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Analysis of the Phytochemical Constituents of *Rauvolfia caffra* to ascertain its Sedatives Potentials

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ABSTRACT

This research focuses on the analysis of phytochemical constituents and the evaluation of sedative potentials of *Rauvolfia caffra*. The phytochemical constituents as well as its sedative potentials and most medicinal values of *Rauvolfia caffra* were evaluated in this work using standard laboratory analytical techniques. This study revealed that the percentage composition of alkaloids (13.37%), tannins (0.28%), saponins (6.30%), phenols (3.30%) in the root were significantly higher ($p<0.05$) when compared to their composition (concentration) in the leaves and stem bark. The percentage of terpenoids (1.73%) and steroids (1.24%) in the stem bark were significantly higher ($p<0.05$) when compared to their composition (concentration) in the leaves and root. The percentage of flavonoids (8.32%) in the leaves were significantly higher ($p<0.05$) when compared to its composition (concentration) in the stem and root. The result also shows that sedative potentials of *Rauvolfia caffra* analyzed from the root have higher percentage of alcohol (31%) when compared to the stem bark (0.29%), while the stem bark of *Rauvolfia caffra* has a higher content of barbiturate (5.30mg) when compare to the root (0.52mg). This research suggests that with the presence of barbiturate and alcohol in the sample evaluated in the laboratory, it implies that R*auvolfia caffra* has sedative potentials that can be extracted and utilized as sedative drug in our hospitals. Therefore, massive propagation and conservation of *Rauvolfia caffra* to enhance the production of sedative herbal modern medicine should be encouraged.

INTRODUCTION

In using herbal medicine against disease, it is possible to limit side effects that manufactured synthetic drugs from the pharmaceutical industry can induce (Briskin, 2000; Lee and Bae, 2017). Available synthetic medicines and their generic forms are expensive, especially as poverty is an increasing concern globally and affects more than five billion people in developing countries (Afzal *et al.,* 2011; Hoang *et al.,* 2017). Naturally available flora, provides inexpensive treatments that benefits a large portion of the global community (Afzal *et al.*, 2011; Amuka *et al.,* 2017).

Since prehistory, plants have been used extensively as medicine for the treatment of various ailments, even today this trend continues. According to WHO, approximately 75-80% of the world's population use plant-based medicines. All plants may not be as useful as claimed, or may have more therapeutic properties than are known traditionally. Therefore, proper scientific knowledge is required to investigate and explore the exact standardization of such medicinally important plants.

Traditional medicine systems form a large part of the health expenditure in Some African Countries. In South African and other Sub-saharan African countries, about eighty percent of the population groups consult traditional healers regularly while other population have specific cultural traditions (Tetyana *et al.,* 2002; Afolayan and Lewu, 2009; van Vuuren, 2008; Xego *et al*., 2016; Petersen *et al.,* 2017). Of the thirty thousand plants that make up the distinctive floral diversity, only about three thousand species are employed in a therapeutic manner (van Vuuren, 2008; Xego *et al*., 2016). The scientific information on these medicinal plants is scarce amidst the considerably recorded written reports on the relationship between the people and the plants they choose to use (Hutchings *et al*., 1996; van Vuuren, 2008). The indigenous medicinal plants of African countries like Nigeria offer a wide array of natural plant compounds for the purposes of treating common illnesses that are prevalent in third world countries (Moteetee and Kose, 2017). The need for confirmation and approval by the scientific method comes from the heavy dependence of the destitute to utilize inexpensive forms of medication (Hutchings *et al.,* 1996; van Vuuren, 2008). In the standard functioning of plants, secondary metabolites or phytochemicals are formed which is not vital in the survival of the plant but may have other functions (Okigbo *et al.,* 2009). Importantly for humans, plants also have compounds that serve as therapy or healing for common and fatal human illnesses such as cancer, tuberculosis and malaria (Ramamurthy and Sathiyadevi, 2017). Existing research has shown that about half of all medicine available commercially is from plants or derived from plant sources.

Many illnesses have come to light in recent years. These diseases are responsible for a large percentage of deaths in African countries. (Motsei *et al.,* 2003). Natural sources have been effective in providing cures for most of these illnesses (Boadu and Asase, 2017; Doffana, 2017; Kinda *et al*., 2017). According to Olukayode *et al.,* (2011) and Ginovyan *et al*., (2017), it is imperative to institute the regulation and reliability of customary medical care, which highlights the importance of testing the antibacterial and antimicrobial activities of the plant species used.

In the Kingdom Plantae, microscopically distinguishable plant structures carry out the activities of either secreting or excreting biochemical substances (Cutter, 1978; Samuel *et al.*, 2018). Secretory components may occur in various locations in plant tissues and cells. By characterizing and identifying secretory structures, distinguishing features may arise and in only a particular family of plants (Cutter, 1978). The cytoplasm may be the site of the production of the substance and its transportation towards the outside of the cell is defined as 'secretion' (Cutter, 1978). Excretion on the other hand refers to the substances that the plant has no benefit from (Cutter, 1978). The substances secreted by plants have potential to be used for medicinal and economic gain (Cutter, 1978). The medicinal components of plant tissue can be found in oil glands and glandular structures of leaf hairs, also called trichomes. The latter are thread-like outgrowths that originate from the epidermal layer of plant tissues (Levin *et al.,* 1973; Barthlott *et al.,* 2017). They occur in many different conformations across the surfaces of leaves, stems and roots. They are also responsible for carrying out a variety of fundamental functions required by the plant to adapt and thrive in its environment (Elzbieta and Chernetskyy. 2005; Barthlott *et al.,* 2017).

Generally, trichomes function to enhance wind protection to prevent the epidermal layer from dissipating water from the tissues, decreasing the absorbance of sunlight and increasing the absorption of moisture (Levin *et al.,* 1973; Dalin *et al.,* 2008). The secretory structures present in plants serve as a vital component of indicating the presence of phytochemicals, secondary metabolites and other secretions that form part of the plants biochemical defense system (Umah *et al.,* 2017). Effectively, the biological activity of the phytochemicals determines the degree of pharmacological assistance (Umah *et al.,* 2017).

It is well known that plants from the Apocynaceae family contain latex and have been used medicinally (Lopes *et al.,* 2014). Similar to other Apocynaceae species, *Rauvolfia caffra* has characteristic white latex which is used to treat stomach problems (Mnxati, 2011). This plant has been used traditionally for its medicinal benefit; however, there is scarce information on its sedative potentials. *Rauvolfia caffra* has been used traditionally in the treatment of sexually transmitted infections and general health

problems such as skin infections, fever and pneumonia (Njau *et al.,* 2014).

Sample Description: *Rauvolfia caffra*

Rauvolfia caffra is a plant species belonging to family Apocynaceae. The Apocynaceae is one of the largest families with 300 genera and around 5000 species (Endress, 2004; Endress *et al.,* 2014). It is commonly known as "quinine tree and is widely used in Africa by natives as a medicine (Freiburghaus *et al.,* 1996). The family comprises large tree, shrubs, wild herbs and even vines (Freiburghaus *et al.,* 1996). In Africa R. *caffra* is widely distributed in riverine Branchiostegal woodlands, lowlands, in dry montane rainforests and in swamps (FAO, 1986). R. *caffra* (Apocynaceae) are rich in indole chemical most of which have been isolated and identified in several literature.

Figure 1: *Rauvolfia caffra* Plant. Source: Researcher's Field Work (2022)

MATERIALS AND METHODS

MATERIALS

The following equipment and apparatus were used; Electronic weighing balance, Heating apparatus (Hot plate), Volumetric flasks, Beakers 250ml, 500ml, 100ml, Boiling Tubes/Test tubes, Conical flask, Mortar/Pestle. Chemical and reagents used include; Petroleum ether, H_2SO_4 Concentration, NaOH Sodium Hydroxide, Anh Na_2SO_4 Sodium Sulphate, Anh, CUSO4, Mayer's reagent, Dragendorff's reagent, Ethyl acetate, Aluminum chloride {$ALCL_3$}, Ammonia solution, Oliver oil, 45% Ethanol, Ferrous chloride, Ferrous sulphate {$FeSO_4.7H_2O$}, Lead Acetate, Bromine water, Fehling's Solution A+B, Chloroform (Abadoni and Ochuko, 2001).

Description of Study Area

The study was conducted in Otuoke, Ogbia Local Government Area of Bayelsa State, Nigeria. The study area is bounded by Latitude 4º43'48.69" N, Longitude 6º20'19.84" E. It is bounded to the north by Elebele Community, to the East by Emeyal 1 and Kolo, to the South East by Akoloman and to the West by Onuebum, Otuogbori, and Otuokpoti, to the South by Ewoi and Otuabula II Communities; all in Ogbia Local Government Area of Bayelsa State. Otuoke Community receives biannual rainfall with short rain in October to December and long rain in March to September.

Collection of Plant Samples

Plant materials of R. *caffra* (Figure 2) were collected from the forest in Otuoke community. Leaves, stem barks and roots were collected, washed with tap water to remove soil debris followed with distilled water. They were then allowed to dry under shade for 2 weeks. The plant materials were grinded to fine powder, packed and sealed in cellophane paper and transported to Bayelsa State Medical University Central Research Laboratories for analysis.

Figure 2: Leaves. Source**:** Researcher's Field work (2022)

Figure 3: Stem bark, Source: Researcher's Field Work (2022)

Qualitative Phytochemical Screening of *Rauvolfia caffra*

Test for Alkaloids

0.2g of dried and powdered leaves were boiled in a boiling tube with 5ml of 2% of HCL on a steam bat for 5min. The mixture was filtered after cooking. The filtrate was divided into 3 test tubes A, B, C. Test A 1ml portion of filtrate was treated with 2 drops of Mayer's reagent, a creamy white precipitate was observed. This was confirmed with 1ml of filtrate treated with 2 drops of Dragendroff reagent which gives a red precipitate to indicate the presence of alkaloids.

Test for Tannins

To 2g of the powdered samples 5ml of 45% ethanol was added and boiled in a water – bath for 5min. The mixture was cooled and filtered. 1ml of the filtrate, 3 drops of lead acetate of solution were added. The formation of gelatinous precipitate indicates the presence of tannins. Also, as a confirmation test, 1ml of the filtrate was treated with 0.5ml of Bromine water and the formation of a pale brown precipitate indicates the presence of Tannins.

Test of Flavonoids

0.5g of powdered sample was introduced into a boiling tube, 10ml of ethyl acetate was added and the mixture was brought to boiling in a water bath for 1min. The mixture was cooled and filtered, 4ml of the filtrate was treated with 1ml of aluminum chloride ($ALCL_3$) solution (1%) and left to stand for 10min. The formation of a yellow coloration in the presence of 1m of chloride ammonium solution (hydroxide) indicates the presence of Flavonoids.

Test for Saponins

0.2g of powdered sample were boiled with 5ml of distilled water in a boiling tube in a water-bath. The mixture was filtered while still hot. 1ml of the filtrate was treated with 3drops of olive oil and the mixture vigorously shaken. The formation of an emulsion was observed. Another 1ml was shaken with 1ml of distilled

water and the formation of a stable frothing on standing indicated the presence of saponins.

Test for Cardiac Glycosides

2g of powdered sample was boiled with 30ml of distilled water for 5min. The mixture was cooled and filtered. 5ml of the filtrate, 0.2ml of Fehling's solution A and B were added and boiled for another 3min. A brick red coloration indicates the presence of glucosides.

Test for Terpenoids (Salkowski Test)

0,5g of sample was treated with 2ml of chloroform, and 3ml of conc H_2SO_4 was carefully added to form a layer. A reddish-brown coloration of the interface indicates the presence of terpenoids.

Test for Phenols

1ml of the aqueous filtrate was treated with 3 drops of ferric chloride solution. The formation of a blue-black color indicates the presence of phenol. Also, 1ml of the filtrate was treated with 3drops of lead acetate solution. The formation of a yellow-colored solution indicates the presence of phenols.

Quantitative Phytochemical analysis of *Rauvolfia caffra*

Determination of Alkaloids

5g of powdered samples were placed in a 250ml beaker, and 200ml of 10% acetic acid was added and covered with aluminum foil and allowed to stand for 4hours. This was filtered and the filtrate concentrated to about ¼ of its original volume on a water-bath. Conc Ammonia solution was added drop-wise to the filtrate until precipitation was completed. The solution was allowed to settle. The ppt was then collected over a Whatman No 1 filter paper and further washed with dilute ammonia solution. The residue was dried in the oven at 65^0C until completely dried, it was then weighted as the alkaloids was obtained.

% Alkaloids = b/a x 100/1
Where: a= weight of sample
b= weight of dried ppt
(Harborne, 1998)

Determination of Tannins

0.5g of sample was weighted into a plastic bottle (100ml) and 50ml of distilled water was added and shaken for 1hr on a mechanical shaker. This was filtered into a 50ml volumetric flask and the solution made up to the mark. 5ml of the filtrate was transferred in the test-tube and mixed with 2ml of 0.1m $Fecl_3$ in 0.1m Hcl and 0.008m potassium ferro cyanide. The spectrophotometer was set at 220nm. The absorbance was measured within 10min. Tannic acid was used to plot the stand curve.

Determination of Saponins

2.0g of powdered samples were put into a conical flask and 100ml of 20% Ethanol was added. The samples were heated over a water bath for 4hrs with constant stirring at 55^0C. The mixture was then filtered and the residue re-extracted with another 100ml of 20% ethanol. The combined extracts were reduced to 40ml over a water bath at 90^0C. The concentrate was transferred into a 250ml separating funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification was repeated. 60ml of n-butanol was added. The combined n-butanol extract was washed twice with 10ml of 5% NaCl solution. The resulting solution was evaporated on a water bath to a constant weight. The saponin content was then calculated.

Determination of Flavonoid

This method is based on the formation of the Flavonoid-Aluminum complex which has an absorptivity maximum at 415.100ul of the sample extract in methanol (10mg/ml) mixed with 100ul of 20% $Alcl_3$ in methanol and a drop of acetic acid and then diluted to 5ml. The absorbance at 415 was taken after 40min to develop the color. A blank was prepared from 100ul of sample extract and a drop of acetic acid and diluted to 5ml with methanol. The absorbance of routine (std for flavonoid) solution 0.5mg/ml in methanol was measured under the same condition.

Estimation of Barbiturate

To estimate the amount of barbiturate present in the samples**,** A Perkin–Elmer Lambda 6 and Spectronic 21D were used for spectrophotometric analysis with 1 cm quartz cells. 2.2. Assay method utilizing spectrophotometer and micro reaction tubes at an absorbance of 530 nm wavelength with UV–V spectrometer (Bartzatt, 2002)**.** Bartzatt, (2002) steps were then used for comparison and interpolating concentrations of test samples.

Estimation of alcohol

The estimation of alcohol was based on the complete oxidation of ethanol by dichromate in the presence of sulfuric acid with the formation of acetic acid. This reaction is highly preferred because potassium dichromate is easily available in high purity and the solution is indefinitely stable in air. The reaction that occurs between alcohol and potassium dichromate is: $2Cr_2O_7$– + $3C_2H_5OH$ + 16H+ 4Cr+++ + $3CH_3COOH$ + $11H_2O$.

Figure 4: Dried stem bark, Source: Researcher's work field (2022)

Figure 5: Dried Root, Source: Researcher's field work (2022)

Figure 6: Grounded root, Source: Researcher's (2022)

Figure 7: Grounded stem and root powder mixed in a chemical mixture, Source: Researcher's (2022)

Statistical Analysis

All experiments were conducted in triplicate and statistical analysis was done by using the SPSS Statistical software (version 25). The data were presented as mean ± standard deviation.

RESULTS

Table 1: Phytochemical screening of *Rauvolfia caffra* (qualitative analysis)

Sample code	Alkaloids	Tannins	Saponins	Flavonoids	Glycosides	Terpenoids	Phenols	Steroids
A	+	+	+	++	+	++	+	-
B	+	-	+	+	+	+	+	+
C	++	+	+	+	+	+	+	-

A - Leaves; B - Stem bark; C - Roots; ++ = abundant; + = moderately present; - = absent, Source: Researcher's (2022)

Table 1 showed the result of qualitative phytochemical screening on extracts of *R. caffra*. The result revealed the *R. caffra* plant parts tested positive for most of the phytochemicals. The leaves had abundant flavonoids and trepenoids, while the roots had abundant alkaloids. The stem bark showed the absence of tanins, while the leaves and roots showed absence of steroide.

Table 2: Phytochemical screening of *Rauvolfia caffra* (quantitative analysis)

	%Alkaloids	%Tannins	%Saponins	%Flavonoids	%Terpenoids	%Phenols	%Steroid
Leave	2.86±0.02*	-	3.97±0.01*	8.32±0.01*	0.30±0.02*	0.42±0.02*	0.02±0.02*
Stem bark	3.76±0.02*	0.25±0.02*	1.86±0.02*	3.54±0.02*	1.73±0.02*	0.48±0.02*	1.24±0.01*
Roots	13.27±0.02*	0.28±0.002*	6.30±0.02*	6.76±0.01*	1.58±0.02*	3.30±0.02*	0.65±0.02*

Values (%) are the means of triplicate measurement (n = 3) ± STD *Significant difference ($p<0.05$), Source: Researcher's (2021)

Table 2 presented the result of quantitative phytochemical analysis on the extracts of *R. caffra*. This result reveals the presence of alkaloids, tannins, saponins, flavonoids, terpenoids, phenols, and steroids. The percentage compositions of alkaloids (13.27%), tannins (0.28%), saponins (6.30%), and phenols (3.30%) in the roots were significantly higher ($p<0.05$) when compared to their composition (concentration) in the leaves and stem bark. The percentage compositions of terpenoids (1.73%), and steroids (1.24%) in the stem bark were significantly higher ($p<0.05$) when compared to their compositions (concentration) in the leaves and roots. The percentage composition of flavonoids (8.32%) in the leaves was significantly higher ($p<0.05$) when compared to its composition (concentration) in the stem bark and roots.

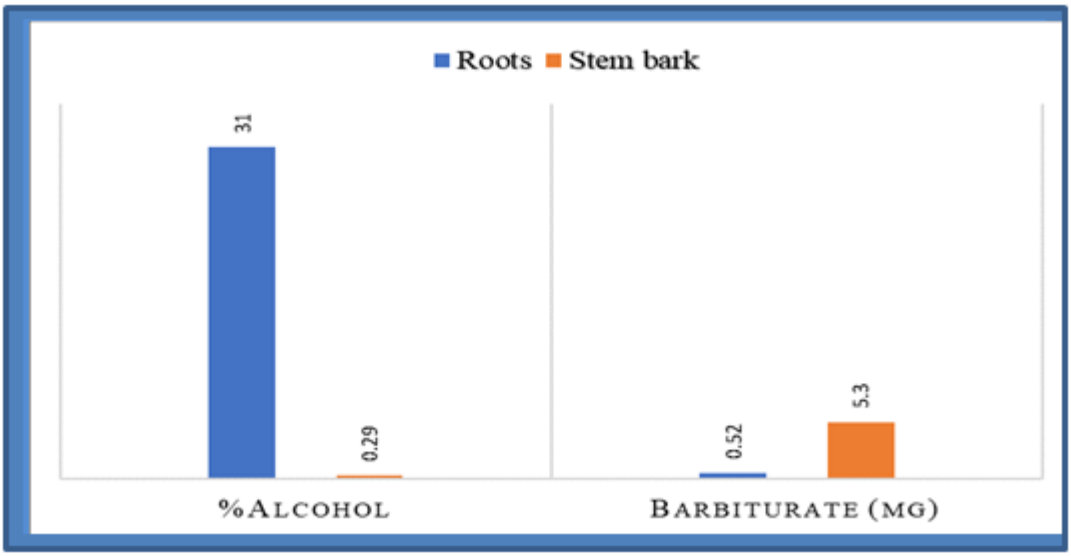

Figure 8: Sedative content of *Rauvolfia caffra*, Source: Researcher's (2022)

Figure 8 shows the sedative content of the extracts of *R. caffra*. This result revealed that the roots of *Rauvolfia caffra* have a higher percentage of alcohol (31%) when compared to that of the stem bark (0.29%). The stem bark of *Rauvolfia caffra* had a higher content of barbiturate (5.30mg) when compared to that of the roots (0.52mg).

DISCUSSION OF FINDINGS

Natural remedies and medicinal plants are a great source of bioactive chemicals that can be exploited to find novel therapeutic agents and treat a variety of disorders (Nesa *et al*., 2018). Phytochemicals occurring naturally in plants can have either positive or negative impacts on human health. The richest bio-reservoirs of different phytochemicals are found in medicinal plants that are used to treat various illnesses and conditions. The phytochemical components of plants determine their therapeutic qualities. Alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes, and other essential phytochemicals are found throughout various plant parts (Nortjie *et al*., 2022; Shaikh and Patil, 2020).

Crude extracts of *R. caffra*'s leaves, stem bark, and roots were screened for phytochemicals, and the following groups of substances were discovered: alkaloids, tannins, terpenoids, saponins, glycosides, flavonoids, phenols, steroids (Table 1 and 2). For each class of substance, these findings held true despite minor variations in abundance. The *R. caffra* extract phytochemicals content results further demonstrate their therapeutic benefit, making them potential agents in preserving the anti-oxidant, anti-inflammatory, antiviral, anti-hypertensive, anti-diabetic, anti-malarial, anti-microbial, analgesic, and anti-carcinogenic properties in living systems (Egbuna and Ifemeje, 2015; Forni *et al*., 2019). The presence of antioxidant-active compounds in the *R. caffra* samples was confirmed by the detection of alkaloids, terpenoids, saponin, cardiac glycosides, and steroids. These results confirm the importance of using indigenous knowledge to identify plants for medicinal purposes. The presence of phytochemicals with documented health advantages supports the use of quinine trees in traditional medicine. *R. caffra* contains cardiac glycosides, which may be the reason why the plant has historically been used to treat heart conditions (Milugo *et al*., 2013).

The results from the qualitative screening of the extracts of *R. caffra* correspond with the previous reports from this plant and other species of *Rauvolfia (*Deshmukh *et al.,* 2012; Ebeh Messanga *et al*., 2018; Jamkhande *et al*., 2013; O'Connor and Maresh, 2006).

According to (Tlhapi *et al*., 2018), high concentrations of reserpine, ajmaline, and ajmalicine, three well-known alkaloid chemicals, can be found in the roots and stem bark extract of *R. caffra*, and this corresponds with the findings from this study. Species of *Rauvolfia* are frequently used to treat a variety of

illnesses. Due to the presence of alkaloids, extracts of *Raulvolfia* are found to be effective in treating neuropsychiatric patients' schizophrenia. They also have a sympatholytic effect and are frequently used to treat hypertension, corneal opacities, epilepsy, skin conditions, chest pains, internal disorders, hair loss restoration, and convulsions (Kadiri and Ayodele, 2021).

The high amount of alcohol and barbiturate reveals the sedative abilities of *R. caffra*. Alcohol has been proven to exhibit sedative effects (Chung and Martin, 2009; Hendler *et al*., 2013). Alcohol has a stimulating and a sedative effect on people (Hendler *et al*., 2013). Alcohol specifically inhibits executive cognitive functioning, a group of higher-order cognitive skills like organizing, planning, abstract reasoning, cognitive flexibility, and monitoring one's own and other's behaviour (Hendler *et al*., 2013). Barbiturates are a class of sedative-hypnotic drugs that are used to treat seizures, newborn withdrawal syndrome, insomnia, preoperative anxiety, and inducing comas in the event of high intracranial pressure. For causing anesthesia, they are also helpful (Skibiski and Abdijadid, 2022). In both outpatient and inpatient settings, barbiturates have historically been a class of medications that are frequently administered. Barbiturates are used therapeutically as intravenous anesthetics, antiepileptic medications, and sedatives or hypnotics. Due to their negative effects, including drowsiness, addiction, and worsening of seizures upon withdrawal, barbiturates are not commonly used nowadays. However, phenobarbital and other barbiturates are still been used as anticonvulsant medications (Maideen, 2019). When barbiturates and alcohol are taken by a patient, the risk of sedation is increased. The complications brought on by mixing alcohol and barbiturates must be explained to the patients (Maideen, 2019). As a result of this, the use of *R. caffra* increases the risk of sedation in the users, because of its high barbiturates and alcohol content.

Sedatives are used to treat anxiety, nervous tension, pain, and to aid in sleep when there is insomnia (Nkundineza *et al*., 2020). *R. caffra* may exhibit sedative effects as a result of the presence of barbiturate, which can also be linked to the presence of alkaloids and flavonoids in its extracts (Nkundineza *et al*., 2020), and these can be used as basic medicinal agents for their analgesic, antispasmodic effects. The roots of *R. serpentina* (a species of *Rauvolfia*) are used as a treatment for a variety of ailments in Ayurvedic medicine, including hypertension, insomnia, mental agitation, gastrointestinal issues, excitation, epilepsy, trauma, schizophrenia, sedative insomnia, and insanity.

CONCLUSION

As a result of the presences of alcohol and barbiturate as well as phytochemicals such as alkaloids, flavonoids, tannins, saponins, glycosides in the root, stem bark and leaves of *Rauvolfia caffra,* this research considered *Rauvolfia caffra* as a viable herbal choice in pharmaceutical industry to be used as a raw material for the production of sedative medicines. Extracts of *Raulvolfia caffra* obtained from its roots and stem barks contained more concentrations of phytochemicals and sedative parameters and as a result, may be suitable for medicinal purposes and to treat a variety of ailments such as hypertension, insomnia, mental agitation, gastrointestinal issues, excitation, epilepsy, trauma, schizophrenia, sedative insomnia, insanity, and various nervous disorders. The ethnomedicinal uses make it one of the most important medicinal plants used in the suppression of skin diseases and infections. The presence of phytochemicals confirms *R. caffra's* historical therapeutic use and demonstrates that traditional medicine is a reliable source of information for the creation of novel medications. The use of sedatives should also be monitored as extensive usage can lead to addiction and drowsiness. Massive propagation of *rauvolfia caffra* should be embarked upon to enhance sedative herbal and modern medicine. In order to obtain a complete picture of its *in vitro* sedative effects in living organisms, additional study should be conducted to extract, identify, define, and elucidate the structures of more of the bioactive chemicals present. Extensive studies should be carried out on the plant so as to determine more medicinal properties of *Raulvolfia caffra*. There is also the need to discover more domestic ways to increase its production and improve its preservation.

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