The first group received the extract (*i.p.*) at a dose of 1000 mg/kg b.wt, group 2 received the extract at a dose of 100 mg/kg b.wt (*i.p.*), while the last group received the extract at the dose of 10 mg/kg body weight. Animals were observed for general signs and symptoms of toxicity including mortality over a period of 24 h. In the second phase 9 mice were divided into 3 groups. The extract was administered at a dose of 1600, 2900, and 5000 mg/kg b.wt (*i.p.*) respectively. Based on the result of the first phase, the final LD_{50} was calculated as the square root of the geometrical mean of the highest non-lethal dose and the lowest lethal dose [6].

Using the formula below, the LD_{50} was calculated:

D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that produced mortality

RESULTS

The present study investigated the phytochemical properties of *Napoleonae imperialis*. The **Table 1** and **Table 2** show the results of the qualitative and quantitative composition of the powdered leaf extract.

Table 1. Qualitative composition of powdered *Napoleonae imperialis* leaf extract.

Phytochemicals	Bioavailability
Saponin	+
Carbohydrates	+++
Reducing sugar	++
Glycosides	++
Acidic compounds	+
Tannins	++
Phenols	+++
Flavonoids	++
Steroids	+
Terpenoids	++
Alkaloids	++

Key:
+ = present in low amount
++ = present in moderate amount
+++ = present in abundance

Table 2. Quantitative composition of powdered *Napoleonae imperialis* leaf extract.

Phytochemicals	Bioavailability
Phenols	5807.79 ± 210.87
Tannins	71.49 ± 4.77
Glycosides	56.97 ± 1.59
Alkaloids	607.22 ± 14.42
Reducing sugar	1853.26 ± 178.63
Saponin	6.06 ± 0.03
Carbohydrates	15547.83 ± 729.84
Flavonoids	2104.38 ± 39.29
Steroids	19.44 ± 1.57
Terpenoids	101.69±2.88

Values are expressed as Mean ± Standard deviation (n = 3)

Acute toxicity and lethality (ld_{50}) test

Oral administration of up to 5000 mg/kg body weight of methanol extract of *Napoleonae imperialis* leaves to mice caused no death in the two stages of the test. Thus, oral LD_{50} of the extract in mice was estimated to be greater than 5000 mg/kg body weight, which suggests that the leaf may be generally regarded as safe with a remote risk of acute intoxication. The high degree of safety is also consistent [7] and its popular use as herbs in some part of Nigeria. The **Table 3** shows the lethal dosage of the extract, using albino rats as experimental animals.

Table 3. Lethal dose (LD_{50}) of the methanol extract of *Napoleonae imperialis* in male albino rats.

Phase I	Dosage (mg/kg b.wt)	Mortality
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
Phase II		
Group 1	1600	0/3
Group 2	2900	0/3
Group 3	5000	0/3

DISCUSSION

These stud(ies)y revealed that, the oral administration of 5000 mg/kg body weight of the *Napoleonae imperialis* methanol leaf extract, did not affect the rat and thus no acute toxicity or instant death in any of the rats treated with high dosage during the observation period. The median lethal dose (LD_{50}) of the present study is in agreement with earlier studies [8,9]. These two earlier studies reported the LD_{50} of this plant to be greater than 4000 mg/kg and 2000 mg/kg b.wt respectively. The results from the present study suggested that oral administration of the methanol extract of *Napoleonae imperialis* could be considered safe, which may be attributed to low or absence of toxic constituents in the methanol extract.

In the present study, phytochemical analysis of the methanol leaf extract of *Napoleonae imperialis* showed the presence of alkaloids, tannins, terpenoids, reducing sugar, glycosides and flavonoids in moderate quantities, while saponins, acidic compounds and steroids were in low amount whilst, carbohydrates and phenols were present in abundance, all these bioactive components have been reported previously to have hepatoprotective and antioxidant activities [10,11,12].

Experimental evidence had shown earlier that flavonoids possess antimicrobial, antibacterial and HIV-inhibitory activities [13]. The alkaloids are responsible for reducing the blood pressure and are thus known for the hypotensive effect of *Napoleonae imperialis* leaf extract [14]. Plants composed of saponins are considered to possess antioxidant, anti-cancer, anti-inflammatory and anti-viral effects [15]. Other studies have attributed some of these activities to the presence of alkaloids, flavonoids, polyphenols and reducing sugars [15]. The presences of these biological molecules justify some of the reasons, for the ethno-medicinal applications of *Napoleonae imperialis* [2].

Phytocompounds as epigenetic regulators

Current understanding clearly indicated that the genome contains information in two forms, i.e., genetic and epigenetic. Whilst the genetic information gives the blueprint for the transcription and translation of all the proteins necessary for both prokaryotic and eukaryotic cells, the epigenetic information provides instructions on the general direction such as; when, where and how the genetic information should be used.

With high throughput screening and available molecular targeting techniques, precision targeting is now possible. Thus, using such, the modulation of DNA methylation, histone and chromatin modification, an epigenetic directional processes in cancer via phytocompounds that altered expression of tumor suppressor genes have been identified and discussed [16, 17].

Foods and herbal medicine high in flavonoids, phenols and other bioactive compound characterized in the *Napoleonae imperialis* leaves, have been implicated in altering histone acetylation, thus modulating gene expression [16]. Gene expression is known to be regulated by epigenes, which occur following post-translational modification (PTM) of histone proteins, involving; acetylation, ADP ribosylation. methylation, ubiquitinylation, phosphorylation and sumoylation. Of these processes, methylation and acetylation of histone, all, which are potential targets for bioactive compound, seems to be the most common PTM event associated with carcinogenesis [17]. These processes, underscore the role bioactive compound could play in managing complex diseases such as cancer (which possess variant phenotypes), if and when properly harnessed. A major drawback of phyto extracts are the likelihood of adulteration with toxic agents such as heavy metals and other toxicants such as insect toxins or animal products etc.

Some classes of enzymes referred to as histone acetyltransferases (HATs) are involved in catalyzing histone acetylation processes whilst, deacetylation is catalyzed by histone deacetylases (HDACs). While HATs transfer acetyl groups onto the ε-amino group of lysine (K); HDACs remove these acetyl groups from K. It is known that acetylation of histones leads to an open chromatin structure

enabling transcription factors to bind to DNA, via some remodeling, whereas deacetylation would leads to transcriptional repression due to chromatin condensation [17]. Phytocompounds are thought to modulate these processes **(Figure 1)**, via activation or deactivation of some cellular enzymes.

The impact of natural compounds on mammalian epigenome (nutri-epigenetics) is rapidly emerging **(Figure 1)**. However, with the advent of genomics, there remain challenges (such as; interpretation of effects and its impacts on genetic variants), in using phytocompound to target genomic Single Nucleotide Polymorphisms (SNPs) that have phenotypic outcome. In many disease processes such as cancer and aging for, which DNA damage is associated, epigenes modifications are implicated, so also, in diseases involving DNA damages due to mutations such as in sickle cell disease (SCD), [see 18 and references therein]. However, the precise role of bioactive compound and the mechanisms by, which they may be involved in the suppression of genetic transcription via epigenetics mechanisms such as hypermethylation at CpG islands and histone modifications remain an area of active research [19]. Here, using the **Figure 1**, we postulate putative areas where and how phyto active compounds could modulate and thereby, be implicated in the complex interplay signaling activities leading to gene expression/regulation **(Figure 1)**.

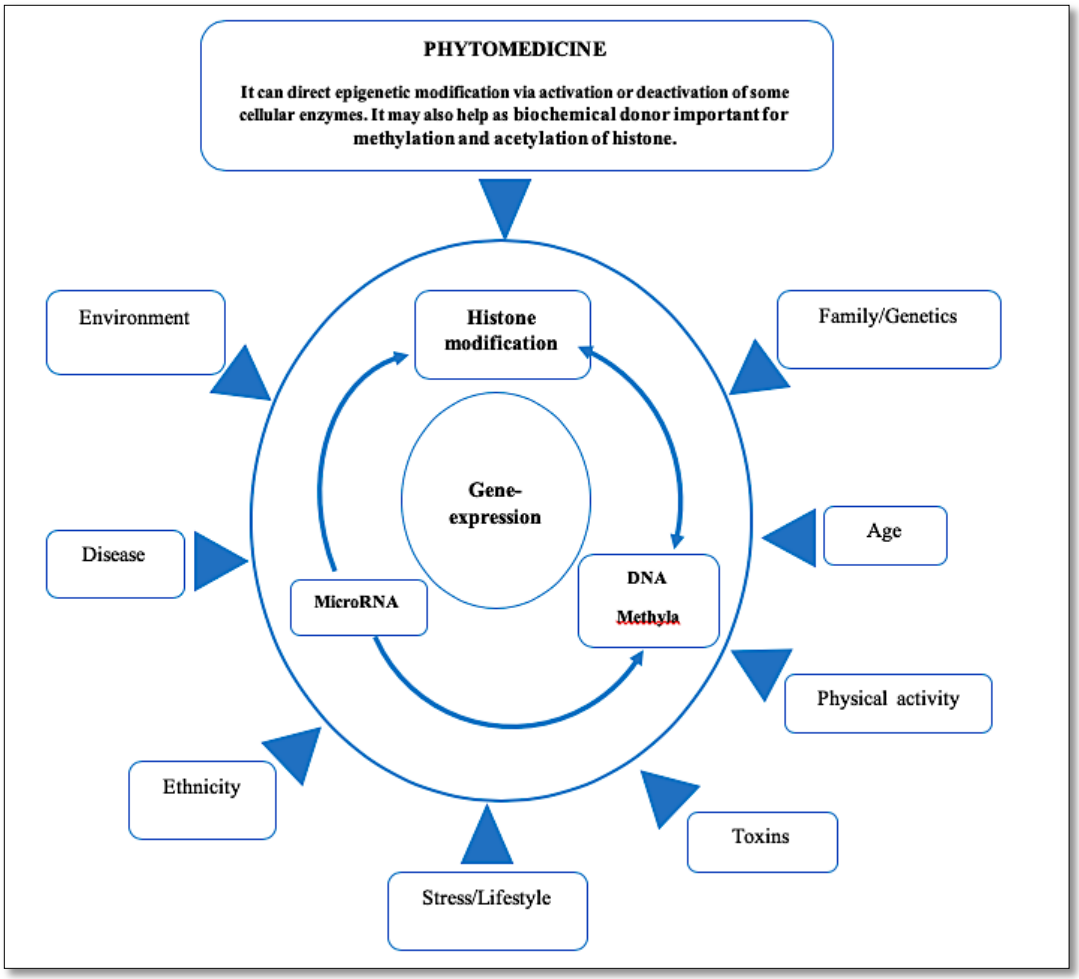

Figure 1. Factors influencing Epigenetics (Modified from [19]: Complex interplay is required for a wholesome gene expression understanding; such complexes will further enhance the use of phytocompounds in disease management).

CONCLUSION

The present study investigated the phytochemical properties of *Napoleonae imperialis*. The study establishes the low or absence of toxic constituents in the methanol extract.

The use of phytochemicals is of particular interests in several, disease management. Many of these phytochemicals establish their disease curative potency via multiple processes and mechanisms. These characteristics, as well as their generally low toxicity, position these phyto-compounds as crucial in attempts to find less expensive curative drugs in both resource poor environment and in developed economics. Moving forward, there are needs for more functional studies to understand the biochemistry that would

reveal the mechanisms and pathways by, which active compounds of plants origin affects candidate genes.

CONFLICT OF INTERESTS

We declare that there is no conflict of interests.

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