

Phytochemical Screening, Antibacterial Effects and Antioxidant Activities of Ethanolic Stem Bark Extract of *Pterocarpus erinaceus*

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ABSTRACT

Background and Objective: In Nigeria, Pterocarpus erinaceus is used as a medicinal plant to treat many illnesses ranging from malaria, ulcer, cough and fever, to rheumatism. The antioxidant, antimalaria, antiulcerogenic and antibacterial properties of stem bark, leaves and root of P. erinaceus have been studied. The aim of this study was aimed to determine the phytochemical compositions, antibacterial effect and antioxidant activity of the stem bark of Pterocarpus erinaceus. Materials and Methods: A Gas Chromatography Flame Ionization Detector (GC-FID) instrument was used for the analysis and quantification of phytochemicals present in the ethanolic stem bark extract of P. erinaceus. The antibacterial test was carried out using the agar well method against standard bacteria: Staphylococcus aureus, Bacillus subtilius, Pseudomonas aeruginosa and Escherichia coli the antioxidant activity was carried out by evaluating the DPPH scavenging activity. Results: The results of GC-FID analysis revealed flavonoids (38.98%), alkaloids (23.96%), steroids (4.86%), tannins (2.63%) and anti-nutrients (12.61%). The ethanolic stem bark extracts of P. erinaceus exhibited significant antibacterial activity against all bacterial strains selected (Bacillus subtilius, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus) at all tested concentrations (100, 150 and 200 µg mL⁻¹). DPPH scavenging activity was found to be 97.53% at a concentration of 1000 μ g mL⁻¹ and 24.26% at a concentration of 40 μ g mL⁻¹. **Conclusion:** The study revealed that the crude ethanolic extract of P. erinaceus stem bark contained pharmacologically active compounds and exhibited significant antibacterial activity and DPPH free radical scavenging activity in a concentration-dependent manner.

KEYWORDS

Antibacterial, Pterocarpus erinaceus, phytochemicals, antioxidant, GC-FID, ethanolic extract

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INTRODUCTION

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The search for new drugs globally has become essential due to so many health challenges bobbing up from resistant species of disease causative agents and the discovery of different species and trends of microorganisms (bacteria and viruses)¹. These have therefore enticed the attention of researchers into sourcing plants with bioactive compounds that can remedy health challenges¹. Thus, plants are considered the key resources with which the well-being and health of humans can be actualized¹.



These various plants on which human and animal life depend for food contain thousands of phytochemicals and allelochemicals which constitute anti-nutritional or beneficial factors to the consumer¹. It was therefore observed from the outcome of research works that the medicinal value of these plants/medicinal plants lies in some chemical substances that produce a definite physiological action in the human body¹.

Pterocarpus erinaceus (P. erinaceus) Poir., which belongs to the family Fabaceae, is a tree usually up to 8 and 15 m in height? This plant grows in savannah and is endemic from West Africa to Central Africa². In savannah, P. erinaceus is a medicinal plant used to heal various diseases. In the central region of Burkina Faso, its stem bark is used to treat inflammatory affections such as ulcers, rheumatism, dermatitis and infections². Pterocarpus erinaceus bark, leaves and stem barks have been studied for their anti-inflammatory, analgesic and anti-plasmodial properties³, antidiarrheal⁴, antiulcerogenic⁵, antimalarial⁶, antioxidant², antimycotic⁷.

Little is known about its phytochemical components despite its widespread usage in traditional medicine. This research aimed to evaluate the phytochemical constituents and antibacterial and antioxidant activities of ethanol extracts of *Pterocarpus erinaceus* stem bark.

MATERIALS AND METHODS

Study area: This study was carried out at Federal University, Wukari, Taraba State, Nigeria, between February to July, 2022. Wukari town is the headquarters of Wukari Local Government Area in Taraba State, Nigeria. It lies between latitude 7.9303°N and longitude 9.8125°E of the equator.

Materials: The stem bark specimens of *Pterocarpus erinaceus* were collected from uncultivated farmland of Federal University Wukari, Wukari Local Government Area of Taraba State, Nigeria. The plant was taxonomically identified and authenticated in the Department of Plant Science of Modibbo Adama University of Technology, Yola, Nigeria.

Stem bark extraction: The stem bark samples were rinsed before being air-dried over 30 days. It was then reduced to powdered form by grinding in a mortar and pestle. One hundred and fifty grams of the powdered stem bark were cold macerated in 500 mL of ethanol inside an Erlenmeyer flask shaken at the interval of an hour and then allowed at room temperature to stand for 48 hrs and filtered using Whatman's filter paper No. 1. The extract was then concentrated to dryness using a rotary evaporator. It was then stored under a frozen condition until required.

GC-FID identification and quantification of phytochemical constituents: For the GC-FID analysis, 1 g of stem bark extract of *P. erinaceus* was weighed and transferred into a test tube. About 15 mL of ethanol and 10 mL of 50% w/v potassium hydroxide were added to the crushed stem bark in the test tube. The test tube was allowed to stand in a water bath at 60°C for 60 min. Then the content of the test tube was carefully transferred into a separatory funnel and the tube was rinsed into the same funnel with 10 mL of cold water, 10 mL of hot water, 20 mL of ethanol and 3 mL of hexane. The extract in the test tube was washed three times with 10 mL of 10% v/v ethanol solution. The extract solution was then dried with anhydrous sodium sulphate and the solvent was evaporated. A sample of the extract was then made soluble in 100 μ L of pyridine of which 20 μ L was transferred into a vial on the gas chromatography machine for phytochemical analysis.

The GC-FID phytochemical analysis was performed on a BUCK M910 Gas Chromatograph (GC) (BUCK Scientific, USA), equipped with a Flame Ionization Detector (FID). A RESTEK 15 m MXT-1 column (15 m×250 μ m×0.15 μ m) was used. The injector temperature was 280°C with a split less injection of 2 μ L

of sample and a linear velocity of 30 cm sec^{-1} , Helium 5.0 Pas was the carrier gas with a flow rate of 40 mL min^{-1} . The oven operated initially at 200°C , it was heated to 330°C at a rate of 3°C min $^{-1}$ and was kept at the temperature of 320°C .

Phytochemicals were determined by the ratio between the area and mass of the internal standard and the area of the identified phytochemicals⁸.

Antibacterial assay: The antibacterial activity of ethanolic stem bark extract of Pterocarpus erinaceus was determined by the agar well diffusion method¹. The 20 mL of molten nutrient agar was poured into each of the Petri dishes and allowed to solidify. The 0.5 McFarland standardized bacterial broth, was spread on the dry nutrient agar with the aid of a spreader pre-sterilized in ethanol and flame overnight. With the aid of a sterile cork-borer, four wells of 6 mm in depth each and about 5 cm apart, were made in the nutrient agar. Three of the wells were filled with 500 µL of the Pterocarpus erinaceus stem bark extract dissolved in sterile distilled water, one well with the water only (the negative control) and the last with 1% standard antibiotic, gentamicin. The positive control was dispensed into the wells in triplicates. After incubating for 24 hrs at 37°C, the antibacterial activities were determined by measuring the diameter of the inhibition zone. The zones of inhibition observed with the extract were compared with that of the standard antibiotic, chloramphenicol. The experiment was carried out in three sets. The measured chloramphenicol inhibition zones' diameters were subsequently matched with the respective standard zones' diameters for Escherichia coli (Gram -ve), Staphylococcus aureus, (Gram +ve), Bacillius subtilis (Gram +ve) and Pseudomonas aeruginosa (Gram -ve). The Pterocarpus erinaceus zone of inhibition from 9-14 mm in diameter was taken as a positive antibacterial activity based on the growth inhibition standard as reported by Umaru et al.¹.

DPPH scavenging assay: Different concentrations (40-1000 μ g mL⁻¹) of the stem bark ethanolic extracts were taken in different test tubes. The volume was adjusted to 250 μ L by adding MeOH. Two millilitres of a 0.18 mM (0.005%) methanolic solution of DPPH were added to these tubes and shaken vigorously. The tubes were allowed to stand in the dark at room temperature for 30 min⁹. The control was prepared as above without any extract and MeOH was used for the baseline correction. Changes in the absorbance of the samples were measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

Scavenging activity (%) =
$$\frac{A_{517} \text{ Control-} A_{517} \text{ treatment}}{A_{517} \text{ Control}} \times 100$$

RESULTS AND DISCUSSION

The result of GC-FID identification of phytochemicals present in the stem bark extract is presented in Fig. 1 and Table 1. Studies have reported that medicinal plants are particularly abundant in a variety of bioactive chemicals¹⁰. The extract is rich in flavonoids. Flavonoids are made up of natural substances with different phenol groups found mainly in vegetables and some grains, stems and flowers. They are well known for their valuable health benefits, especially for their antioxidant, antimutagenic, anti-inflammatory, anti-cancer and enzyme-regulating properties⁸. Flavonoids found include naringenin, proanthocyanin, anthocyanin, flavanones, flavone, flavan-3-ol, rutin and kaempferol.

Rutin has the highest concentration (11.79 μ g mL⁻¹ or 6.52%), while, the lowest concentration was proanthocyanin (1.94 μ g mL⁻¹ or 1.07%) in Table 1. Because of their powerful antioxidant activity, proanthocyanin and anthocyanin are widely dispersed pigments in land plants, where they function as stress protectants and health-promoting components⁸. Naringenin is found mostly in citrus fruits and tomatoes and has been demonstrated to be effective in the treatment of cancer, cardiovascular disease

Fig. 1: Chromatogram showing the phytochemical constituents of ethanolic stem bark extract of *Pterocarpus erinaceus**

Table 1: Phytochemical components identified in stem bark extract of *P. erinaceus* by GC-FID

	Phytochemical				Concentration	า
Types of phytochemical	constituents	Retention	Area	Height	$(\mu g m L^{-1})$	Composition (%)
	Anthocyanin	0.25	2355.11	56.34	6.27	3.47
	Catechin	2.39	12524.97	167.27	3.74	2.07
	Rutin	6.02	18996.71	245.37	11.79	6.52
	Flavan-3-ol	12.97	5160.74	77.24	3.06	1.69
Flavonoids (38.98%) Alkaloids (23.96%)	Naringin	17.96	10309.33	146.73	8.51	4.71
	Flavonones	20.31	11976.97	166.76	7.09	3.92
	Kaempferol	25.65	9642.03	133.81	8.68	4.80
	Flavone	33.00	14523.77	193.10	10.82	5.98
	Epicatechin	34.58	5536.62	81.88	8.58	4.75
	Proanthocyanin	42.28	3276.26	46.82	1.94	1.07
	Lunamarin	0.16	246.40	58.60	0.54	0.30
	Sparteine	10.37	18469.84	258.64	7.80	4.31
	Epihedrine	44.17	10577.20	142.52	34.97	19.35
Steroids (7.92%)	Steroids	22.73	8952.56	125.63	14.32	7.92
Saponins (4.86%)	Sapogenin	15.46	3987.36	60.51	2.79	1.54
•	Sapogenin	4.11	5998.39	87.17	5.99	3.32
Tannins (2.63%)	Tannin	7.42	7129.17	114.77	4.76	2.63
Anti-nutrients (12.61%)	Phytate	29.86	5189.67	193.10	6.80	3.76
	Oxalate	36.88	6833.21	93.57	15.99	8.85
Other phenolic (3.17%)	Resveratrol	39.20	10062.84	137.48	5.72	3.17
	Cyanogenic glycoside	27.53	11266.58	153.82	10.60	5.86

and osteoporosis^{8,11}. It has recently been proven to generate a considerable reduction in collagen fibre formation in rats with liver injury⁸. Other positive features of naringenin include its capacity to minimize oxidative stress, as well as its anti-inflammatory, anti-diabetic, anti-hyperlipidaemia, antioxidant and antidepressant properties⁸.

Flavones and flavanones are other important forms of flavonoids. Flavones are found mainly in leaves, flowers, fruits, celery, parsley and red peppers, while flavonones are found in all citrus fruits such as lemons, grapes and oranges⁸. Flavones can interact with proteins and bind to human serum albumin to facilitate plasma-mediated transport¹². On the other hand, flavonones are known to have antioxidant, antihyperlipidemic and anti-inflammatory effects⁸. Rutin (11.79%) which is the most abundant flavonoid in the extract, is found mainly in plants such as passionflower, buckwheat, tea and apples. Rutin's pharmacological activities include antioxidant, cytoprotective, angioprotective, cardioprotective, neuroprotective and anticarcinogenic properties^{8,13}. Likewise, kaempferol is another flavonoid commonly found in many other vegetables and plants such as grapes, green tea, potatoes, onions and cucumbers. Like other flavonoids, they may have antidiabetic, antitumor and anti-inflammatory activities¹⁴. It has been reported to regulate several key factors of cell signalling pathways involved in apoptosis, angiogenesis, inflammation and metastasis, thereby potentially inhibiting the growth of cancer cells and angiogenesis by inducing cancer cell apoptosis¹⁵.

Catechin and epicatechin are also among the flavonoids present in the ethanolic extract of the stem bark of P. erinaceus. While, catechin is found in various foods and herbs such as apples, grapes, berries and tea, epicatechin is mainly found in green and black tea with the highest levels of epicatechin found in $\cos a^{16,17}$. Catechin possesses great health benefits such as anti-obesity, anti-cancer, hepatoprotective, anti-diabetic and neuroprotective effects while, epicatechin is known to have cardioprotective, anti-inflammatory activities. Antioxidant, anti-diabetic and anti-cancer⁸. Epicatechin-rich green tea has also been shown to have antiplatelet effects *in vivo* and increase insulin sensitivity⁸. These protective effects of flavonoids in organic systems are generally attributed to their ability to donate electrons to free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α -tocopherol radicals and prevent oxidases⁸. Oxidative stress and inflammation are frequent response which contributes to tumour development by stimulating defective cells to promote and progress tumours, initiating direct damage to genomic nucleic acids, initiating aberrant cell proliferation and altering intracellular signalling the normal reaction to do¹⁸. The rich flavonoid content of ethanolic extract of the stem bark of P. erinaceus confers many pharmacological activities such as anti-inflammatory, antipyretic, hypoglycemic, antifungal, antibacterial, antitumor and wound healing properties^{8,19,20}.

Plant alkaloids are one of the largest groups of natural products, composed of structurally distinct and biogenetically unrelated molecules⁸. They have a wide range of pharmacological activities and have been used as components of many herbal remedies²¹. These include narcotic analgesics, morphine and codeine⁸. They have also been shown to have potent antimalarial, antibacterial and antiprotozoal properties²². The results of the present study indicate that ethanol extracts of *P. erinaceus* stem bark contain significant amounts of alkaloids (23.96%), with epihedrine which is the most abundant of all phytochemicals in Fig. 2 in the extract (34.97 µg mL⁻¹ or 19.35%) having the highest concentration, followed by sparteine (7.80 µg mL⁻¹ or 4.31%), then lunamarin (0.54 µg mL⁻¹ or 0.30%) (Table 1). Lunamarin has been reported to have free radical scavenging properties⁸. In addition, lunamarins have anticancer, immunomodulatory, antiestrogenic and antiamoebic properties⁸. Sparteine is a quinolizidine alkaloid and has been reported to have bactericidal activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus thuringienis*²³. It has also been reported to induce uterine contraction as well as exhibit diuretic and anti-inflammatory activity²³.

Fig. 2: Concentrations of phytochemicals in ethanolic stem bark extract of *Pterocarpus erinaceus* identified by GC-FID

Saponins comprise a group of structurally related natural compounds that include either steroidal or triterpenoid aglycones (sapogenins) and are found primarily in plants and other lower marine animals (including some bacteria)⁸. They are found in both a wide range of plants and crops, with triterpenoid saponins being more common as they are found in many legumes such as soybeans, beans and peas. Pharmacological effects attributed to saponins include immunomodulatory, anti-inflammatory, antifungal, antiviral, antibacterial, hypercholesterolemic and anticarcinogenic properties^{8,24}. Saponins accounted for 4.86% of the total phytochemicals obtained from the ethanol extract of *P. erinaceus* stem bark (Table 1). Sapogenins are known for many beneficial properties, but other harmful properties have also been documented. For example, their hemolytic and cytotoxic effects have been observed⁸. It has also been observed to significantly impair protein digestion and vitamin and mineral absorption in the small intestine, causing hypoglycemia⁸.

Tannins are primarily water-soluble polyphenols found in various plant foods. They are found in tea, cocoa, vegetables, legumes and some immature fruits²⁵. The extract of *P. erinaceus* stem bark was found to contain low levels of tannins (4.76 µg mL⁻¹), accounting for 2.63% of total phytochemicals (Table 1). Tannins play an important role in traditional Asian medicine and tannin-rich plant extracts are used as both astringents and diuretics⁸. It is also used to treat diarrhoea, gastrointestinal ulcers and tumours. It also has anti-inflammatory and antioxidant properties⁸. However, tannin-rich diets have been reported to be responsible for reduced feed intake and efficiency in experimental animals and are therefore usually considered to be of low nutrient density. It is thought to inhibit their conversion into substances in the body^{26,27}. Tannin-protein complexes can lead to the inactivation of digestive enzymes and reduced protein digestibility caused by interactions with protein substrates and ionized iron⁸.

Nutrients have been thought to have beneficial effects on human health, whereas anti-nutrients are not considered beneficial because they interfere with mineral absorption. Blockage of nutrient absorption is known to cause headaches, rashes, nausea, bloating and malnutrition²⁸. While, some antinutrients bind to essential micronutrients and prevent the body from absorbing them, others inhibit the optimal functioning of digestive enzymes and prevent food from being broken down properly⁸. Antinutrients are mostly organic or synthetic structures that are highly reactive and thus can have toxic effects. Phytate and oxalates are some of the well-known anti-nutrients found in the ethanolic extract of *P. erinaceus* stem bark (Fig. 2). Phytic acid (Myo-inositol hexaphosphate) is found in a variety of foods, including nuts, seeds and

Table 2: Effect of ethanol stem bark extract of Pterocarpus erinaceus on bacteria

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	Bacillus subtilis	Escherichia coli	Pseudomonas aeruginosa	Staphylococcus aureus
	(Gram +ve) inhibition	(Gram -ve) inhibition	(Gram -ve) inhibition	(Gram +ve) inhibition
Extract (µg mL ⁻¹)	zone (mm)	zone (mm)	zone (mm)	zone (mm)
Gentamicin (µg mL ⁻¹)	20.37±0.25 ^d	20.33±0.35 ^d	30.23±0.32 ^d	24.17±0.15 ^b
100	2.20±0.35°	3.47 ± 0.42^a	1.53±0.47 ^a	-
150	5.57±0.42 ^b	4.57±0.31 ^b	3.30 ± 0.10^{b}	-
200	8.10±0.21 ^c	8.83±0.29°	7.10±0.26 ^c	2.80±0.06 ^a

Result is Mean \pm SD, value with the same superscript within the column are statistically not significant while, values with different superscripts within a column are statistically significant (p<0.05)

whole, grains. It also contains significant amounts in roots and tubers. Phosphorylated inositol, especially phytic acid, has been suggested to be involved in insulin secretion by pancreatic beta cells⁸. It has also been suggested that phytic acid inhibits plaque development and lowers serum cholesterol and triglycerides^{8,29,30}. Oxalates, on the other hand, are known to interfere with the absorption and utilization of calcium by forming calcium oxalate crystals that lead to the formation of kidney stones. They also irritate and swell in the mouth and throat⁸. Oxalate is found in raw cruciferous vegetables such as kale and broccoli, spinach and soybeans⁸.

The ethanolic stem bark extract of *P. erinaceus* exhibited different levels of antibacterial activity against the tested bacterial strains. The bacterial strains used were clinical and laboratory isolates. All these bacterial species are known to cause serious human infections. From the clinical point of view, *Escherichia coli* causes septicemias and can infect the gall bladder, surgical wounds, skin lesions and the lungs³¹. *Staphylococcus aureus* causes dermatitis and sialadenitis³². *Bacillus subtilis* is known to cause disease in severely immune-compromised patients³¹ and *Pseudomonas aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds and also causes other blood infections. All assayed bacteria were sensitive to the extract.

The results shown in Table 2 depict that the ethanolic stem bark extract of P. erinaceus exhibited significant growth inhibition of all bacterial strains at all concentrations, except in Staphylococcus aureus at the 100 and 150 mg mL⁻¹ concentration where, no inhibition was observed. The extract showed an increasing inhibitory activity across all concentrations (100, 150 and 200 µg mL⁻¹) in a dose-dependent manner in the two bacteria in which it was found active. The highest inhibition was exhibited in Escherichia coli with a mean growth inhibition zone of 8.83 mm at a concentration of 200 µg mL⁻¹ while, the lowest inhibition was observed in Staphylococcus aureus with a mean growth inhibition zone of 2.80 mm at 100 μ g mL⁻¹ concentration. When compared to the mean inhibition zone of the standard drug, the values of the mean inhibition zone of all organisms (Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus) are found to be statistically significant when compared to the values of mean inhibition zone of the standard drug (gentamicin). They found reported that the methanolic extracts of P. erinaceus stem bark exhibited inhibitory effects against Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus. With the highest inhibition against Escherichia coli with an inhibition zone of 30 mm, while, the lowest inhibition against Staphylococcus aureus with an inhibition zone of 3 mm. Tittikpina et al.³³, also reported methanolic-dichloromethane extract was active against all the bacteria tested with MICs ranging from 32-256 µg mL⁻¹ on six out of the seven bacterial strains under investigation (i.e., E. faecalis, S. aureus, P. aeruginosa, A. baumannii, E. coli and E. cloacae).

The DPPH method used for studying the antioxidant activity of the stem bark extract of P. erinaceus revealed that the extract has a strong radical scavenging activity which is dose-dependent. With the highest activity of 97.53% at the concentration of 1000 μ g mL⁻¹ with IC₅₀ of 149.78 in Table 3 while, the lowest activity of 24.26% at the concentration of 40 μ g mL⁻¹ with IC₅₀ of 1.38.

Table 3: DPPH scavenging activity of various concentrations of ethanol stem bark extract of Pterocarpus erinaceus

Extract concentration (µg mL ⁻¹)	Control	Average	RSA (%)	IC ₅₀	
40.00	0.65	0.52	24.26	188.99	
100.00	0.65	0.47	27.36	964.19	
200.00	0.65	0.38	41.42	2256.18	
400.00	0.65	0.19	70.89	4840.16	
1000.00	0.65	0.02	97.53	12592.09	

CONCLUSION

This study was conducted to evaluate the phytochemicals composition, antibacterial potential and antioxidant activity of ethanolic stem bark extract of *Pterocarpus erinaceus*. The results of the study on the phytochemical GC-FID analysis of ethanolic stem bark extract of *Pterocarpus erinaceus* revealed the presence of some phytochemicals which are pharmacologically significant in the plant extract. This plant part could serve as a potential source of lead molecules with pharmacological activities for the development of new novel pharmaceutical products for the treatment of malaria and other diseases. Also, the presence of compounds with biological activities justifies the traditional use of stem bark of *P. erinaceus* for the treatment of malaria and other diseases. However, there will be the need for further studies into the isolation and identification of the individual bioactive compounds responsible for their therapeutic activity and the elucidation of their mechanism(s) of action.

All bacterial strains used are found to be sensitive to the stem bark extract of *Pterocarpus erinaceus* at different concentrations. Hence, the stem bark is a promising therapeutic agent that can be used in combating infectious diseases caused by drug-resistant bacteria. Furthermore, the stem bark extract of *P. erinaceus* proved to have a strong DPPH-scavenging antioxidant potential. Further study is needed to isolate and structurally characterize the pure compounds and evaluate their antimicrobial activity against multidrug-resistant microbial strains.

SIGNIFICANCE STATEMENT

The phytochemical contents, antibacterial activities and DPPH scavenging activity of ethanol extracts of *Pterocarpus erinaceus* stem bark were discovered in this work. This study will assist researchers in understanding a vital part of the plant that many researchers have not been able to investigate. As a result, a new theory about the plant's therapeutic activity may be developed.

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