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Mikailu Suleiman

Department of Pharmacognosy
and Phytotherapy, Faculty of
Pharmaceutical Sciences,
University of Port Harcourt,
Nigeria

Nkeiru Chidinma Nze

Department of Pharmacognosy
and Phytotherapy, Faculty of
Pharmaceutical Sciences,
University of Port Harcourt,
Nigeria

Corresponding Author:**Mikailu Suleiman**

Department of Pharmacognosy
and Phytotherapy, Faculty of
Pharmaceutical Sciences,
University of Port Harcourt,
Nigeria

Antimitotic and free radical scavenging properties of a herbal preparation used in the treatment of fibroid

Mikailu Suleiman and Nkeiru Chidinma Nze

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Abstract

The research aimed at evaluating the antimitotic and antioxidant property of a herbal preparation and its component plant used in the treatment of fibroid. Phytochemical and physicochemical parameters were assessed using standard methods. *Allium cepa* anti-mitotic assay was used for anti-proliferative investigation of the aqueous methanol extract of the herbal powder and *Rauwolfia vomitoria* fruits at a concentration range of 1.25-20 mg/mL. Vincristine (0.01 mg/mL) was used as positive control. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to assess free radical scavenging activity. Flavonoids, Tannins, carbohydrates, phenols and terpenoids were present in the products. The product showed 3.1, 2.4, 0.45, 5.5 and 1.4% value in total ash, acid insoluble ash, water soluble ash, sulphated ash, and moisture content respectively. The aqueous extract of the herbal powder and *R. vomitoria* demonstrated a dose and time-dependent anti-mitotic activity with complete inhibition of mitosis. The IC₅₀ of 17.0 µg and 62.2 µg for ascorbic and the herbal preparation respectively on DPPH was obtained. The study suggests that the anti-fibroid herbal drug have antioxidant and Antimitotic potentials.

Keywords: Anti-proliferative, Physicochemical, *Rauwolfia vomitoria*, *Allium cepa*

1. Introduction

Another name for uterine fibroids is uterine leiomyomas. Uterine leiomyomas, or fibroids, are benign tumors of the uterine smooth muscle and extracellular matrix and are extremely common in women of reproductive age. They are in fact, among the most common diseases in women of reproductive age. Cumulative incidence is as high as 70 percent among white women and more than 80 percent among African-American women by age fifty. This disease has a profound impact on health care delivery and costs worldwide. Uterine fibroids are the most common tumors in women of reproductive age and are asymptomatic in at least 50% of afflicted women^[1]. This shows that most fibroids are asymptomatic; however, in those women with symptoms such as pain or heavy menstrual bleeding, there are limited treatment options; in fact, about 30 percent of them experience severe symptoms such as abnormal uterine bleeding, anemia, pain in the pelvis and pressure, back pain, urinary frequency, constipation, or infertility, and will require intervention. Uterine fibroids have also been linked with poor obstetrical outcomes. Uterine fibroids come in three different main categories. The location of fibroids affects the symptoms they may produce and the treatment options available to them. According to where they are, fibroids are categorized as submucosal (SM), intramural (IM), and subserosal^[2-4]. The uterine cavity is protruded into by submucosal fibroids. Broadly speaking, they are those that alter the endometrial cavity. They can be splitted into three different subtypes: Pedunculated fibroids of type 0 without any intramural extension; Type I sessiles have an intramural extension of less than 50%, whereas Type II sessiles have an intramural extension of more than 50%^[3]. Intramural fibroids develop inside the uterine wall's musculature. By definition, intramural fibroids do not enlarge the uterine cavity, and less than half of their maximum diameter does not extend into the serosal cavity at the uterine surface. Like intramural fibroids, subserosal fibroids do not cause distortion of the endometrial cavity. However, more than 50% of the fibroid protrudes beyond the uterine serosal surface. Sessile or pedunculated subserosal fibroids are two possible types^[2-4] Subserosal fibroids protrude from the uterus's wall. Like they have been further characterized above, some submucosal or subserosal fibroids may be pedunculated-hanging from a stalk inside or outside the uterus. The limitation of the existing classification method is that it does not account for the size of the fibroids. The average myometrium is roughly 15-20 mm thick^[5]. According to this

categorization, even if a fibroid is present throughout the full thickness of the myometrium and is greater than 4 cm but does not alter the uterine cavity, it would be considered subserosal. At the National Institutes of Health (NIH), a new classification system that considers the location and size of uterine fibroids is being developed. This system could aid in determining the impact of fibroids on fertility. There are three possible treatment plans for the disease and they include expectant, medical, and surgical management, and interventional radiology procedures [6]. The increase in fibroid amongst African women has stimulated use of Herbal Product in the treatment of fibroid. Recent research from the United State has shown that fibroids are detected by ultrasound in more than 80% of women of African ancestry and nearly 70% of white women by the age of 50 [7]. *Rauwolfia vomitoria* is a shrub or small tree up to 8 m belongs to the family of Apocynaceae. *Rauwolfia vomitoria* has been used across its range in tradition medicine. A decoction or extract of the root is used for diarrhea, jaundice, venereal diseases, rheumatism, snake bite, colic, and fever, to calm people with anxiety or epilepsy and to lower blood pressure. The macerated root or sometimes the pulp fruit is used for variety of skin conditions, and the bark and leaves are used as a purgative and emetic.

Rauwolfia vomitoria is used as hypnotic, sedative and antihypertensive. It is specific for reducing blood pressure and cure pain due to affection of the bowels. It is given in labour to increase uterine contractions and in certain neuropsychiatric disorders. Ajmaline, which has pharmacological properties similar to those of quinidine, is marketed in Japan for the treatment of cardiac arrhythmias which substantially decreases the rate of depolarization of atrial and ventricular cells. Reserpine is an antihypertensive and tranquilizer.

There is paucity of report on the antimutagenic and antioxidant effect on *Rauwolfia vomitoria* fruit which is the reason this research focuses on *Rauwolfia vomitoria*. The curiosity in this investigation is the prevalence of fibroid in Nigeria and the claim of efficacy of this herbal powder. Plant products are prepared for the treatment of fibroid without scientific proof of efficacy. This study focuses on evaluating the antioxidants and anti-mutagenic effect of Herbal Powder that is obtained from Kuby's Global Concept, used in treatment of fibroids.

2. Materials and Methods

2.1 Plant material: The Herbal Powder was obtained from Kuby's Global Concept in Kwara State. *Rauwolfia vomitoria* fruits were also obtained from Oja Oba in Ilorin, Nigeria as well. The fruits were picked to remove obvious impurities and then powdered using milling machine. The powder was kept in an air tight container for investigation.

2.2 Preparation of methanol extract

The Herbal Powder and the *Rauwolfia vomitoria* fruits powder (200 g each) were separately extracted with absolute methanol for 72 hours. In each 24hours of the 72 hours, each of the extracts were shaken with an electric orbital shaker for 6hours and the solvent was replaced after every 24 hours. The extracts were filtered and evaporated to dryness *en vacuo* in a rotary evaporator at 40 °C to obtain a solid mass from the Herbal powder and *Rauwolfia vomitoria* fruits.

2.3 Phytochemical Screening

The Herbal Powder and the *Rauwolfia* fruits were screened for the presence or absence of secondary metabolites using standard methods [8].

2.4 Physicochemical Screening

The powdered *Rauwolfia* fruits and the Herbal powder were evaluated for total ash, acid insoluble ash, sulphated ash, alcohol extractible, water extractible, chloroform extractible and moisture content as described by WHO [9].

2.5 Antimitotic assay

This was done using the *Allium cepa* anti-mutagenic assay model [10]. Approximately equal sized (32.3 g) bulbs of the onions (*Allium cepa* L.) were obtained from the local vegetable market at Choba, Rivers State. Onion bulbs that were dry, moldy or have started shooting green leaves were discarded. The chosen bulbs were grown in the dark for 48 hours in a 10 ml capacity beaker containing tap water at ambient temperature until the roots have grown to approximately 1-2 cm. The viable bulbs were then selected and used for subsequent studies. The bulbs with root tips greater than 1 cm were removed from the tap water and placed into different concentrations of the aqueous methanol extract of the herbal preparation and *Rauwolfia vomitoria* (20, 10, 5, 2.5, 1.25 mg/mL), negative control (tap water) and positive control (Vincristine sulphate 0.01, 0.005, 0.0025 mg/mL). The onions bulbs root tips were grown in the dark for 96 hours in a 10 ml capacity beaker at ambient temperature and the elongations were measured after every 24 hours. The root tips were harvested and placed in micro tubes, they were placed on a water bath for 12 minutes, and 0.1 N HCl was added to the micro tubes to fill two-third of the tubes. They were then placed on a water bath for 12 minutes, after which the 0.1 N HCl was removed. The root tips were rinsed severally with distilled water and a small section was cut and placed on a microscope slide. The root tips were then flooded with acetocarmine stain, and the slides were rinsed with distilled water. The slides were covered with a cover slip, mounted on a compound microscope and viewed at x10 and x40 objectives, and also on a photo microscope to obtain the pictures of structural features observed. The results obtained were recorded.

2.6 Anti-oxidant assay

This was done using 2,2-diphenyl-1-picrylhydrazyl (DPPH) [10]. A 0.1g of the dried extract was mixed with 10 ml of methanol in a clean 50 ml beaker until a clear solution was obtained. The solution was carefully transferred into a 10 ml volumetric flask and was further diluted into five (5) different concentrations which ranges from 10,000 ug/ml to 625 ug/ml. The negative control sample was also prepared. A standard solution of ascorbic acid was prepared and was diluted into six (6) different concentrations which ranges from 100 ug/ml to 3.125 ug/ml. A methanolic DPPH solution was prepared by dissolving 2.4 mg of DPPH in 100 ml volume of methanol and was allowed to dissolve completely. A 2.5 ml of the methanolic DPPH solution was transferred into each of the diluted concentration of the extract and ascorbic acid. The diluted concentration mixtures were shaken vigorously and were carefully kept in the dark at room temperature for 30 minutes. The absorbances of the reaction mixtures were measured at 515 nm spectrophotometrically including the absorbance of the negative control and recorded. The absorbance of the standard solution of ascorbic acid was also recorded. The radicals scavenging capability of the extract and ascorbic acid were plotted on a graph and IC₅₀ was extrapolated.

2.7 Statistical analysis

All data obtained from the study were expressed as mean+ standard deviation and percentages. They are presented in

tables, charts and graph using GraphPad Prism 9.

3. Results and Discussion

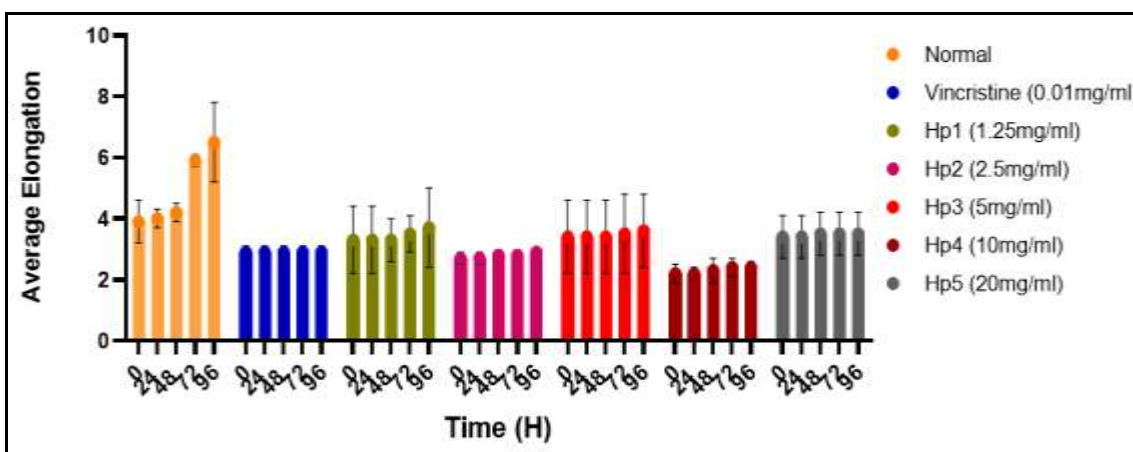
Table 1: Phytochemical constituents

Secondary metabolite	Herbal Powder	<i>Rauwolfia vomitoria</i> fruit
Alkaloids	+	+
Anthraquinones	+	+
Cardenolides	+	+
Flavonoids	+	+
Saponins	-	-
Tannins	+	+
Triterpenoids	+	+

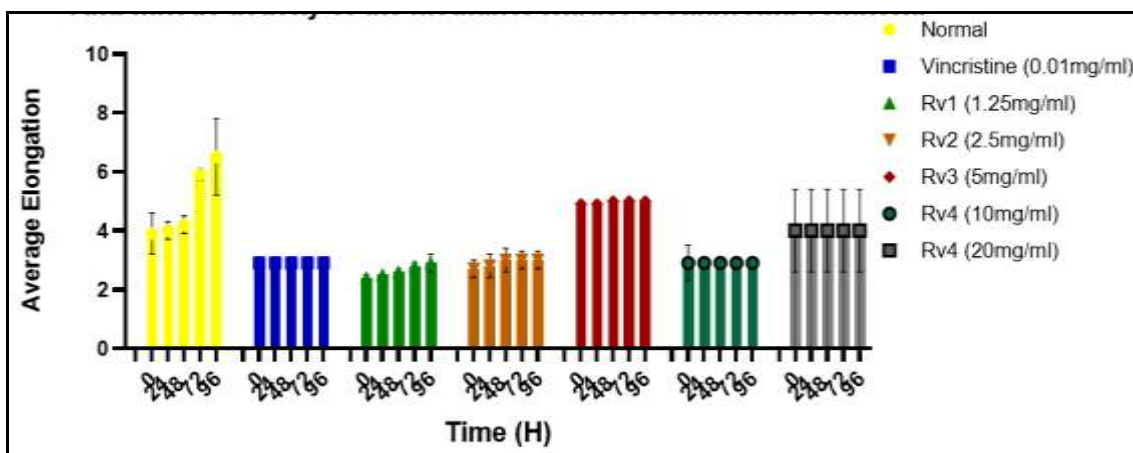
+ means present; - means absent

Table 2: Physicochemical parameters

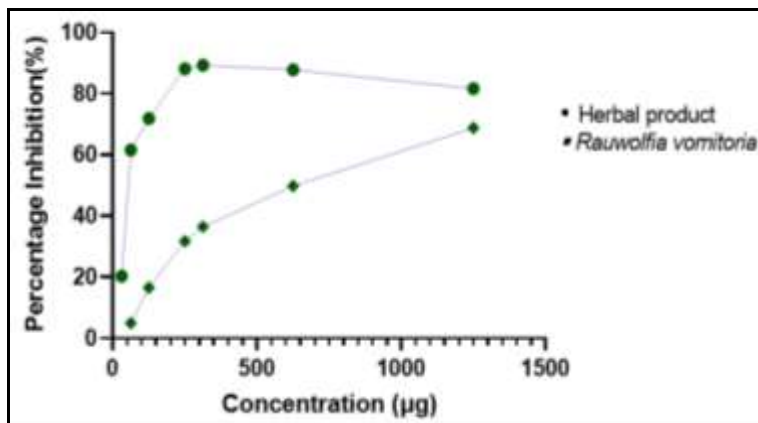
S/N	Parameters	Weight after ash(g)	Herbal product	<i>Rauwolfia vomitoria</i>
1	Moisture content		1.44	17.8
2	Alcohol extractive value			66.2
3	Chloroform extractive value			38.4
4	Hexane extractive value			51
5	Total ash	29.97	3.1	32.46
6	Acid Insoluble ash	38.15	2.4	16
7	Water Soluble ash	29.91	0.45	5.2
8	Sulphated ash	38.07	5.5	5.2



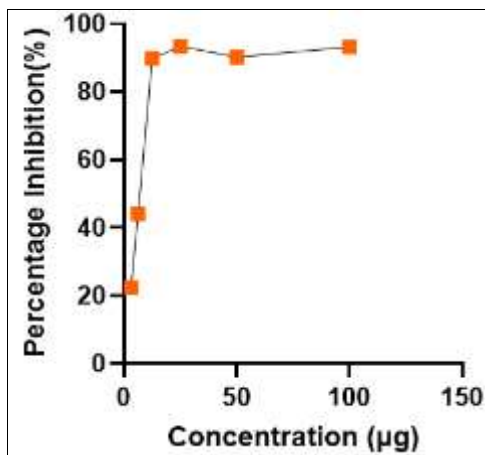
Anti-mitotic activity of the methanol extract of the Herbal powder



Anti-mitotic activity of the methanol extract of *Rauwolfia vomitoria*



Effect of Herbal Product and *Rauwolfia vomitoria* on DPPH



Effect of Herbal Product and *Rauwolfia vomitoria* on DPPH



Fig 3: Photomicrograph of *Allium cepa* root tip after 96 hours exposure to extract of *Rauwolfia vomitoria* 20mg/ml showing absence of dividing cells



Fig 1: Photomicrograph of *Allium cepa* root tip after 96 hours exposure to tap water (negative control) showing rapidly dividing cells



Fig 4: Photomicrograph of *Allium cepa* root tip after 96 hours exposure to extract of *Rauwolfia vomitoria* 1.25mg/ml showing absence of dividing cells



Fig 2: Photomicrograph of *Allium cepa* root tip after 96 hours exposure to Vincristine 0.01mg/ml showing absence of dividing cells

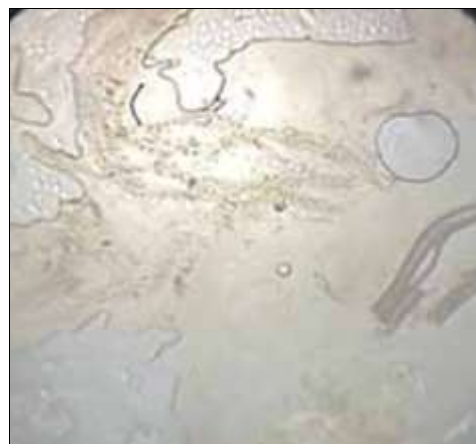


Fig 5: Photomicrograph of *Allium cepa* root tip after 96 hours exposure to extract of Herbal Powder 20mg/ml showing absence of dividing cell

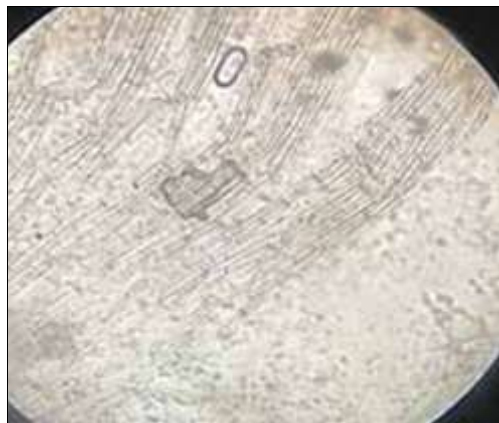


Fig 6: Photomicrograph of *Allium cepa* root tip after 96 hours exposure to extract of Herbal Powder 1.25mg/ml showing absence of dividing cell

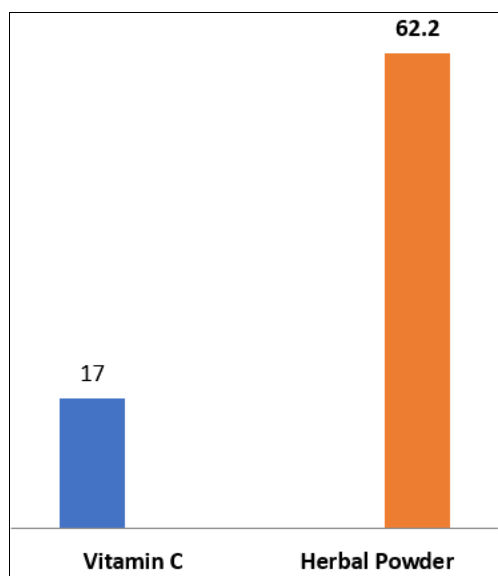


Fig 9: A chart showing IC₅₀ for the Herbal Powder extract and vitamin C

Herbal products are taking turns now in the present-day treatment of ailments in the world. Ailment such as fibroid are not exempted from herbal cures. The Herbal Powder claimed to contain *Sorghum bicolor* and *Rauwolfia vomitoria*. However, *in vitro* studies have been reported on the strong antioxidative and anti-tumor capacity of sorghum compounds^[11] which showed a significant suppression of cell proliferation and induction of apoptosis in human cancer cells, which were associated with the up- and downregulation of cell cycle and apoptosis-regulating genes^[12].

The Herbal Powder showed a percentage yield of 9.6% although the dose is the whole powder not its extract. This can serve as a means of quality measure.

The phytochemical screening result shows that the extract of the Herbal powder contain starch in difference to the other secondary metabolite present in *Rauwolfia vomitoria* fruit extract (Table 4.2 and 4.3) and *Rauwolfia vomitoria* showed the presence of cardiac glycosides. The Herbal powder and *R. vomitoria* contain flavonoids and tannins that have been shown to have the ability to prevent cancer through the induction of cell cycle arrest, antiproliferation and apoptosis^[13]. Flavonoids have a broad spectrum of biological activities that is deregulated during tumor progression^[14, 15]. Similarly, tannins can limit the mutagenic properties of compounds inhibiting the initiation of tumors^[16, 7, 18]. The phytochemical

compounds contained in *Rauwolfia vomitoria* were also detected in the herbal powder which suggests the claim of the producer of the herbal powder.

The moisture content of the herbal powder was observed to be 1.4% which propose that the herbal preparation contains low moisture content which will not support the growth of microorganisms that can deteriorate herbal powder since the recommended maximum limit is 10-14%^[19]. Ash values suggests the presence of carbonates, phosphates, oxides, silicate and silica present in the drug. The percentage of ash values obtained for the Herbal Powder are within the permissible limit as presented by WHO, this could be used in the quality control of the drug.

In the evaluation of the anti-mitotic activity of the methanol extract of *Rauwolfia vomitoria* fruits and the herbal product using *Allium cepa* model, it was observed that their methanol extract showed a dose dependent inhibition of the elongation of the root tips. The higher concentrations of the extracts (Table 4.6 and 4.7) showed significant inhibition as there was no further growth in the length of the root tips when compared to the standard vincristine. The inhibition observed might be as result of cell death (Apoptosis) of onion cells. Surprisingly, the lowest concentration of the test samples (1.25gml) also showed inhibited elongation when compared to the negative control after 96 hours of growth incubation.

Further confirmatory evidence of cytotoxic activity was seen from the microscopic examination of the meristematic cells using acetocarmine stain. The absence of dividing cells in the root tips as seen in Fig. 3,4,5,6 with the absence of stained chromatids at the highest and lowest concentration of the methanol extract of the Herbal Powder, *Rauwolfia vomitoria* extract and the reference drug vincristine (Fig. 2). This is in contrast to the observed presence of dividing cells in the negative control (Fig. 1). The trend in the results suggest that the methanol extract of the herbal powder extract has anti-mitotic potentials which could be harnessed in the development of drugs and nutraceuticals for the treatment of tumors and related cancerous ailments. Mitosis is a method of cell division in which a cell divides and produces identical copies of itself. The primary mechanism by which organisms generate new cells is through cell division. Cancerous cells are known to proliferate through rampant and unregulated cell divisions. If mitosis occurs, it means that cells are actively dividing. Actively dividing cells may lose control (Mutation) and when this happens, cancer can set occur.

This study also evaluated free radical scavenging properties of the Herbal Powder which was determined using the DPPH radical scavenging method. From the result obtained, the IC₅₀ of ascorbic acid which is the standard and the Herbal Powder value was found to be 17.0 µg and 62.2 µg/ml respectively. The result showed a significant difference in IC₅₀ when compared with standard. The antioxidant potential of the Herbal powder might be as a result of the bioactive phenolic compounds, such as flavonoids, and tannins as seen in (Table 4.2 and 4.3) which are known to be associated with reducing the risk of many chronic diseases, such as diabetes, obesity, cancer, and cardiovascular disease. Free radicals are produced due to oxidative stress in the body causes the progression of mitosis, the scavenging property of the herbal preparation could aid in scavenging free radicals and thereby help in reduction in mitotic activity^[20].

4. Conclusions

It could be concluded that the study revealed the inherent characteristics of the fruit, the anti-mitotic and anti-oxidant

properties of the Herbal powder using the *Allium cepa* model and DPPH scavenging method respectively which also suggest its antifibroid potentials as claimed by the producer.

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