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PHYTOCHEMICAL SCREENING, FTIR CHARACTERIZATION, AND ANTIMICROBIAL ACTIVITY OF DOUM PALM (*HYPHAENE THEBAICA*)

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ABSTRACT

The doum palm fruit (Hyphaene thebaica), a native of northern Africa, is an oval, edible fruit that grows on desert palm trees. It grows extraordinarily well in the northern part of Nigeria as well. It is a member of the palm family Arecaceae. The optimal process for making an aqueous extract from raw doum fruit was determined using the extraction methods. The crushed doum fruit is soaked for 12 days at room temperature in a solution of methanol and ethanol. The plant's crude pericarp powder was used in the new study's phytochemical screening procedure using conventional phytochemical techniques. Tannins, carbohydrates, flavonoids, terpenes, and terpenoids were found in the extract in low and moderate concentrations, according to the phytochemical examination. To get high-spectral-resolution data over a broad spectral range, Fourier-transform infrared spectroscopy (FTIR) is utilized. Doum palm plants are a highly significant source of bioactive components that may be helpful in the creation of novel chemotherapeutic drugs. The results of FTIR analysis confirmed the presence of O-H, C-H, C=C, C=O, C-O, and C-H functional groups. The result shows the frequencies of 3268 cm^{-1}, 2925 cm^{-1}, 1719 cm^{-1}, 1607 cm^{-1}, 1443 cm^{-1} and 1287 cm^{-1} from Methanol extract and 3283 cm^{-1}, 2925 cm^{-1}, 1719 cm^{-1}, 1611 cm^{-1}, 1104 cm^{-1} and 992 cm^{-1} from Ethanol extract. The microbial activity of the doum palm fruit extract has shown a greater effect on Salmonella typhi and Escheria coli at high concentration of about both microbial organism but no effect at lower concentration of 25, 12.5, 6.25 and 3.125 respectively.

Keywords: Antimicrobial, Bioactive, FTIR, Phytochemicals

INTRODUCTION

Natural goods, including plants, animals, microbes, and marine organisms, have been utilized by humans as remedies to treat and alleviate illnesses since prehistoric times (Shi et al., 2001). Fossil evidence suggests that humans have been using plants as medicine for thousands of years (Fabricant et al., 2001). Traditional medicine is the oldest method of treating diseases and infections, and throughout history, people have used a variety of plants to treat diseases and infections (Vineela & Elizabeth, 2005). Additionally, differ ent plant parts have been employed to treat different types of illnesses. There have been reports of the Doum palm trees being utilized in various folk remedies in Asia and Africa. The compounds that are referred to as phytochemical substances or active principles include anthrax-quinones, tannins, saponins, cyanates, oxalate, terpenes, flavonoids, bioflavonoids, and xanthenes (Asaolu, 2002).

Hyphaene thebaica, often known as doum, is a kind of palm tree that is a member of the mint family (Arecaceae) and has tasty oval fruit. They go by a number of colloquial names, including, zembaba, mkoma, arkobkobai, doum palm, and goruba hausa (Vandenbeldt et al., 1992). The northern portion of Africa is home to the doum palm. It grows from Senegal and Mauritania in the west to Egypt, Kenya, and Tanzania in the east. It typically grows in regions with groundwater along the Egypt and Sudan river. Additionally, the Arabian Peninsula (Saudi Arabia, Yemen, and the Levant) are home to it (Vandenbeldt et al., 1992).

The wood has a high fiber content, making it challenging to cut with an axe. The wood that the male palm produces is said to be stronger to that of the female (El-beltagi et al., 2018). It is frequently used in construction to provide raft construction, fence posts, planks, water ducts, railroad sleepers, and support and ratters for dwellings. A black dye for leather goods is made from dried bark 8, bilharzia is treated with roots, and controlling hypertension can be aided with fruit pulp (Sherifa et al., 2011). The hard seed inside the fruit, known as (vegetable ivory) is used to treat sore eyes in livestock using charcoal from the seed kernel as well as making buttons and small carvings, and artificial peals (Orwa et al., 2009)

Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies. During the past decade, traditional systems of medicine have become a topic of global importance (Hussain et al., 2012). Medicinal plants are frequently used as raw materials for the extraction of active ingredients which used in the synthesis of different drugs. Medicinal plants are an integral component of research developments in the pharmaceutical industry. Such research focuses on the isolation and direct use of active medicinal constituents, or on the development of semi-synthetic drugs, or still again on the active screening of natural products to yield synthetic pharmacologically-active compounds (Aamer, 2016). The plants are applied in different forms such as poultices, concoctions of different plant mixtures, infusions as teas or tinctures or as component mixtures in porridges and soups administered in different ways including oral, nasal (smoking, snuffing or steaming), topical (lotions, oils or creams), bathing or rectal (enemas). Different plant parts and components (roots, leaves, stem barks, flowers or their combinations, essential oils) have been employed in the treatment of infectious pathologies in the respiratory system, urinary tract, gastrointestinal and biliary systems, as well as on the skin (Aamer, 2016).

Figure 1 Fresh- Doum Palm Fruit. Kangire, 12.12.2020

Medicinal plant represents a rich source of antimicrobial agents. Plants that are used medicinally in different countries are the sources of many potent and powerful drugs. A wide range of plant parts are used for extract as raw drug and they possess various medicinal properties (Koseck et al., 2007).

Phytochemicals

Phytochemicals are the chemicals that present naturally in plants. Now- a-days these phytochemicals become more popular due to their countless medicinal uses. Phytochemicals play a vital role against number of diseases such as asthma, arthritis, cancer etc. unlike pharmaceutical chemicals these phytochemicals do not have any side effects. Since the phytochemicals cure diseases without causing any harm to human beings these can also be considered as "man-friendly medicines". This paper mainly deals with the collection, extraction, and qualitative and quantitative analysis of phytochemicals (Smith-Warner et al., 2003).

In general, with their established antibacterial qualities, the application of phytochemicals and plant extracts can be very important to medical interventions. Numerous research demonstrating the effectiveness has been carried out in various nations in the past few years. Numerous plants have been employed for their antibacterial properties, which are primarily derived from compounds that are created during the plant's secondary metabolism (Prusti, 2008). Certain bioactive chemicals found in medicinal plants, such as tannins, alkaloids, carbohydrates, terpenoids, steroids, and flavonoids, have defined physiological effects on human bodies (Edoga, et al., 2005; Mann, 1978). A primary metabolite is directly involved in normal growth, development and reproduction, for example, proteins, carbohydrates, carbon dioxide. It usually performs physiological functions in the organism. Secondary metabolites are organic compounds that are not directly involved In the normal growth, development or reproduction of an organism and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property for example, alkaloids, saponins, terpenes, tannins, flavonoids, steroids, cardiac glycosides (Bremer, 2003).

MATERIALS AND METHODS

This research analyzed the phytochemicals present in the doum palm crude extract, the exploration of phytochemical screening with methanol and ethanol extract of *Hyphaene Thebaica* was used reveal the presence of Alkaloids, Flavanoids, Carbohydrates, steroids, Tannins, and Terpenoids compounds which are known to have remedial activity against diseases producing pathogens. The FTIR (Fourier-Transform Infrared Spectroscopy) Spectrum was used to determine the various functional groups of the active components present in the extract based on the peak values in the region of IR radiation

Plant collection

Fresh pericarp of *H. thebaica* was sampled from Kangire village, Birnin-kudu (11.4531^0N, 9.4756^0E), Jigawa State, and North-West Nigeria. In February 2021. The seeds were authenticated by a taxonomist at the Department of Biological Science, Sule Lamido University Kafin Hausa. Specimen of this plant was deposited at the Biological Sciences Laboratory, Sule Lamido University Kafin Hausa for reference purpose.

Preparation of aqueous *Hyphaene thebaica* pericarp extract

Fresh pericarp of H. thebaica collected were grounded into uniform powder using mortar and pestle and stored in a glass container. The Methanol, and Ethanol extract were prepared by soaking 150 g of the dry powdered plant materials in 350 ml Ethanol and Methanol respectively at room temperature for three weeks. The extracts were filtered through a No.1 whatmann filter paper. The extract was concentrated at room temperature by being subjected to air drying in the laboratory

to give a dried crude extract. The extract was weighed and the weight was recorded.

Phytochemicals screening

The aqueous extract obtained from the pericarp of the plant was subjected to a phytochemical test using standard methods. The metabolites test for Alkaloids, Tannins, Flavonoids, Reducing sugar, Saponins, and Steroids.

Test for tannins (Ferric chloride test)

A 2 ml of the aqueous solution of the extract was added to a few drops of 10% ferric chloride solution (light yellow). The occurrence of blackish-blue colour showed the presence of Gallic tannins and a green-blackish colour indicated the presence of catechol tannins.

Test for Saponins (Frothing test)

A 10 ml of distilled water and a 3 ml of the aqueous solution of the extract were mixed in a Test tube. The Test tube was stopped and shaken vigorously for about 5 min; it was allowed to stand for 30 min and observed for honeycomb froth, which is indicative of the presence of saponins.

Test for alkaloids (Wagner's Reagent)

Filtrate was treated with Wagner's reagent (Iodine in potassium iodide). The formation of a brown/reddish precipitate indicates the presence of alkaloids.

Tests for carbohydrates (Benedict's test)

Filtrate were treated with Benedict's reagent and heated gently. Orange-red precipitate indicates the presence of reducing sugar.

Test for Flavanoids (Alkaline Reagent Test)

Filtrate was treated with a few drops of NaOH. The colour changes to intense yellow.

HCl is added for confirmation which causes the intense yellow to disappear.

Antimicrobial susceptibility studies

Inhibition of microbial growth was tested by using the well method (Kirby-Bauer Method as described by (Drago et al., 1999). Standard aseptic microbiological methods were followed throughout this antibacterial study.

Serial Doubling Dilution

Stock solution was prepared using 1 g of the solid plant extract dissolved in 10mls of normal saline making a stock of 100 mg/ml. Serial doubling dilution was employed to obtain concentrations of 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.25 mg/ml, 6.125 mg/ml and 3.125 mg/ml respectively.

Well method for antibacterial activity

The well method was employed to assay the plant extracts for antimicrobial activity. Petri dishes were plated with Muller – Hinton agar and prepared according to the manufacturer's manual was allowed for 30 minutes to solidify. The test organisms were then spread on the surface of the media using a sterile swap stick. A cork borer (3 mm) was used to bore wells in the media, six/petri-dish. The different concentration of the plant extracts were dispensed into the wells using a sterile syringe and needle. These were then allowed a diffusion time of 1 hour after which it was incubated at 37^0C for 24 hour. Zones of inhibition were measured using a metre rule and the mean was was recorded in millimeter as described by (Mukherjee et al., 1995).

RESULTS AND DISCUSSION

In this research, I analyzed the future of *H. thebaica* as a medicinal plant, particularly as a potential antimicrobial crude drug as well as a source for natural compounds. In the present study, the exploration of phytochemical screening with methanol and ethanol extract of *Hyphaene Thebaica* revealed the presence of Alkaloids, Flavanoids, Carbohydrates, steroids, Tannins, and Terpenoids compounds which are known to have remedial activity against diseases producing pathogens. Therefore it can be used pharmacologically to develop new compounds for health benefits as shown in Table 1 and Table 2.

The FTIR Spectrum was used to identify the functional group of the active components present in the extract based on the peak values in the region of IR radiation. When the extract was passed into the FTIR the functional group of the components was separated based on its peak ratio. The results of FTIR analysis confirmed the presence of O-H, C-H, C=C, C=O, C-O, and C-H functional groups (Figure 3 and Figure 4).

The activity of crude pericarp extract of this plant was tried in vitro on some bacterial isolates and revealed some level of activity on *S. Typhi* and *E.coli*, indicating various zones of inhibition for *S. Typhi* and *E.coli* least zone of inhibition (6 mm). Methanol extract of *Hyphaene T.* exhibits a characteristic band at 3283cm^{-1} for (O-H stretch) due to a hydroxyl group. It also showed characteristic absorption at 2970, 2925cm^{-1} for (C-H stretch), 1719 cm^{-1} for carbonyl group (C=O stretch), 1611 cm^{-1} for alkene (C=C), the peak at 1201, 1104cm^{-1} for C-O Stretch indicate alkyl group and 992,881,825 and 773cm^{-1} indicates the presence of (C=C) for alkyl. Also, Ethanol extract of *Hyphaene T.* exhibits a characteristic band at 3283 cm^{-1} for (O-H) due to a hydroxyl group. It also showed characteristic absorption at 2925 cm^{-1} for (C-H stretch), 1719 cm^{-1} for the carboxylic group (C=O stretch), 1607 cm^{-1} for alkene (C=C), the peak at 1443 cm^{-1} for (C-H Stretch) indicate alkyl group and finally,1287 cm^{-1} indicates the presence of (C=C) for alkyl.

Finally, the microbial activity of the doum palm fruit extract has shown a greater effect on *Salmonella typhi* and *Escheria coli* at high concentration of about both microbial organism but no effect at lower concentration of 25, 12.5, 6.25 and 3.125 respectively.

Determination of Percentage Yield

The percentage yield of the extract was calculated using the formula below:

Percentage yield (%) = weight of extract/weight of powdered × 100

Mass of extract (g) = (mass of crucible + mass of extract) - mass of crucible

Mass of extract (g) = (119.1 g - 108.100 g) = 11.79 g

% yield = 11.79 g /150 g × 100 = 7.86%

Result of Phytochemical Screening of plant *Hyphaene Thebaica*

The results of the phytochemical screening of the plant extract of *Hyphaene Thebaica* is shown below in Table 1 and 2. The preliminary phytochemical screening of the plant extract of *Hyphaene Thebaica* showed the presence of secondary metabolites such as carbohydrates, alkaloids, Flavonoids and Tannins were present in the extract. While saponins is absent from both extracts.

Table 1: Result of phytochemical screening of Methanol extract of Hyphaene Thebaica

S/N	Constituents	Name of test	Result
1	Tannins	Ferric chloride test	+
2	Carbohydrate	Benedict's test	+
3	Saponins	Foam test	-
4	Alkaloids	Wagner's test	+
5	Flavonoids	Alkaline Reagent Test	+

Key: + = present, - = absent ME= Methanol extract

The percentage yield of the extract was calculated using the formula below:
Percentage yield (%) = weight of extract/ weight of powdered × 100
Mass of extract (g) = (mass of crucible + mass of extract) - mass of crucible
Mass of extract (g) = 119.1 g - 109.98 g = 9.98 g
% yield = 9.98 g /150 g × 100 = 6.65 %

Table 2: Result of phytochemical screening of Ethanol extract of *Hyphaene Thebaica*

Constituents	Name of test	Result
Tannins	Ferric chloride test	+
Carbohydrate	Benedict's test	+
Saponins	Foam test	-
Alkaloids	Wagner's test	+
Flavonoids	Alkaline Reagent Test	+

Key: + = present, - = absent ME= Methanol extract
Mass of the powdered fruit used = 150 g

Table 3: characteristics of plant extract

Solvent	Weight of extract (g)	Percentage extract (%)
Methanol	11.79	7.86
Ethanol	9.12	6.65

Result of the activity test on the microbial organism plant *Hyphaene Thebaica*

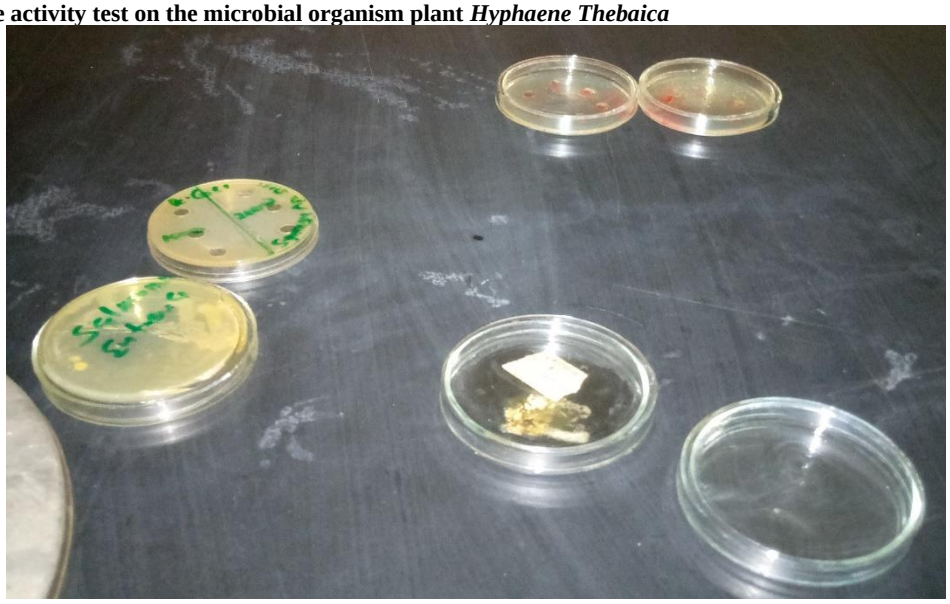

Figure 2: Antimicrobial test of *Salmonella Typhi* and *E. coli.* 2021

Table 4: Determination of minimum inhibitory concentration of *H. thebaica* crude seed extract

Organism	100	50	25	12.5	6.25	3.125
Salmonella Typhi	-	-	-	-	-	+
E. Coli	-	-	+	+	+	+

+, Growth observed; -, growth inhibited.

Table 5: Determination of minimum inhibitory concentration of *H. thebaica* crude seed extract

Organism	100	50	25	12.5	6.25	3.125
Salmonella Typhi	-	-	+	+	+	+
E. Coli	-	-	-	+	+	+

+, Growth observed; -, growth inhibited.

Result of Fourier Transform Infrared spectroscopic (FTIR) Analysis

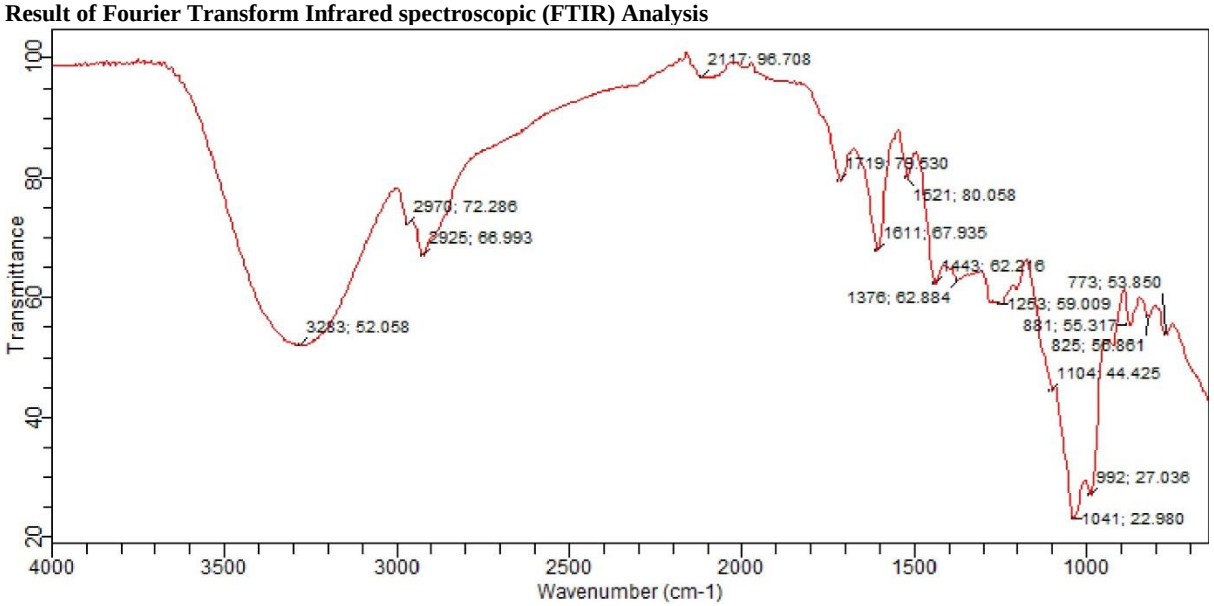

Figure 3: FTIR Spectra of Methanol extract of *H. thebaica.*

Table 6: FTIR Peaks Value of Ethanol extract of *H. thebaica.*

S/No	Frequencies	Functional Groups	Compound Class
1	3283	O-H Stretching	Alcohol
2	2970, 2925	C-H Stretching	Alkane
3	1719	C=O Stretching	Carbonyl
4	1611	C=C Stretching	Alkene
5	1253, 1104	C-O Stretching	Alkyl, Aryl, Ether
6	992, 881, 825, 773	C=C Bending	Alkene

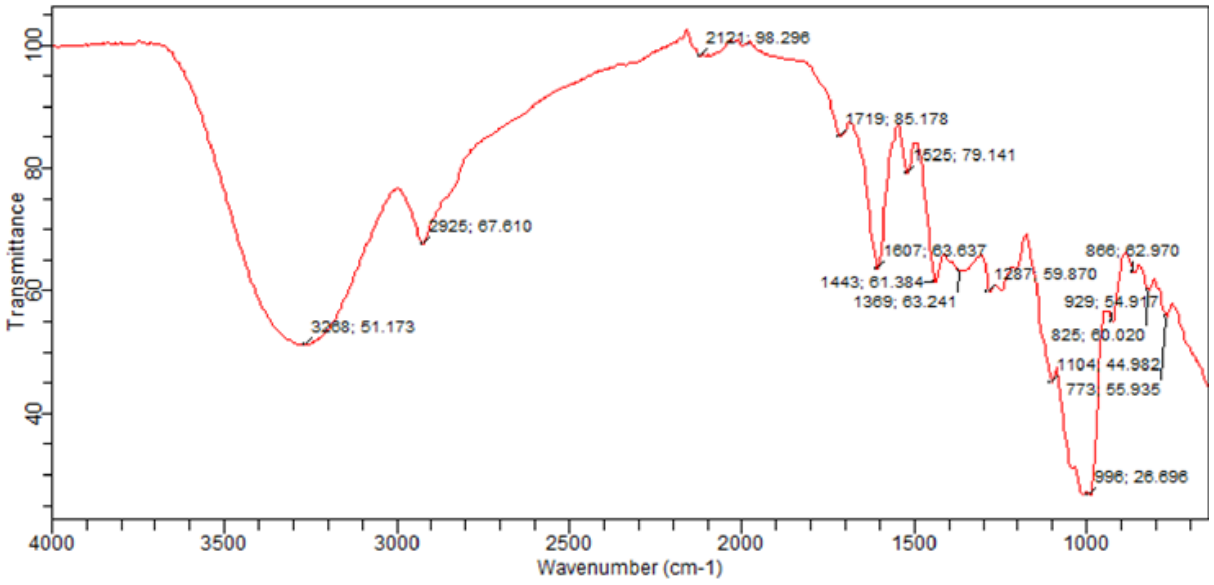

Figure 4: FTIR Spectra of ethanol extract of *H. thebaica.*

Table 7: FTIR Peaks Value of Ethanol extract of *H. thebaica*

S/No	Frequencies	Functional Groups	Compound Class
1	3268	O-H Stretching	Alcohol
2	2925	C-H Stretching	Alkane
3	1719	C=O Stretching	Carboxylic acid
4	1607	C=C Stretching	Alkene
5	1443	C-H Bending	Alkane
6	1287	C-O Stretching	Alkyl aryl ether

CONCLUSION

In conclusion, this study comprehensively investigated the phytochemical composition, molecular structure via FTIR characterization and antimicrobial potential of Doum Palm (*Hyphaene thebaica*). The phytochemical screening revealed the presence of the Tannins, carbohydrates, flavonoids, terpenes and terpenoids were found in the extract in low and moderate concentrations, according to the phytochemical examination. FTIR analysis shows the frequencies of 3268; O-H, 2925 cm^{-1}; C-H, 1719 cm^{-1}; C=O, 1607 cm^{-1}; C=C, 1443 cm^{-1}; C-H and 1287 cm^{-1} C-O. Additionally, the microbial activity of the doum palm fruit extract has shown a greater effect on *Salmonella typhi* and *Escheria coli* at high concentration of about both microbial organism but no effect at lower concentration of 25, 12.5, 6.25 and 3.125 respectively. These findings collectively emphasize the therapeutic potential of Doum Palm, warranting further exploration for its applications in pharmaceuticals and healthcare, and highlighting its significance in traditional medicine and natural product research.

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