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# Preliminary Screening of the Phytochemical Composition, Antioxidant and Antimicrobial Activity of the Twigs of *Prosopis africana* Used as Chewing Stick in Benin Republic

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**Abstract** The present work was devoted to the evaluation of the chemical and antimicrobial potential of the stems of *Prosopis africana*. Standard techniques described in the literature were used to analyse the phytochemicals. The antioxidant activity was carried out by DPPH and FRAP methods and antimicrobial activity, by the diffusion method. Many secondary metabolites like tannins, steroids, coumarins, flavonoids, mucilage and alkaloids were identified in the different extracts of this plant. The semi-ethanolic extract presented the highest total phenolic content which is of 124.142 ± 3.535 mg eqGA/gCE whereas the ethanolic extract had the highest content of flavonoid with values of 124.708 ± 5.595 mg eqC/gCE. The ethanolic extract of this plant exhibited a good ability to inhibit the DPPH radical and a good ability to reduce ferric $Fe^{3+}$ ions to ferrous $Fe^{2+}$ ion and this could be explain by it good content in phenolic compounds. The ethanolic extract was the only one that was able to inhibit the strains used in this study. MICs and MBCs ranged from 12.500 to 25.000 mg / mL.The same extract was able to inhibit *Candida albicans*, a strain that is known to be implicated in oral diseases with a MIC of 12.500 mg/mL.

**Keywords** Phytochemical; Antimicrobial; Antioxidant; Chewing stick; Oral hygiene

## 1. Introduction

For many decades now, plants have played an important role in oral hygiene. Most societies recognize that the cleaning of teeth is a desirable social habit and also a means of keeping diseases away. Plants have been used in oral health as chewing sticks, mouthwash, dental floss and to cure tooth ache and infections [1]. Even though many people have abandoned the traditional use of vegetable toothbrushes, and have adapted to the conventional method of brushing teeth, some peoples still use chewing stick as a daily ritual to maintain oral hygiene. This is particularly true in developing countries where the economy, customs, religion and the availability of oral hygiene tools play a role in their continued use. Chewing stick have long been used in the Greek, Roman, Jewish and Islamic empires [2]. The stem of *Prosopis africana* is one the multiples plant parts

that are still been used as chewing stick in the central region of Benin Republic. *P. Africana* is a *Perennial leguminous* tree and is mostly found growing in the savanna regions of Western Africa [3]. The *Leguminosae* family consists of large number of trees and herbs with diverse locations and habit. The family includes approximately 700 genera and 17,000 species that are widely distribution in tropical, subtropical and temperate zones. This family is the most important in the Dicotyledonous group as they contain chemically diverse compounds such as: alkaloids, terpenoids, flavonoids and glycosides which are of interest for their biological activities [4]. For this raison, the phytochemical analysis and biological testing of traditionally used medicinal plants has become an important research area as it promotes the use of herbal medicine through confirmation of their efficacy and safety. It also determines their potential as sources of new drugs [5].

Although the World Health Organization has promoted the use of toothbrush sticks and has encouraged further research of their efficacy, few studies have been undertaken on the potential antimicrobial properties of chewing stick [1]. This is particulary true in Benin Republic where just few work has been overtaking in the determination of chemical composition and antimicrobial activity of chewing stick. For that purpose, the present study is a contribution to the knowledge of the antibacterial activities and the phytochemical potential of the stem of *Prosopis africana* used for oral hygiene in Benin Republic.

## 2. Materials and Methods

### 2.1. Plant material

The stem of *Prosopis Africana* were collected in a rural zone of the central Benin Republic (latitude/longitude: 8° 52' 60 " North / 2° 36' 0 " east) in September 2017.

### 2.2. Microorganisms

The used bacteria strains were obtained from the Bacteriology section of the National Laboratory of the Ministry of Health (LNMSP). They were constituted of reference strains namely:

- gram positive cocci: *Enterococcus faecalis* ATCC 10240, *Staphylococcus aureus* ATCC 29223
- Bacillus negative gram: *Proteus mirabilis* ATCC 24974, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922
- Fungus: *Candida albicans* IP 4872

### 2.3. Plant extracts preparation

The plants, once collected, were left at room temperature (20°C) in the laboratory for two weeks to dry. They were then grounded to a fine powder to make three types of crude extracts, namely semi ethanolic 50% macerate (eth), aqueous macerate (aq) and aqueous decoction (de). The ethanolic and aqueous macerate were obtained by maceration for three successive days, taking care to renew the solvent each day while the decoction was prepared by boiling the vegetables' powder for 30 minutes in distilled water. The filtrates obtained were concentrated on a rotary evaporator and then in an oven at 40 ° C for 3 days.

### 2.4. Phytochemical Screening

The phytochemical screening was done according to the standard techniques described by Harborne [6] and Noudogbessi et *al*., [7] and table 1 gives a summary of these methods. The plant extracts were screened for presence of alkaloids, tannins, flavonoids, coumarins, mucilage, saponins, terpene, triterpene, anthocyans and leuco-anthocyans.

**Table 1**: Standard techniques used for the phytochemical screening

| Phytochemical | Name of test | Observations |
|---|---|---|
| **Tannins** | Braemer's test | Dark blue or greenish grey coloration of the solution |
| **Flavonoids** | Shinoda test | Orange, red or purple coloration |
| **Anthocyans** | Test with 10% $H_2SO_4$ and 50% $NH_4OH$ | Appearance of a coloration after acidification which turns to purplish blue in basic medium |
| **Coumarines** | Lactone test | Intense UV fluorescence at 365 nm |
| **Alkaloids** | Meyer and Dragendorff | Appearance of a precipitate |
| **Sterol** | Liebermann-Burchardt test | Reddishbrowncolor of Interface |
| **Mucilages** | Test withabsoluteethanol | appearance of a flakyprecipitate. |

**2.5. Determination of total phenolic content**

The determination of total polyphenols was carried out with the Folin-Ciocalteu reagent according to the method presented by Ainsworth and Gillespie [8]. This method consists in adding to 20 µL of each diluted ethanolic extract obtained by maceration of the crude extracts (eth, aq, de) in ethanol for 24 hours, 200 µL of Folin-ciocalteu (10%) in a tube. The mixture obtained is vigorously vortexed before adding 5 min after 800 µl of sodium carbonate $Na_2CO_3$ (7%). The absorbance is read at 765 nm after 2 hours against a blank (ethanol). Gallic acid was used as standard for the calibration curve and the content is expressed in milligram equivalent of Gallic Acid per gram of crude extract (CE) (mg eq AG / g CE).

**2.6. Determination of flavonoid content**

The estimation of the total flavonoid content was carried out by the method used by Kim et *al*., [9]. The extract used were obtain from dilution of each the crude extracts (ethanolic, aqueous, and decoction) in ethanol. A volume of 100 µl of the extract of each plant was mixed with 0.4 ml of distilled water and subsequently with 0.03 ml of a solution of 5% sodium nitrite. After 5 minutes, 0.02 milliliters of a 10% solution of $AlCl_3$ was added. After 5 minutes again, 0.2 ml of $Na_2CO_3$ solution (1 M) and 0.25 ml of distilled water were added to the mixture. The whole was vortexed and the absorbance measured at 510 nm. The flavonoid content is calculated from a standard curve using catechin as a standard. The results are expressed in milligram-equivalent of catechin per gram of the crude extract (mg eq EC/ g CE)

**2.7. Antioxidant activity**

**2.7.1. DPPH method**

This test was carried out by adding 50 µl of the diluted extracts at different concentration to 1950 µL of DPPH at 130 µM, Discoloration of DPPH was measured at 516 nm against the blank (1950 µl of DPPH at 130 µM and 50 µl of ethanol) after 45 min [10]. The scavenging percentage was calculated by the following formula:

$$P = [(Ab - Ae) / Ab]*100$$

With P: percentage of trapping; Ab: absorbance of the blank; Ae: Absorbance of the sample

The extract concentration necessary for trapping 50% of free radicals of DPPH ($IC_{50}$) is calculated graphically by linear regression of the plotted graphs of DPPH free radical scavenging percentages as a function of the concentrations of extracts tested.

**2.7.2. Ferric Reducing Antioxidant Power (FRAP) method**

FRAP method is based on the ability of extracts to reduce ferric ion ($Fe^{3+}$) to ferrous ($Fe^{2+}$) ion. The total antioxidant capacity of each plant extract and reference compounds was determined by the method used by Chung *et al*., [11] with a slight modification. Thus 2 ml of an aqueous solution of each extract was mixed with 2 ml of phosphate buffer (0.2 M, pH 6.6) and 2 ml of the aqueous solution (1%) of potassium hexacyanoferrate [$K_3Fe(CN)_6$]. After 20 min incubation at 50°C; 2 ml of trichloroacetic acid (10%) was added. The mixture was then centrifuged at 3000 rpm for 10 min. 2 ml of the supernatant were then mixed with the same volume of water and 20 µl of a freshly prepared aqueous solution of $FeCl_3$ (0.1%) was added. Absorbances were read at 700 nm against a calibration curve obtained from gallic acid and catechin and an absorbance increase can be correlated to the reducing ability of the plant extract. The reducing power of the plant extracts were expressed as function of gallic acid equivalent per gram of crude extract (mg eq GA / g CE) and also as function of catechin equivalent per gram of crude extract (mg eq C/ g CE).

**2.8. Antimicrobial activity**

**2.8.1. Preliminary antimicrobial screening of the extracts**

It was carried out by the diffusion method of the extracts placed in wells dug in Mueller Hinton agar plates. Thus bacteria mentioned above were suspended according to the recommendations of the Antibiogram Committee of the French Society of Microbiology [12]. The suspensions thus made were seeded by swabbing on Mueller Hinton square agar plates. The three extracts of plants previously prepared at a concentration of 100 mg / ml in DMSO were filtered using 0.4µm multi-pore membranes in order to obtain sterile extract solutions.

16 wells of about 6 mm were dug in the agar plates as described by Agbankpe et *al*., [13] and 50 µl of each of the sterile extract solutions were deposited in each well. DMSO solution was used as the negative control. The positive control was conventional Vancomycin antibiotic discs for Gram-positive cocci and Imipenem and Colistin discs for Gram-negative bacilli. The different Petri dishes were left at room temperature for one hour for prediffusion and then incubated at 37 ° C for 18h as described by Oke et *al*., [14]. Each test was conducted three times for quality control purposes. Inhibition diameters were measured and compared to the standards indicated in the Table 2.

**Table 2:** Standard values used to interpret the results of the susceptibility tests of the plant extracts [15-16]

| Inhibition zone diameter (Δ) | Degree of susceptibility of the germ | Symbol |
|---|---|---|
| Δ <7 mm | Resistant | – |
| 7 mm ≤ Δ <8 mm | Susceptible | + |
| 8 mm ≤ Δ <9 mm | Fairly Susceptible | ++ |
| Δ ≥ 9 mm | Very Susceptible | +++ |

**2.8.2. Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)**

The determination of the MIC was performed according to the microwell methodology used by Lagnika et *al*., [17]. Different successive dilutions of 180 µl of the extract at initial concentrations 50 mg/mL prepared in Mueller Hinton broth were distributed in the wells. 20 µl of a 10% dilution of a suspension of 0.5 Mc Farland strains in Mueller broth Hinton were distributed in all wells. On each plate, bacterial suspension + Mueller Hinton broth served as a positive control and Negative control was DMSO + Mueller Hinton broth. The plates were then stirred for 5 minutes and placed in an oven at 37 ° C. for 18 h. 40 µl of a solution of 0.2% p-iodonitrotetrazolium (INT) in distilled water was added to each well. The plates were then deposited for 20 minutes in the dark. The presence of a red color in a well indicates the presence of viable bacteria. The MIC is the first concentration for which viable bacteria are present. Wells that did not show a red color (subjects are missing here) are seeded on Mueller Hinton agar. CMB is the first concentration for which there is a colony presence.

**2.9. Statistical Analysis**

All experiments were conducted in triplicate, and results, analysed using SPSS Statistics 17.0 software, were reported with means ± standard deviation (S.D.). An analysis of variance (ANOVA single factor) was used to compare the means of the inhibition zone diameters of the same plant on different strains, and also the inhibition zone diameters of plants extracts with reference antibiotic. The level of significance was defined at 5%.

## 3. Results & Discussion

### 3.1. Phytochemical Screening

The phytochemical screening of the three differents extracts of *P. africana* has revealed the presence of many secondary metabolites like tannins, steroids, coumarins, flavonoids, mucilage and alkaloids. The analyses of the results as presented in table 3 indicate that the presence of some phytochemical in this plant depends on the type of extract used. For exemple, coumarins were found only in the aqueous and ethanolic extracts and in the decoction of *P. africana*, whereas they were not reveled in the total extract of plant (the direct use of plant powder for screening without any initial extraction. We could not highlight the presence of alkaloids in this plant. On the contrary, Kolapo et *al*., [18] reported that saponins, tannins, alkaloids and steroids were highly concentrated in the stem and root of *P. africana*.

**Table 3**: Result of the phytochemical screening

| Plant Extract | Phytochemicals | | | | | | |
|---|---|---|---|---|---|---|---|
| | Mucilage | Tannin | Anthocyan | Steroid | Coumarin | Flavonoid | alkaloid |
| Total extract | + | + | - | + | - | + | - |
| Aqueous | - | + | + | + | + | + | - |
| Decoction | + | + | + | + | + | + | - |
| Ethanolic | + | + | + | + | + | + | - |

+ : Present ; - : Absent

### 3.2. Total phenolic contents

The total phenolic contents of the semi-ethanolic extracts which 124.142 ± 3.535 mg eq GA / g CE were generally and logically above those of the aqueous extracts and decoction. Indeed, countless studies have already shown that ethanol or methanol alone or mixed with water extract the phenolic compounds better than water alone. Total flavonoids content of the three extracts of the plant were also determine and results appear in table 4. The ethanolic extracts of both plants had the highest content of flavonoid with values of 124.708 ± 5.595 mg eq C / g CE. Flavonoids have vitamin P properties in the capillaries, which they reinforce and make it possible to reduce the frequency of minor hemorrhages [19]. Catechins, the most reduced form of the $C_3$ unit in flavonoid compounds, deserve special mention. These flavonoids have been extensively researched due to their occurrence in oolong green teas and their antimicrobial activity against oral microbes implicated in oral infection like *Streptococcus mutans* [20].

**Table 4**: Total Phenolic Content

| **Phenolic compounds** | **Plant extracts** | | |
|---|---|---|---|
| | **Aqueous** | **Decoction** | **Ethanolic** |
| **Total phenolic(mg eq GA / g CE)** | 51.107 ± 6.313 | 61.464 ± 6.313 | 124.142 ± 3.535 |
| **Flavonoid (mg eq C / g CE)** | 76.060 ± 3.948 | 52.934 ± 4.196 | 124.708 ± 5.595 |

### 3.3. Antioxidant activity

#### 3.3.1. DPPH method

The inhibitory concentration of each extract of plant and positive control necessary for trapping 50% of free radicals of DPPH ($IC_{50}$), graphically calculated are shown in figure (1a) and figure (1b). The ethanolic extract of *P. africana* was the most effective with an $IC_{50}$ of 0.5 ± 0.084. The aqueous extract and the decoction were less effective against free radicals although the aqueous extract was more effective than the decoction. In fact the aqueous extract presented an $IC_{50}$ of 1.160 ± 0.056 mg/mL whereas the $IC_{50}$ of the decoction was 1.7 ± 0.141 mg/mL. The results where compare to gallic acid and catechin with $IC_{50}$ of 0.028 ± 0.001 mg/mL and0.071 ± 0.012 mg/mL respectivily. As result, the reference compound where far active than all the plant extracts. The results suggest that this two plants could increase the antioxidant capacity of saliva when used as chewing stick and this could explain the fact that they are used by rural populations of Benin Republic. The antioxidant activity of these two chewing sticks may be attributed to their phenolic content. Indeed Lee and *al*. [21] showed that delivery of tea polyphenols by holding green or black tea in the mouth for 2-5 minutes increases the antioxidant capacity of saliva. In the same logic, Chinsembu et *al*., [22] reported that green tea as an excellent source of the polyphenol andantioxidant epigallocatechin-3-gallate plays a beneficial role in alleviating oral oxidative stress and inflammation.

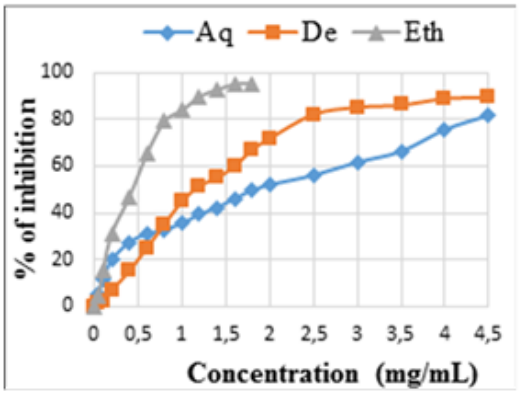


**Figure (1a) : Scavenging activity of *P. africana***

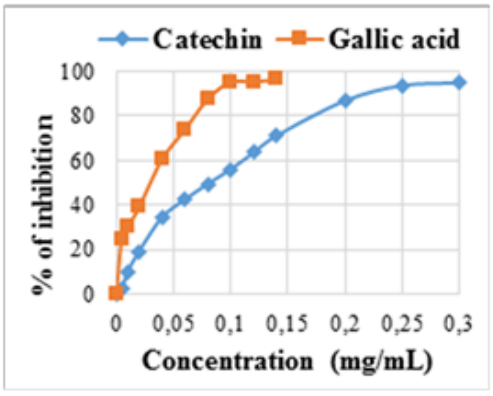


**Figure (1b) : Scavenging activity of references**

*Figure 1: Radical DPPH scavenging activities of extracts of P. africana (Aq: aqueous; De: decoction; eth: ethanolic), catechin and gallic acid*

### 3.3.2. Ferric Reducing Antioxidant Power method

In other to confirm the DPPH free radical scavenging test, the reducing power of iron $Fe^{3+}$ by the extracts of the plants studied was evaluated using the FRAP method describe in the experimental section. The results of this study presented in figure 2 show that the ethanolic extract have the best reducing power which is express as 78.7629 ± 1.361 mg eqGA/g CE and 165.877 ± 3.696 mg eqC/g CE. This could be justify by the high phenolic content previously indicated. The aqueous extract presented a medium reducing power of 44.762 ± 1.676 mg eqGA/g CE and 75.599 ± 1.706 mg eqC/g CE whereas the decoction extract present a low reducing power. Results interpretation simply means that 1g of the ethanolic extract of *P. africana* would have the same reducing power with about 78.7629 mg of gallic acid and 165.877 mg of catechin approximately. We could then conclude that this plant have a relatively good reducing power towards ion Fe (III) as compare with pure reference compounds and this reducing power could be improved by farther purifications of this extracts.

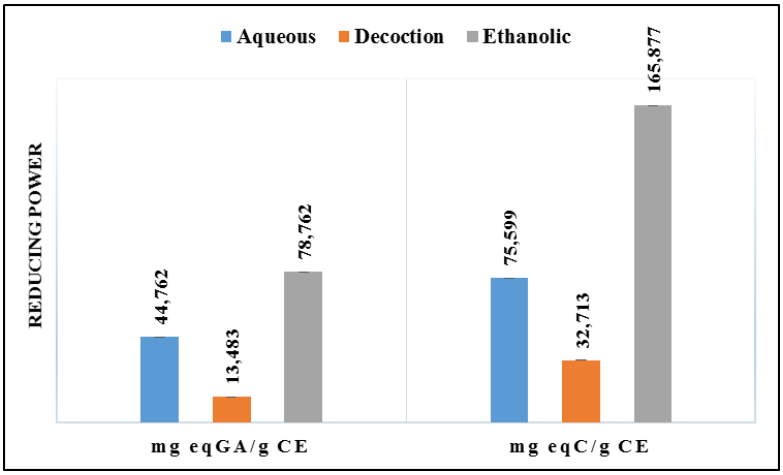


*Figure 2: Ferric reducing power of the extracts of P. africana*

### 3.4. Antimicrobial activity

### 3.4.1. Preliminary screening

The determination of the inhibition zones diameter of the extracts of the selected plants on the strains tested reveals that only the ethanolic extract of *P. africana* was effective against the microorganisms tested (table 5). This extracts was effective against the microorganisms tested with inhibition zones diameter ranging from 11.000 ± 1.000 mm to 13.333 ± 0.577 mm. This extract were able to inhibite *Candida albicans*, known to be implicated in oral infection, with remarkable inhibition zone diameters of 13.333 ± 0.577 mm. Though active, the ethanolic extract of *P. africana* was less active against the microoganisms comparatively to synthetic antibiotics. Kolapo et al., [3] reported that both ethanol and aqueous extracts of the stem and root of *P. africana* had inhibitory effect on the growth of the tested microorganisms like *Candida albicans*. This fact led them to conclude that the stem and root of *P. africana* could be potential candidate in the production of dentifrice and natural chewing gums for oral hygiene and to treat toothache, gingivitis and periodontal disease as early reported by kerry [23]. All plant extracts that were active on the microorganisms studied were submitted to the determination of MICs and MBCs to better highlight their antimicrobial activity.

**Table 5**: Inhibition Zone Diameters of plant extracts and antibiotics

| **Strains** | **Plant extraxts** | | | **Antibiotics** | | |
|---|---|---|---|---|---|---|
| | Aq. | De | Eth. | Vancomycin | Imipenem | Colistin |
| *S. aureus* | Na | Na | 12.333 ± 0.577 | 17.666 ± 0.577 | ND | ND |
| *E. coli* | Na | Na | 13.000 ± 1.000 | ND | 26.666 ± 1.154 | 19.333 ± 0.577 |
| *E. faecalis* | Na | Na | 11.333 ± 0.577 | 17.666 ± 0.577 | ND | ND |
| *P. mirabilis* | Na | Na | 11.000 ± 1.000 | ND | 26.666 ± 1,154 | 19.333 ± 0,577 |
| *C. albicans* | Na | Na | 13.333 ± 0.577 | ND | ND | ND |
| *P. aeruginosa* | Na | Na | 11.000 ± 1.000 | ND | 26.666 ± 1.154 | 19.333 ± 0.577 |

*Aq : aqueous ; De : decoction ; Eth : ethanolic ;* ND : Not Done ; na : not active

### 3.4.2. Determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

The most effective plant extracts against the various microorganisms tested were farther inquired about their MICs and CMBs to better appreciate their activity and the results are recorded in Table 6. Thus, only the ethanolic extract of *P. africana* was used for this study. From the analysis of these results, *P. africana* ethanolic extract, MICs range from 12.500 to 25.000 mg/mL whereas the MBCs range from 25.000 to 50.000 mg/mL. The same extract was able to inhibite *candida albicans*, a strain that is known to be implicated in oral diseases with a MIC of 12.500 mg/mL. Ayanwuyi *et al* [24] reported an oral median lethal dose of the methanolic extract of *P. africana* at 3.808 g/kg in mice and >5 g/kg in rats and the study results support the traditional claim of the use of *P. africana* for the analgesic and anti-inflammatory activities.

At this stage we can suppose that natural compounds, especielly phenolic compounds in the chewing sticks are responsible for the observed antimicrobial activities of the chewing sticks studied. This hypothesis seems to be confirmed by Matsumoto *et al* [20], who showed that administration of the oolong tea extract and its chromatographically isolated polyphenol compound into diet and drinking water resulted in significant reductions in caries development and plaque accumulation in rats infected with *mutans streptococci*. Since they are known to be synthesized by plants in response to microbial infection, it should not be surprising that they have been found *in vitro* to be effective antimicrobial substances against a wide array of microorganisms [25].

**Table 6 :** MICs et MBCs of different plant extracts

| **MIC and MBC (mg/mL)** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Extracts** | | **Strains** | | | | | |
| | | ***S. aureus*** | ***E. coli*** | ***E. faecalis*** | ***P. mirabilis*** | ***C. albicans*** | ***P. aeruginosa*** |
| **Ethanolic** | MIC | 25.000 | 12.500 | 25.000 | 25.000 | 12.500 | 25.000 |
| | MBC | 25.000 | 25.000 | 25.000 | 25.000 | 50.000 | 25.000 |

## 4. Conclusion

The results of the present study revealed that the ethanolic extract of *Prosopis Africana* contain an important quantity of phenolic compounds. It has also exhibited a relative good antioxidant and antimicrobial activity which could be related to their phenolic content. Further study have to done in other to find out the bioactive compound of this plant and to evaluate the toxicity this plants extract for further use.

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