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Nutritional Composition of Tumeric (*Curcuma longa*) and its Antimicrobial Properties

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Abstract: The studies on the nutritional composition of turmeric and its antimicrobial properties; proximate, vitamin, mineral and phytochemical compositions of the turmeric were determined using standard methods. The results of the analysis shows that it contains 8.92 % moisture content, 2.85 % ash, 9.42 % crude protein, 4.60 % crude fibre, and 6.85 % fat. The methanolic extract of the plant exhibited significant inhibitory actions against *Escherichia coli, Streptococcus, Staphylococcus, Bacillus cereus, Micrococcus, Pseudomonas, Aspergillus* and *Penicillium* at a final concentration of 20 mg/ml. The zone of inhibition exhibited by the plant extracts against the tested organisms ranged between 7.0 - 20.0 mm. The zones of inhibition exhibited by the standards, *Ampicilin* and *Fungabacter* ranges between 15.0 - 28.0 mm and 13.0 - 30.0 mm respectively. The plant extract compared favourably with the standard antibiotic used. The phytochemical results show that the plant contains 0.45 % saponin, 1.08 % tannin, 0.40 % flavenoid, 0.08 % phenol and 0.03 % sterol.

Key words: Antimicrobial, nutritional, phytochemical, tumeric

INTRODUCTION

Development of bacterial resistance to the available antibiotics and increasing popularity of traditional medicine has led researchers to investigate the bacterial compounds in plants. Turmeric is a medicinal plant that botanically belongs to *Zingiberacea* family (Chattopadhyan *et al*., 2004). Turmeric is widely used as a spice and colouring agent and is known for its medicinal properties (Luthra *et al.*, 2001). Components of turmeric are named curcuminoids which include mainly curcumin (diferuloylmethane, demethoxycurcumin, and bismethoxycurcumin) (Chainani - Wu, 2003). Curcumin is the important fraction which is responsible for the biological activities of turmeric. The melting point of curcumin, ($C_2H_{52}OO_6$) is 184.2 ^oC. It is soluble in ethanol and acetone but insoluble in water (Joe *et al*., 2004). Curcumin, a potent antioxidant is believed to be the most bioactive and soothing portion of the herb turmeric and posses the antioxidant, anti - inflammatory, anti - platelet, cholesterol lowering, antibacterial and antifungal effects (Peter, 2000).

Moreover, nutrients found in turmeric do more than just prevent deficiency diseases. It has a high nutritional status that can be exploited. The curcumin contain vitamins or vitamin precursor which produces vitamin C, beta - carotene as well as polyphenol coupled with fatty acid and essential oil. Turmeric is a good source of spice compared with other spices. Though consumed in Africa and some sub - Saharan countries, it has been regarded as an under exploited spice. It has probably been one of the most underutilized tropical crops. The leaves are known as great source of vitamin and minerals (Chattopadhyan *et al.*, 2004). Introduction of the plant as part of diet has been successful despite the fact that new foods are very often difficult to introduce (Henry, 1998). Tumeric has been used traditionally as household remedy in curing various diseases such as anorexia, cough, rheumatism and intestine disorder. There is a need to investigate tumeric scientifically so that it would not be used only traditionally but industrially in food and drug production. This study will give an insight of the nutritional, phytochemical and microbial properties of tumeric plant which could be a gate way to different ways in which tumeric could be used. The objectives of this work are to determine the proximate, vitamin, phytochemical and mineral compositions of turmeric plant and to determine the antimicrobial activities of tumeric plant.

MATERIALS AND METHODS

SOURCE OF PLANT MATERIAL

The harvested rhizomes of turmeric plant used in the work were obtained from Eastern farm and were identified by Genetic resource unit of National Root Crops Research Institute, Umudike, Abia State. The analysis was carried out in the Food Science Department of Biotechnology and Nuclear Agriculture Research Institute (BNARI) which is under Ghana Atomic Energy Commission (GAEC), Accra, Ghana.

Preparation of plant material

The harvested rhizomes were carefully washed with clean water. It was then peeled, steamed for 10 minutes to remove the raw odour. It was later dried in the oven at a temperature of 65 ^oC. The dried rhizomes were polished to remove rough surface by handpicking before it was finally milled into powder and packaged for analysis.

DETERMINATION OF PROXIMATE COMPOSITION

Moisture, dry matter, protein and crude fibre contents were determined by the method described by James (1995). Total ash was determined by AOAC (2000). Fat content of the sample was determined by Pearson (1976).

DETERMINATION OF PHYTOCHEMICAL

Alkaloids and sterol were determined by the method described by Haborne (1998). Saponin was determined by the method described by AOAC (2000). Hydrogen cyanide was determined by the method described by Onwuka (2005). Flavonoid was determined by the method described by Haborne (1998). Phenol and Tannins were determined by the method described by Person (1976).

QUALITATIVE TEST

Methanol extract of the samples were used for the test. The dried sample were soaked in the solvent overnight and filtered before heating to one quarter volume of flask (Harbone, 1998).

Test for alkaloid

The extract (1.0 ml) was shaken with 5.0 ml of 2 % HCl on a steam bath and filtered. To 1ml of the filtrate, Wagner's reagent (iodine in potassium iodide solution) was added. A reddish brown precipitate confirms that its presence.

Test for saponin

One millilitre of the filtrate was diluted in 1ml of water and shaken vigorously. A strong Frothing confirms presence of saponin.

Test for tannins

Five millilitres of the extract was added to 2.0ml of 1% HCL. Deposition of a red precipitate shows the presence of tannin.

Test for hydrogen cyanide

This was done using spot paper test. One millilitre of the extract was added with 2 - 3 drops of toluene solution. A change from the yellow colour of the paper to brick red colour is a positive result for

hydrogen cyanide.

Test for sterol

The extract (1 ml) was dissolved in 2.0 ml of chloroform in a test – tube, and then 1 ml of conc. H_2So_4 was added. Formation of reddish brown colour at the inter - phase confirms the presence of steroid.

Test for phenol

The extract (1.0 ml) was added with 1.0 ml of 10 % ferric chloride. The formation of a greenish brown or black precipitate or colour is taken as positive for a phenolic nucleus.

Test for flavenoid

The extract (1.0 ml) was diluted in 1.0 ml of diluted NaOH. Formation of precipitate shows the presence of flavonoid.

DETERMINATION OF VITAMINS

Riboflavin, thiamine and niacin were determined by the method described by Onwuka (2005).

DETERMINATION OF MINERALS

Calcium, phosphorous, potassium and iron were determined by the method described by James (1995).

MICROBIAL ANALYSIS

Preparation of microorganism for the experiment

The pure culture of the microorganisms was obtained from the Microbiology Department, of Biotechnology and Nuclear Agriculture Research Institute under Ghana Atomic Energy Commission, Accra, Ghana. The bacteria isolates include Gram positive: *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus faecalis* and Gram negative bacteria; *Escherichia coli* and *Pseudomonas aeroginosa.* The fungi were *Aspergillus niger* and *Pencillium citrinum* . The stock cultures of bacteria were sub - cultured in nutrient agar (NA) slants while that of yeast and mould on Sabour and Dextrose Agar (SDA) slants and stored at 4^0C.

Sensitivity test of turmeric extract on the microorganisms

The sensitivity test of the plant extract was determined using agar well diffusion method as described by ICMSF (1998) with little modifications. The bacteria isolates were first grown in nutritional broth for 18 hours before use. The isolates were later sub cultured in Mueller Hinton Agar (Oxoid Ltd). Wells were then bored into the agar medium using a sterile 6 mm cork borer. The wells were then filled up with the solution of the extract and care was taken not to allow the solution to spill to surface of the medium. The plates were allowed to stand on the laboratory bench for between 1 - 2 hours to allow proper inflow of the solution into the medium before incubating the plates in incubator at 37 ^{0}C for 24 hours. The plates were later observed for the zones of inhibition. The effects of the extract on bacteria isolates were compared with those of standard antibiotics, ampicillin at a concentration of 1 mg/ml.

Isolation and characterization of bacteria and fungi

One millilitre aliquot of fresh sample solution was aseptically collected and serially diluted. Six folds after I ml was pour-plated using PDA and Nutrient Agar respectively for fungi and bacteria. The plates were gently rotated to distribute the inoculums evenly in the plate and left to solidify under laminar airflow. Each of the inoculated plate was incubated at 30 - 37 ^{o}C for 48 - 72hours. After good growth of the colonies, distinct colonies were sub-cultured with fresh PDA and Nutrient Agar respectively by streak plate technique and incubated again at 30/37 ^{0}C for 24 - 72 hours. Each colony was confirmed to indentify the specific microorganisms present by examining it under a light microscope using oil immersion objective after staining with lactophenol in cotton blue dye, while the bacteria colonies were gram stained before viewing. Pure isolates were placed on PDA/Nutrient Agar slants and stored at 4 ^{o}C until needed. The isolates were characterized based on their cultural and biochemical characteristics.

RESULTS AND DISCUSSION

PROXIMATE COMPOSITION OF TURMERIC PLANT

The result in Table 1 shows that the turmeric contains 8.92% moisture, 2.85% ash, 4.60 % crude fibre and 6.85 % fat. It also contains 9.40 % crude protein and 67.38 % carbohydrate. This implies it could be good source of protein and carbohydrate. Turmeric 67.38 % had higher carbohydrate content than *Acalypha racemosa* (45.26 %) and *Acalypha marginata* (38.24 %) (medicinal plants) but Turmeric (4.60 %, 9.40 % and 2.85 %) was lower in crude fibre, crude protein and ash than *Acalypha racemosa* (7.20 %, 16.19 % and 13.14 %) and *Acalypha marginata* (10.25 %, 18.15% and 10.32 %) respectively (Iniaghe *et al.*, 2009), which implies that *Acalypha racemosa* and *Acalypha marginata* will have more mineral content than turmeric because of higher ash content. The 2.85 % ash content of turmeric shows that turmeric will contain reasonable amount of mineral. The fibre (4.60 %) presents in turmeric will help to cleanse the digestive tract of its consumer by removing potential carcinogens from the body and prevents the absorption of excess cholesterol. Fibre also adds bulk to the food and prevents the intake of excess starchy food and may therefore guard against metabolic conditions such as hypercholesterdemic and diabeties mellitus (Bamishaiye *et. al*., 2011).

Table 1: Proximate composition of turmeric plant

Parameter	Composition (%)
Moisture Content	8.92±0.02
Dry Matter	91.00±0.01
Ash Content	2.85±0.02
Crude Fibre	4.60±0.01
Crude Protein	9.40±0.02
Fat	6.85±0.00
Carbohydrate	67.38±0.01

Values are means ± standard deviation of three determinations

PHYTOCHEMICAL COMPOSITION OF TURMERIC PLANTS

The result in Table 2 shows that tumeric plant had 0.76 % alkaloid, 0.45 % saponin, 1.08 % tannin 0.03 % sterol, 0.82 % phytic acid, 0.40 % flavenoid and 0.08 % phenol . Alkaloid (0.76 %) in tumeric plant shows that tumeric could be used in curing headache associated with hypertension, management of cold, chronic catarrh and migraine (Gill, 1992). Tumeric plant could be necessary in the management of inflammation, improve sex hormone, lowering cholesterol, preventing deleterious and cytotoxins and could have antioxidant property as it had saponin (0.45 %) and flavenoid (0.40 %). Tannin (1.08 %) in tumeric pant shows it could be used as antioxidant and in treatment of intestinal disorder such s diarrheal and dysentery (Okwu and Josiah, 2006).

The phytochemical results of the extract reveal that it contains 0.40 % flavenoid. Flavenoids exhibits a range of biological activities, one of which is their ability to scavenge for biological radicals and superoxide anions radicals and thus health promoting in action. Flavenoids also exhibits anti - inflammatory, antiangionic, anti - allergic effects, analgesic and antioxidant properties (Gill, 1992). These observations support the usefulness of turmeric plant in

remedies for treatment of various infections.

Turmeric also contains 1.08 % tannin. Tannin exerts antimicrobial activities by iron deprivation, hydrogen bonding or specific interactions with vital proteins such as enzymes in microbial cells (Scalbert, 1991). Herbs that have tannins as their components are astringent in nature and are used for treatment of intestinal disorders such as diarrhea and dysentery (Dharmananta, 2003). This finding support the reasons why turmeric plant has positioned among medicinal plants used for the treatment of diseases. Tannin has been observed to have remarkable activity in cancer prevention and anti cancer. In addition to this; tannins could be used in treatment of inflamed or ulterated tissues thus, the presence of tannins in turmeric support the traditional medicinal use of this plant in the treatment of ailments caused by micro-organisms (Scalbert, 1991). Just *et al*. (1998) revealed inhibitory effect of saponins on inflamed cells. Saponin also supports the usefulness of turmeric in managing inflammation. Steroidal compound also present in turmeric extract are of importance and interest due to their relationship with such compounds as sex hormone (Okwu and Josiah 2006). Saponins have been shown to possess both beneficial (Cholesterol- lowering) and deleterious cytotoxic; permeabilization of the intestine property. Although saponin was found to be high in turmeric, processing may reduce it to a non-toxic level. More research is required on the isolation, structural determination, quantitative and biological effect of saponins in a wide range of Nigerian food spices and medicinal plants. The extract also contains 0.76% alkaloid which is capable of reducing headaches associated with hypertension. It also helps in the management of cold, chronic catarrh, persistent headaches and migraines (Gill, 1992).

Table 2: Phytochemical composition of turmeric plants

Phytochemicals	Composition (%)
Alkaloid	0.76±0.01
Saponin	0.45±0.00
tannin	1.08±0.02
Sterol	0.03±0.01
Hydrogen cyanide	0.82±0.00
Flavenoid	0.40±0.01
Phenol	0.08±0.03

Values are means ± standard deviation of three determinations

THE QUALITATIVE TEST ON PHYTOCHEMICAL COMPOSITION OF THE TURMERIC PLANT

The result in Table 3 shows that there is presence of alkaloid, saponin, tannin, cyanogenic glycoside, sterol, phenol and flavenoid. The presence of this phytochemicals confirmed the medicinal properties of the turimeric plant. Saponins are a special class of glycosides which have soapy characteristics. It has also been shown that saponins are active antifungal agents. Tannins are also known antimicrobial agents. Tannins are water soluble plant polyphenols that precipitate proteins (Prasad *et al.,* 2008). Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional proteins unavailable for them. The growth of many fungi, yeasts, bacteria, and viruses was inhibited by tannins. Tannins are reported to have various physiological effects like anti - irritant, antisecretolytic, antiphlogistic, antimicrobial and antiparasitic effects. Phytotherapeutically tannin-containing plants such as turmeric plant, *acalypha racemosa* and *acalypha marginata* are used to treat nonspecific diarrhoea, inflammations of mouth and throat and slightly injured skins (Iniaghe *et al.,* 2009; Prasad *et al.,* 2008). The potent antioxidant activity of flavonoids their ability to scavenge hydroxyl radicals, superoxide anions and lipid peroxy radicals may be the most important function of flavonoids (Iniaghe *et al.,* 2009).

Table 3: The qualitative test on phytochemical composition of the turmeric plant.

Phytochemical components	Test	Observation	Inferences
Alkaloid	Wagners Dragendi offs Test	Reddish Brown Test	+
Saponin	Frothing Test	Stable Froth Emulsion	+
Tannin	Acid Test	Reddish Brown	+
Cyanogenic Glycoside	Sodium Picrate	Yellow to Reddish brown	+
Sterol	Salkowsk is Test	Red Color Interface	+
Phenol	Ferric Chloride	Greenish brown precipitate	+
Flavenoid	Sodium hydroxide	Yellow Color	+

MINERAL AND VITAMIN COMPOSITION OF TURMERIC

The result in Table 4 shows that tumeric plant had 0.89 % thiamine, 0.16 % riboflavin, 2.30 % naicine, 0.20 % calcium, 0.63 % phosphorus, 0.46 % potassium and 0.05 % iron. Constant feeding on tumeric plant could be important in sustaining strong bone, muscle contraction and relaxation, blood clothing, reduce blood pressure, and help in haemoglobin formation (Latunde - dada, 1980; Kubmarawa *et al*., 2007) as it had thiamine (0.59 %), riboflavin (0.16 %), potassium (0.46 %) and iron (0.05 %) respectively. Calcium is a major factor sustaining strong bones and plays a part in muscle contraction and relaxation, blood clotting and absorption of vitamin B_{12} potassium and magnesium are known to reduce blood pressure. Potassium plays a role in controlling skeletal muscle contraction and nerve impulse transmission. Patients with soft bone problems are usually placed on high calcium and potassium meals (Kubmarawa *et al.*, 2007). The iron content present in the extract can help in heamoglobin formation (Latunde - dada, 1980) and hence recommend for iron deficiency anaemia. Various minerals are also co - enzymes in certain biochemical reactions in the body which underscores the importance of the plant in metabolic reactions.

Table 4: Mineral and vitamin composition of turmeric

Parameter	Composition (%)

Riboflavin	0.59±0.02
Thiamine	0.16±0.00
Niacin	2.30±0.00
Calcium	0.21±0.01
Phosphorus	0.63±0.02
Potassium	0.46±0.03
Iron	0.045±0.02

Values are means ± standard deviation of three determinations

ANTIMICROBIAL ACTIVITY (ZONE OF INHIBITION, MM) OF TURMERIC EXTRACT AGAINST PATHOGENS.

The antimicrobial activity of the plant was studied using the extracts of the turmeric as shown in Table 5. Agar well diffusion method was used to determine the zone of inhibition of bacterial growth. The results revealed that turmeric plant possess antimicrobial activities against tested bacterial and fungal isolates at a concentration of 1 mg/ml. The turmeric extract was active against eight bacterial isolates comprising both gram - positive and gram negative organisms which include *Bacillus subtilis*, *Pseudomonas aerigunosa*, *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger*, *Pencillium citrinin*, *Micrococcus* and *Streptococcus faecalis*.

Ampicillin and fungobacter were used as standards for both bacteria and fungi respectively. Ampicillin (21 cm) had significantly ($p < 0.05$) higher zone of inhibition than tumeric extract (14 cm) but not significantly ($p > 0.05$) higher than that of fungarbacter (18 mm) on *Bacillus subtilis* (Table 4.5). Tumeric extract (15 mm) had the same value of zone of inhibition with ampicillin (15 mm) but higher zone of inhibition when compared to fungabacter which were not significantly ($p < 0.05$) different on *Pseudomonas aerigunosa*. Ampicillin had significantly ($p < 0.05$) the highest zone of inhibition followed by fungabacter and then tumeric extract in *Escherichia coli* (24 mm, 19 mm and 9 mm*)* and *Streptococcus faecalis* (26 mm, 20 mm and 12 mm) respectively. Ampicillin (18 mm) also had significantly ($p < 0.05$) higher zone of inhibition than tumeric extract (7 mm) but not significantly ($p < 0.05$) higher from fungabcter (13mm) on *Staphylococcus aureus*. Fungabacter (28 mm) had significantly ($p < 0.05$) higher inhibition zone than ampicillin (20 mm) and tumeric extract (16mm) on *Micrococcus lusteus*. Fungabacter had significantly ($p < 0.05$) higher zone of inhibition from tumeric extract but not significantly ($p > 0.05$) different from that of ampicillin on *Aspergillus niger* (24 mm, 23 mm and 20 mm) and *Penicillium citrinin* (30 mm, 20 mm and 13 mm) respectively.

In all indications, the result in Table 5 shows that tumeric extract had the lowest microbial zone of inhibition as compared to ampicillin and fungabacter standard antibiotics. There is no doubt that if the crude tumeric extract becomes purified, it could have higher zone of inhibition than most standard antibiotics.

Table 5: Antimicrobial activity (zone of inhibition, mm) of turmeric extract against pathogens.

Microorganism (1mg/ml)	Turmeric extract (mm)	Ampicillin (mm)	Fungabacter (mm)
Bacillus subtilis	$14.00^{a} \pm 0.70$	$21.00^{b} \pm 0.90$	-
Pseudomonas aerigunosa	$15.00^{a} \pm 0.80$	$15.00^{a} \pm 0.80$	-
Staphylococcus aureus	$7.00^{a} \pm 1.41$	$18.00^{b} \pm 0.70$	-
Escherichia coli	$9.00^{a} \pm 0.14$	$24.00^{c} \pm 0.28$	-
Aspergillus niger	$20.00^{a} \pm 0.71$	-	$24.00^{b} \pm 0.78$
Pencillium citrinin	$16.00^{a} \pm 0.90$	-	$30.00^{b} \pm 0.50$
Micrococcus lutes	$13.00^{a} \pm 0.70$	$20.00^{b} \pm 1.41$	-
Streptococcus faecalis	$12.00^{a} \pm 0.14$	$26.00^{c} \pm 0.70$	-

Values are means ± standard deviation of three determinations. Means with different superscript in the same row are significantly different ($p<0.05$).

MORPHOLOGICAL CHARACTERISTICS OF THE ISOLATES

The result in Table 6 shows the morphological characteristics of the isolates used to study the anti-microbial effect of the turmeric extract. *Escherichia coli*, *Bacillus cererus* and *Micrococcus* were positive to Motility test while *Streptococcus*, *Staphylococcus* and *Pseudomonas* were negative. *Escherichia coli* and *Pseudomonas* were negative to Gram stain reaction test while other microorganisms were positive. The cell arrangement of the characterised micro-organisms (Table 4.6) was similar as reported by Madigan and Martinko (2008) and Mcdevitt (2009).

Table 6: Morphological characteristics of the isolates

Organisms	Motility	Gram stain reaction	Spore	Cell arrangement
Escherichia coli	+	-	-	cell shaped
Streptococcus	-	+	-	short cocci
Staphylococcus	-	+	-	cocci in irregular cluster
Bacillus	-	+	+	blunt end singly in pairs
Micrococcus	+	+	-	cocci rods
Pseudomonas	+	-	-	small cocci rods

BIOCHEMICAL CHARACTERISTICS OF THE ISOLATES

The result in Table 7 shows the biochemical characteristics of the isolates used to study the anti-microbial effect of the turmeric extract. *Streptococcus* was positive to catalase test while other organisms such as *Escherichia coli*, *Staphylococcus*, *Bacillus*, *Micrococcus* and *Pseudomonas* were negative. Many organisms make the enzyme catalase to remove hydrogen peroxide. The catalase test measures the ability of an organism to produce the enzyme catalase that degrades hydrogen peroxide to water and

oxygen. The catalase test is useful to differentiate between the *Staphylococci/Micrococci* and the Streptococci (Madigan and Martinko, 2008). *Staphylococcus* was positive to coagulase test while other microorganisms were negative. *Micrococcus* and *Pseudomonas* were positive to oxidase test while other microorganisms were negative. Coagulase test is a protein enzyme produced by several microorganisms that enables the conversion of fibrinogen to fibrin. In the laboratory, it is used to distinguish between different types of *Staphylococcus* isolates. The enzyme oxidase is part of the electron transfer system used by some organisms that use molecular oxygen as at terminal electron acceptor. The oxidase test is a useful test for distinguishing between the Gram negative rods *Pseudomonas* and the *Enterobacteriaceae* (Madigan and Martinko, 2008). Only *Pseudomonas* was negative to Methyl red test**.** When the culture medium turns red after addition of methyl red, because of a pH at or below 4.4 from the fermentation of glucose, the culture has a positive result for the methyl red test. *Bacillus* and *Pseudomonas* were positive to Vogas Proskauer test while only *Escherichia coli* was positive to Indole test **(**Mcdevitt, 2009**).** Testing for indole production is important in the identification of enterobacteria. Most strains of *Escherichia coli, P. vulgaris, P. rettgeri, M. morganii*, and *Providencia* species break down the amino acid tryptophan with the release of indole (Madigan and Martinko, 2008; Mcdevitt, 2009).

Table 7: Biochemical Characteristics of the isolates

Organisms	Catalase	Coagulase	Oxidase	Methyl red	Vogas Proskauer	Indole
Escherichia coli	+	-	-	+	-	+
Streptococcus	-	-	-	+	-	-
Staphyloccus	+	+	-	+	-	-
Bacillus	+	-	-	+	+	-
Micrococcus	+	-	+	+	-	-
Pseudomonas	+	-	+	-	+	-

CONCLUSION

The data obtained showed that turmeric contains potential antimicrobial components that may be of great use for the development of pharmaceutical industries as a therapy against various diseases. The turmeric extract possess significant inhibitory effect against tested pathogens. The extract is nutritionally rich as it contains some essential vitamins and minerals needed for body growth. The results of the study support the development of new antimicrobial drugs from the plant.

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