



Antibacterial study of guava leaves on some enteric bacteria (*E. coli* and *Shigella dysenteriae*) from Sokoto, Nigeria

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ABSTRACT

This study conducted identification of phytochemicals in guava and expunge the antimicrobial capacity possessed by the plant on some bacteria. Ethanol and water were utilized to make the plant extracts that are used against *Escherichia coli*, and *Shigella dysenteriae* all isolated from clinical isolates. The results showed that the phytochemicals were present in leave extracts of *P. guajava*. The plant contains alkaloid, saponins, flavonoids, tannins, steroid. The antibacterial activity of aqueous and ethanolic leaves extract of *P. guajava* revealed the mean diameter of zone of inhibition of extract on the test isolate with *E. coli spp* being the most susceptible isolate at 200 mg/ml concentration (20 mm). The ethanol extracts revealed the highest activity against the test bacteria, *Escherichia coli* (20mm zone of inhibition, MIC of 12.5mg/ml, and MBC of 25mg/ml) followed by *Shigella* (18.3mm zone of inhibition, MIC of 6.25mg/ml and MBC of 25mg/ml). The aqueous extracts showed slightly lower activity on the test organisms compare to the water extracts. *Escherichia coli* (8.6mm zone of inhibition, MIC of 12.5mg/ml and MBC of 12.5mg/ml), followed by *Shigella* (5mm zone of inhibition, MIC of 12.5mg/ml and MBC of 25mg/ml). The obtained result displayed that both extracts impede the growth of the test isolates using 6.25 - 25mg/ml concentration. In turn, the leave contains of the plant can be improved to benefit from its antibacterial and phytochemical compounds.

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INTRODUCTION

The famous guava plant in Hausaland is called in botanical and biological language as *Psidium guajava*. It is a renowned plant that bore fruits and belongs to the family called *Myrtaceae* (Qa'dan *et al.*, 2005; Egga *et al.*, 2014). Guava is a plant growing in almost all parts of the Nigeria and is famous in its nature of reaching about 1500 meter in height and many people across the various regions of the country utilized it as a commercial fruit. The plant elucidated with its smooth, thin, copper hued bark that is usually detaching, and shows a green coloured sheet beneath (Egga *et al.*, 2014). In a large chunk of areas spreading the tropic regions, guava trees are available for the human beings and other organisms to tap benefits and to enrich the nature of the environment. This has been due its ability to withstand and succeed in a diverse soil types of the region, its capacity to be subjected to propagation methods easily, and can bore fruits in an easier manner (Egga *et al.*, 2014). Many animals like fowls, monkeys, humans, appreciate the fruits of guava and make an important impacts seen or cultivated in many parts of the tropics and rainforest areas (Egga *et al.*, 2014). Additionally, many parts of the guava plants including bark, fruits, seeds, have been utilized in the traditional medicine applications for a very long period of time in history and many successes have been reported. Parable, a concoction is prepared from leaves, bark of the plant for the therapy against a diverse array of diseases including, vomiting, sore throats, dysentery, diarrhoea; and also been used to cure menstrual issues (Chanda and Kaneria, 2010). In similar vein, many tribes in Africa apply the concoction made from guava to treat labour issues, tightening of vagina, gum bleeding, mouth sores, and virginal illnesses. Nutritionally, apart from other components, potassium, fibre, and retinoic acid can be obtained from the guava plant (Chanda and Kaneria, 2010). In tandem with guava, the applications of other plants in the prevention and treatment of diseases, more especially the infectious type have been successful since from the ancient time and nowadays the area is being studied by scientists worldwide (Falodun *et al.*, 2006). Diverse studies are carried out to expand and enrich this area for the benefit of mankind and sustainable development in the world; therewith medicinal plants are being critically examined and their yield of phytochemicals through the aid of information gathered by locals (Upadhyay *et al.*, 2013).

Additionally, nowadays there is rise in emergence of resistance against the presently churn out conventional medicines, aggravated by the rising cost of healthcare services around the world especially in developing countries and rural areas or in socially disadvantaged populations; therefore, it is imperative to surf for brand new, efficient, cheap, and cost effective strategies and ways to address the issues of infectious disease (Kaware and Ismail, 2021). Therein, the guava (*Psidium guajava Linnaeus*) is a good candidate for studying, a tree plants that hails from the family dubbed as *Myrtaceae*. It is a plant that lives in the tropics as hardwood type and can attain about 10meters in height; albeit it is regarded as native plant in areas like Mexico, it can be found in Africa, Asia, South America, and Europe. (Abdul Wadood *et al.*, 2013). The guava plant is beneficial medicinally in many aspects such as treatment of inflammation, pain, fever, stomach disorders, hypertension, and act as a sterilization agent. Its leaves are useful on wounds, joints, ulcers, and toothache (Abdul Wadood *et al.*, 2013). In Traditional methods, locals appreciate plant materials owing to their active ingredients that are important and can also serve as components in making modern drugs that are indeed beneficial to humanity (Kaware and Ismail, 2021). In this vein, The World Health Organization (WHO) has unveiled about 200,000 species of plants that have the potentials to be utilized in medicine to treat problems like bronchitis, respiratory infections, pneumonia, and diarrhoea among others (Umar *et al.*, 2022ab). In a similar streak, plants are utilized as a remedy by consumption of their metabolites through using extraction methods that churn-out the active compounds housed by leaves, deeds, flowers, tubers, bark, and other parcels of plants that may have medicinal potentials (Umar *et al.*, 2022ab).

However, enteric bacteria happen to be a scion of a renown Enterobacteriaceae family, a huge, and motely group that has Gram-negative organisms that abode the intestine of animals and humans as the natural habitat. This same streak family has many genera with the famous bacteria, the *Escherichia coli* that abode the intestine in humans as a normal flora and when an incidence take place can lead to infection; while *Salmonellae*, *Shigella*, are parts of the organisms that affect humans (Umar *et al.*, 2022ab).

Luckily, the natural plants embedded several substances that have potentials to cure infections due to the microorganisms especially in the developing nations where herbs made humongous contributions in the human

therapeutic approaches; a situation that has to be escalated because of growing resistance to drugs, and cost of hospital treatment among other factors (Kayode and Kayode, 2011). Tentatively, plants are going to be very beneficial as appreciable radix that when subjected to manipulations could make more desirable chemotherapy (Devika, 2021). This is a welcome value, because nowadays microbial resistance to drugs is an enormous hitch to doctors, pharmacists, public health scientists, and industry. Thus, there has to be an effort to circumvent the problem, a move that involves a wide range screening of plants for medicinal uses through a scientific fashion, with a motive to churn-out safer, cheaper, better, and effective therapy (Natarajan *et al.*, 2003). This study has an aim to identify the phytochemicals in leaves of guava and its antimicrobial properties on some clinical bacteria.

OBJECTIVES OF THE STUDY

- i. To determine the antibacterial efficacy of guava leaves extract on some enteric bacteria.
- ii. To identify the phytochemicals housed by guava plant leaves.

MATERIALS AND METHODS

2.1 Collection of Sample

Plant Sample: Fresh leaves of *Psidium guajava* were collected from Alu Magatakarda farms More Area of Kware local Government Area of Sokoto State, Nigeria.

2.2 Test Organisms: Clinical isolates of *E. coli* and *Shigella spp*, were obtained from Department of Microbiology of Specialist Hospital for further experiment. Identification and characterization of the isolates were conducted thereby using three procedures namely Gram staining and biochemical characterization. The pure isolates of each of the test organism were inoculated in sterile slants containing Nutrient agar and transported to the department of Microbiology and refrigerated at 4°C before use.

2.3 Preparation of plant material

The collected leaves were thoroughly washed under tap water, dried in the shade to air dry and then ground into coarse powder with the help of mortar and pestle. These powders were stored in airtight brown bottles at 4°C until needed for future use.

2.4 Extraction of plant material

The shade dried 100 gm coarse powder of leaves of *P. guajava* plant was immersed in 200 ml of different solvents (ethanol and aqueous) contained in 500 ml sterile conical flasks and covered with cotton wool separately. It was placed aside with intermittent shaking for one week. They were firstly filtered with double layered muslin cloth and then through Whatman No. 1 filter paper. The filtrate was subjected to evaporation by treating at 40°C in an oven to obtain a dried extract. The dried extract was stored at 4°C until used for further study (Atata *et al.*, 2003).

2.5 Phytochemical screening of leaves crude extracts

Phytochemical analysis tests for the screening and identification of bioactive chemical constituents of extracts of the fruits leaf were performed with the standard methods with modifications.

2.5.1 Test for saponins

About 1 ml aliquots of the various plant leaf extracts were combined with 5 ml water which is at 60°C then shaken for 2 minutes as saponins are known to process frothing activity, the volume of froth produced in this experiment was observed (Rathore and Bhatt, 2012).

2.5.2 Test for flavonoids

The plant leaf crude extract was treated with drops of 20% sodium hydroxide. Yellow appears which become colourless on adding dilute HCL (Rathore and Bhatt, 2012).

2.5.3 Test for alkaloids

The plant leaf crude extract was evaporated to dryness in a boiling water bath. The residue was dissolved in 2N HCl. The mixture was filtered and the filtrate was treated with equal amount of Wagner's reagent. The reaction shows the appearance of brown precipitate indicates the presence of respective alkaloids (Wagner and Bladt, 2001).

2.5.4 Test for Glycosides

One ml of glacial acetic acid, 3 drops 5% W/V ferric chloride and concentrated sulphuric acid were added to test tubes containing 2 ml of extracts and observed. The disappearance of reddish brown colour at the junction of two layers and bluish green in upper layer indicates the presence of glycosides (Tona and Kambu, 2000).

2.5.6 Test for Tannins

Extracts were treated with 1ml of 5% ferric chloride. The presence of tannin was indicated by the formation of bluish black or greenish black precipitate (Firdouse and Alam, 2011).

2.5.7 Test for Anthroquinones

2ml of the extract were added and shaken with 2ml benzene, 2ml of ammonia solution were added and shaken. The presence of pink, red or violet colour in the ammonia (lower) phase indicate the presence of anthraquinones (Rathore and Bhatt, 2012).

2.5.8 Test for steroids

5ml of the extract in 2ml of chloroform were added, then 2ml of sulfuric acid at the side of the test tube. A reddish brown ring interface indicates the presence of steroids.

2.6 Sensitivity Test of Antimicrobial activity

The method of Agbaje *et al.* (2006). Mueller Hinton agar was prepared according to the manufacturer's instructions. The inoculum of each test organism were prepared by picking discrete colonies of each organisms in the nutrient agar and transferring into sterile distilled water in a bijou bottle to obtain a turbidity equivalent 0.5 McFarland turbidity standards which gives an approximate density of 3.0×10^8 cfu/ml of the test organism (Cheesebrough, 1994). The suspension was seeded evenly unto the surface of Mueller Hinton agar plates in duplicates with a sterile swab stick. Using 6mm diameter sterile cork borer, 4 well were made in the agar and labelled as 12.5% (v/v), 25% (v/v), 50% (v/v) and 100% (v/v) representing the guava leaves extract. The labelled wells were aseptically filled with their corresponding concentrations of honey. A control was made using ciprofloxacin. The plates were incubated at 37°C for 24hrs and were examined for zones of inhibition (Agbaje *et al.*, 2006).

2.6.1 Minimum Inhibitory Concentration (MIC)

The Muller-Hinton broth was prepared and autoclaved at 121°C for 15mins. 5mls of Muller –Hinton broth was pipetted into 5 sets of test tube for each of the two organism, serial dilution was carried out using 5mls of the least concentration that showed zone of inhibition. 5sets of test tube was set to the dilution of 50% (v/v), 25%(v/v), 12.5%(v/v), 6.2% (v/v) and 3.125%(v/v). A loopful of each of the test organism was inoculated into their respective 5set of labelled tubes and incubated at 37°C for 24hrs.

RESULTS AND DISCUSSION

Phytochemical Screening

The Table 3.1 showed that the phytochemicals present in leaves of *P. guajava* are alkaloid, saponins, flavonoids, tannins, steroid.

Antibacterial Activity

Table 3.2 and 3.3 divulged the antibacterial activity of the guava plant extracts (ethanol and water) in forms of mean diameter of zone of inhibition of extract on the test isolate with *E. coli spp* being the most susceptible isolate at 200 mg/ml concentration (20 mm). The ethanol extracts showing the highest activity against the test bacteria, *Escherichia coli* (20mm zone of inhibition, MIC of 12.5mg/ml, and MBC of 25mg/ml) followed by *Shigella* (18.3mm zone of inhibition, MIC of 6.25mg/ml and MBC of 25mg/ml). The aqueous extracts showed slightly lower activity on the test organisms compare to the water extracts. *Escherichia coli* (8.6mm zone of inhibition, MIC of 12.5mg/ml and MBC of 12.5mg/ml), followed by *Shigella* (5mm zone of inhibition, MIC of 12.5mg/ml and MBC of 25mg/ml).

Minimum inhibitory concentration (MIC)

Tables 3.2-3.7 display the antibacterial result, showing that all the plant extracts (aqueous and ethanolic) are able to inhibit the growth of the test isolates at concentration of 6.25 - 25mg/ml.

Table 3.1: Phytochemical tests on solvent fractions of *P. guajava* leaves extract

Fraction	Tannins	Flavonoids	Alkaloids	Saponins	Glycoside	Antraquinone	Steroids
Ethanol	+++	+	+++	+++	-	-	+++
Aqueous	+++	+	+	++	-	-	++

Key:- =Absent, + = present in moderate quantity, ++ = present in large quantity

TABLE 3.2: Activity of the crude ethanol extract of *Psidium guajava* on the test organisms

ORGANISMS	MEAN OF ZONES OF INHIBITION				CPX(10Ug)
	50	100	150	200	
<i>E. coli</i>	17.3	14.8	16.3	20.0	18.0
<i>Shigella</i>	11.3	13.3	14.3	18.3	18.6

KEY: - CPX= ciprofloxacin

6-8mm = weak activity

8-12mm= moderate activity

Greater than 12mm = high activity

TABLE 3.3: Activity of the aqueous extract on the test organisms.

ORGANISMS	MEAN OF ZONES OF INHIBITION				CPX(10Ug)
	50	100	150	200	
<i>E. coli</i>	0.11	9.0	6.3	8.6	7.0
<i>Shigella</i>	4.2	7.0	10.0	5	7.0

KEY: - CPX= ciprofloxacin

6-8mm = weak activity

8-12mm= moderate activity

Greater than 12mm = high activity

Table 3.4: Minimum inhibitory concentration (MIC) of ethanolic extract against the test organisms

CONCENTRATION (mg/ml)	TEST ORGANISMS	
	<i>E. coli</i>	<i>Shigella</i>
100	+	+

50	-	+
25	-	-
12.5	-	-
6.25	+	-

KEY: - Growth= +
No Growth= -

The MIC of the ethanolic extract against the test organisms as shown in table 3.4 above was 12.5 mg/ml for *E. coli* and 6.25 mg/ml for *Shigella*.

TABLE 3.5: Minimum bactericidal concentration (MBC) of ethanolic extract against the test organisms

ORGANISMS	MBC (mg/ml)
<i>E. coli</i>	25
<i>Shigella</i>	25

TABLE 3.6: Minimum inhibitory concentration (MIC) of aqueous extract against the test organisms

CONCENTRATION (mg/ml)	TEST ORGANISMS	
	<i>E. coli</i>	<i>Shigella</i>
100	+	+
50	-	-
25	-	-
12.5	-	-
6.25	+	+

KEY: - Growth= +
No Growth= -

The MIC of the aqueous extract against the test organisms as shown in table 3.6 above was 12.5 mg/ml for *E. coli* and 12.5 mg/ml for *Shigella*.

TABLE 3.7: Minimum bactericidal concentration (MBC) of aqueous extract against the test organisms

ORGANISMS	MBC (mg/ml)
<i>E. coli</i>	12.5
<i>Shigella</i>	25

In table 3.1 phytochemicals present in the *P. guajava* plant leaves were determined both from aqueous and ethanolic extracts. The aqueous portion of the plant leaves house tannins, flavonoids, alkaloids, and steroid; therewith, no glycosides and anthraquinone were found, but the tannins are present in very large quantity and saponins are in large amount. The phytochemical analysis of ethanolic extract of the plant expunge the tannins, alkaloids, saponins and steroids in very much amount; little amount of flavonoids, absence of glycoside and anthraquinone. This is similar to a study from Sokoto by Adamu (2021) that equally found much tannins, flavonoids, alkaloids, some steroids, and no glycosides in Guava leaves subjected to phytochemical screening. Offor (2015) from the Southern part of Nigeria reported phytochemicals from guava, viz, alkaloids, flavonoids, saponins, tannins, which are all reported in this finding; only differed in reporting the presence of glycosides. A corroborated study in Ethiopia reported by Oncho *et al* (2021) found considerable amount of alkaloid, saponins, and some levels of phenol, tannin in both bark and leaves of guava plant from Ethiopia. In another Indian study, Anbuselvi and Jeyanthi (2017) reported a significant levels of alkaloids, some levels of tannins, flavonoids in guava leaf extracts of different solvents. The mentioned studies have confirmed the presence of diverse array of useful phytochemicals in guava. However, the little observed variation, like absence of glycosides in majority of the mentioned studies, and presence in Offor (2015) and some slight differences in concentrations of chemicals might be due the variation of locations of the plants studied, variations in

terms of varieties, and methods of cultivation or the portion of the plant been studied (Tiwari and Cummins, 2013). The findings in this study as in table 3.1 and the past studies have disgorged that, the guava plant contains elevated level of secondary metabolites that exert many useful activities like antioxidants, anti-inflammatory, and antiviral. That is why this plant in its different forms have been useful in the treatment diarrhoea, cough, oral ulcers, wound, etc. It is a good source of natural compounds that are applicable in industry and utilized by locals in many means in many countries across the globe (Devika, 2021).

To know the exact nature of the phytochemicals embedded in the guava leaves was the reason why phytochemical analyses was done, that is to know the presence or absence of flavonoids, tannins, alkaloids, steroids, etc; because plants play a vital role in medicine produced by scientists and traditionalists. They have good ingredients needed by pharmaceutical industry to avail the overwhelming demands of the global market, and to seriously use plants, and used scientific methods to validate plants that can benefit the locals and the scientists (Offor, 2015; Khare *et al.*, 2021). Nowadays, there has been much concern about the resurgence of antimicrobial resistance to conventional drugs, therefore alternatives ought to be sourced (Devika, 2021); and considering the believe that natural compounds are better than the synthetic ones with regards to some idiosyncrasies (Khare *et al.*, 2021). Surf of past works has confirmed that plants are sources of medicine in traditional and scientific fashions due to their chemical constituents that inhibit microbes (Ogu *et al.*, 2012; Upadhyay *et al.*, 2013). Phytochemicals are derived from plants in order to serve as secondary metabolites instead of the nutritive purposes. They are there to confer protectiveness, and disease fighting ability so that the plant having them or humans that are taking them are protected against microbes (Offor, 2015). Consequently, many works were conducted with a motive to expunge the several bioactive compounds that can be used to inhibit microbes as a way of posing alternative strategies to the old-fashioned synthetic drugs that are no longer effective because of antimicrobial resistance resurgence (Akinpelu and Onakoya, 2006; Ogu *et al.*, 2010; Firdouse and Alam, 2011). Pertaining the *Psidium guajava*, this study disgorged that it possessed more antibacterial activity at a 200mg/ml concentration on *Shigella spp* and *Escherichia coli*. Equally, guava leave chemicals extracted with ethanol have better activity than the ones extracted with water, because ethanol is better solvent than the water to reveal embedded compounds. Other past studies have similar findings with those of this study as in (Nwanneka *et al.*, 2013) that studied the guava leaf antibacterial effect, therein, the plant is effective to inhibit the microbes, but ethanolic extract is better. This has also been in the same streak with findings of (Pandey and Shweta, 2011) that discovered that extracts from ethanol are better in terms of activity than those of water. Similarly, Egga *et al* (2014) reported flavonoids, tannins, saponins, terpenes, alkaloids, anthraquinones and concentration dependent antimicrobial activity in the same plant from Jos Plateau, Nigeria. Moreover, a recent study in Katsina by Kaware and Ismail (2021) revealed a similar finding of antibacterial activity, and the efficacy of ethanolic extract than the aqueous extract.

In table 3.2, the ethanolic extract of guava shows a powerful activity (zone of inhibition greater than 12mm) on *E. coli* and *Shigella* bacteria as denoted by mean zones of inhibition. This shows that the plant is very effective in inhibiting the growth of the two examined bacteria, thus showing the reason behind its effectiveness when applied by the local people for treatment of microbes. However, the results of inhibition denoted in table 3.3 of the aqueous guava extract has shown a weak activity on *E. coli*, and likewise weak activity on *Shigella* except in one case that it denotes moderate activity. This has shown that the exanolic extract is better in terms of effectiveness than the aqueous extract. This is because ethanol is better solvent than the water medium, allowing more chemicals to be divulge for activity (Vieito *et al.*, 2018). The MIC of the ethanolic extract against the test organisms as shown in table 3.4 above was 12. 5mg/ml for *E. coli* and 6 .25 mg/ml for *Shigella*. This has also shown that the extract is more powerful on *E. coli* than the *Shigella*. In table 3.5 the revealed MBC was 25 mg/ml for all the two microbes under study. In table 3.6 the MIC of the aqueous extract against the test organisms as was 12. 5mg/ml for *E. coli* and 12.5 mg/ml for *Shigella*. By implications, the ethanolic extract is more active than the water extract as similarly reported in (Vieito *et al.*, 2018). The values of minimum bactericidal concentration due to aqueous extract for *E. coli* and *Shigella* are 12.5 mg/ml and 25mg/ml respectively as depicted in table 3.7, showing that the *E. coli* is more affected than the *Shigella* bacteria. In similar studies against antibiotic resistance, flavonoids, alkaloids, and tannins in methanolic/other extracts have been reported to have antimicrobial effects against *E. coli* and other related Gram

negative bacteria, and *Shigella* as well. Other metabolites are equally essential in combating antibiotic resistance as well (Khare *et al.*, 2021; Arsene *et al.*, 2022).

The mechanism by which the plant metabolites can be able to circumvent the microbes are explained in various terms. For example, basically in most of the cases the metabolites/ phytochemicals affect the microbes by inhibiting enzymes that are essential in life of the microbes such as those required for synthesis of wall/ membrane, and enzymes required for synthesis of deoxyribose nucleic acid (DNA) or ribonucleic acid (RNA). When DNA gyrase or RNA polymerase enzymes are inhibited the life of the microbe is at stake, and when membrane or wall synthesis is inhibited their cell components are disorganized and in turn death occur (Kumar, 2015; Khare *et al.*, 2021). Other ways of suppressing the microbes using the phytochemicals include the alleviation of virulent factors (inhibition of enzymes, inhibition of toxins, inhibition biofilm formation). Therefore, a possible cheap, effective, and safe antimicrobial agent is available in guava plant with possibly low side-effect and adverse effects (Barbieri *et al.*, 2017). Other means by which phytochemicals specifically inhibits enteric microbes includes, inhibiting the growth of microbes, modulation of signal transduction pathways, modulation of gene expression pathways, and interfering with certain metabolic processes (Godstime *et al.*, 2014).

With the recent resurgence of antibiotic resistance across the world leading to more failure of conventional antibiotics by 2050 and leading to spending of 100 trillion dollars globally, more hospital visits, much cost of treatment; a threat that can lead to 10 million deaths every year; there is need to seek for alternatives (Tiwari and Cummins, 2013; Aresene *et al.*, 2022). Phytochemicals and antimicrobials found from guava are a scientific portent and motive behind its utilization in treatment of several disorders especially those that were due to bacterial pathogens like *E. coli* and *Shigella dysentery*. The findings of this work serve as a tentative justification on why locals and traditionalists used the guava for medicine on many infections. It is pertinent to make more analysis and studies regarding this very plant to clear all the untouched areas.

CONCLUSION AND RECOMMENDATION

This study after performing a phytochemical screening has revealed saponins, alkaloids, flavonoids, tannins, and steroids. It is a critical support on why the guava has been presented with positive outcomes when using the plant for therapeutic purposes by locals. The plants equally, has proven to have an antibacterial effect on *Shigella* and *E.coli* microbes, but the better activity was found using the ethanol extract than the aqueous extract; a phenomena that is due to the phytochemicals therein. 6.25 – 50 mg/ml was the range of MIC and MBC of the plants found. The following are recommended upon completion of the research:

1. There is need for more studies to reconfirm the capacity of the guava on the studied bacteria
2. There is need for study regarding the toxicology of the plant
3. More studies are needed by pharmacological industries on the plant to unveil its more active compounds
4. It is expected that, the leaves can be used by the locals to treat intestinal and urinary disorders

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