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*Research article*

# PHYTOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF SEED AND PULP OF MONKEY COLA (*Cola millenii)* ON SOME SELECTED CLINICAL AND FOOD BORNE ISOLATE

*Giwa O. E[1]., Onileke F. O[1]., Adesina I. A.[1] and Adebote V. T

Department of Science Laboratory Technology[1], Food Science Technology[2], Rufus Giwa Polytechnic, Owo, Ondo State, Nigeria. Corresponding author e-mail address: giwa_muyiwa@yahoo.com

**ABSTRACT:** The phytochemical analyses of pulp and seed of *Cola millenii* K. Sckhum showed that the extract contained alkaloids, tannins, saponins, cardiac glycosides, carbohydrate, sterol, resin and terpenes that could be responsible for the observed antimicrobial activities. The bioactives compounds of pulp and seed extract of *Cola millenii* K. Sckhum were extracted, using both aqueous (water ) and organic (ethanol) solvents, and were investigated for antimicrobial activity some pathogenic and food spoilage microorganisms using agar well diffusion method. The aqueous extract did not show significant difference in their activities against the various organisms but the ethanolic extract had a significant activity demonstrating the highest activity against the test organisms using analysis of variance $p<0.05$. The seed extract demonstrated higher activity against both the gram positive and gram negative organisms tested, and also against the selected test fungi with the highest activity (1.73±0.06 cm) zone of inhibition against *Aspergillus niger*. The minimum inhibitory concentration (MIC) and minimum bactericidal/ fungicidal concentration (MBC/MFC) of the extract ranges between 40-160 mg/ml for both the seed and pulp of the plant. The antimicrobial property shown by the plant extract is an evidence of the ethno-medicinal uses of the plant. *Cola millenii* seed and pulp bioactive content may therefore be extracted and use as antibiotics and/or preservative in foods as the result is comparable with commercially available antibiotics and chemical preservatives.

**Keywords:** Antimicrobial, Phytochemical, Hemolytic, Antibiotics, Preservatives

## INTRODUCTION

The use of wild foods as a component of local response to increasing food insecurity is orderly documented. Variations in food intakes from different wild sources have direct effect on nutritional status (Abitogun *et al.* 2010).

Tropical Africa sub-region is home to many potential valuable fruit species whose potentials have not been fully realized. A good number of this fruit species are not yet domesticated. Nevertheless, tangible economic produce are been harvested from their wild (Abitogun *et al.* 2010). Wild plants which refers to trees, flowers, and grasses, the vines found in the bushes and even the weeds in the lawn, they are what our great grand parents used to doctor the family. Consider the food on the table as what we need as renewable source of oil, fuel, food, pharmaceuticals and more enhanced the need to discover the potentials present in these wild plants.

Plants have basic nutritional importance by their content of protein, carbohydrates, fats and oil minerals, vitamins and water responsible for growth and development in man and animals. Report shows that the greatest sources of these phytochemicals are fruits and vegetables. (Onyeka, and Uwambeke, 2007).

*Cola millenii* has been chosen as case study because of its rampant growth and produce in the wild. Thus, the objective of this research is to analyze the phytochemical and the antimicrobial properties of the seed and pulp of *Cola millenii* (Monkey cola).

This phytochemicals reduces the low density lipoprotein, i.e. the cholesterol involved in depositing fat in the arteries, prevent blood clotting which can reduce risk of heart attack or stroke. They do this either by working alone or in combination with other vitamins. (Onyeka, and Uwambeke, 2007).

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## MATERIALS AND METHODS

This study was carried out using random sampling and was divided into six experimental phases.

(1) The collection, pulverization of plant materials
(2) The extractions and phyto-chemical screening of the extract.
(3) The antimicrobial screening of both the pulp and seed ethanolic and aqueous extract of the plant.
(4) The minimum bacteriocidal/fungicidal concentration and the heamolytic assay of both the pulp and seed of the extract.

**Source of materials**

The monkey cola (*Cola millenii*) was collected freshly in the wild, at Giri village, Abuja from its tree and was further authenticated by professor Olorode of biological Science, University of Abuja before transferred to laboratory for analysis.

**Test microorganisms**

Clinically isolated bacteria (*Escherichia coli, Klebsiella spp, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Aspergillus niger and Aspergillus flavus)* from National Hospital Abuja were cultured at 37 °C on nutrient medium in aerobic conditions for 24 hours.

**Phytochemical extraction**

**Drying**

For extraction, the freshly collected seed and pulp of *Cola millenii* were thoroughly washed with tap water followed by sterile distilled water. The material was air dried followed by grinding into a fine powder (Lin and Lineback, 1990).

**Preparation of ethanolic/aqueous plant extracts**

An extract is a mixture of phytochemicals from any plant which is obtained by extraction of seed and pulp of *Cola milenii* (Loew, 1997). Ethanol (95%) and aqua solvent were used for the phytochemical extraction of both parts. For extraction with ethanol, 25 g of powdered plant material was dissolved in enough sterilized ethanol to make 100 ml of ethanol extract (25% w/v). The mixture was kept undisturbed at room temperature for 24 hrs in a sterile flask covered with aluminium foil to avoid evaporation and subjected to filtration through sterilized Whatman no.1 filter paper. After filtration, the extract was evaporated in water bath until 25 ml extract was left in the container. The same was done for aqua extraction. Ethanolic/aqueous extracts thus obtained were immediately evaluated for antibacterial using agar well diffusion method and antifungal activities (Chen *et al.,* 1987, Barreto *et al.,* 2002).

**Phytochemical Screening**

The Ethanolic extract of the plant was tested for the presence of phytochemicals qualitatively. Secondary metabolites such as phytosterols, polyphenols (tannins, flavonoids), saponins, alkaloids, saponin glycosides, steroids and triterpenoids, glycosides, hydrolysable tannins, phenols and volatile oils were screened for using wet reactions following the procedures described by Sofowora (1993) and Harbone (1998).

**Agar Well Diffusion Method**

The antimicrobial activity of two crude ethanolic extracts of seed and pulp of Cola millenii against some clinical isolated bacteria and mold of food origin was evaluated by using agar well diffusion method (Ahmad and Beg, 2001, Srinivasan *et al.,* 2001). Molten agar plates were inoculated with 1 ml of standardized inoculums ($1.5x10^8$ CFU/ml) of each selected bacterium and fungi (in triplicates) and spread with sterile wire loop. Wells or cups of 7 mm size were made with sterile borer into agar plates containing the bacterial and fungi inoculums. 1 ml volume of the plant extract was poured into a well of inoculated plates. Chemical preservative, (sodium metabisulphate) and antibiotics (gentamycin) were used as a positive control which was introduced into a well instead of plant extract. Solvent, ethanol was used as a negative control which was introduced into a well instead of plant extract. The plates prepared were left at room temperature for ten minutes allowing the diffusion of the extract into the agar (Rios *et al.,* 1988). After incubation for 24 hrs at 37ºC, the plates were observed. If antimicrobial activity was present on the plates, it was indicated by an inhibition zone surrounding the well containing the plant extract. The zone of inhibition was measured and expressed in millimetres (Maneemegalai and Naveen, 2010). The mean and standard deviation of the diameter of inhibition zones were calculated.

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**Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of Pulp and Seed Ethanolic Extracts against some Bacteria/Fungi Isolates**

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation. MIC and MBC/MFC of pulp and seed ethanolic extracts were determined by macro-dilution broth methods (Thongson *et al.,* 2004).

Macro-dilution broth method, a two-fold serial dilution of the seed and pulp ethanolic extracts were prepared in sterile Nutrient broth to achieve a decreasing concentration ranging from 160 to 1.25mg/ml in eight sterile tubes labelled 1 to 8. Each dilution was seeded with 1ml of the standardized bacterial inoculums (1.5 X $10^8$CFU/ml). The inoculated culture tubes were incubated at 37°C for 24 hrs. A set of tubes containing only seeded broth (i.e. without plant extract) was kept as control. The lower concentration that did not permit any visible growth when compared with the control was considered as the MIC. The turbidity was monitored by spectrophotometer.

The minimum bactericidal/fungicidal concentration (MBC/MFC) is the lowest concentration of antimicrobial agent that will prevent the growth of an organism after subculture on to antibiotic-free media. To determine the MBC, a 1ml aliquot from the tube showing MIC was placed on NA plate antibiotic free and was spread over the plate. After incubation at $37^0$C for 24 hours, the plates were examined for the growth of a bacterium to determine the concentration of the extract at which 99.9% killing of bacterial isolates was achieved. (Irkin and Korukluoglu, 2007).

## TEST FOR HEAMOLYSIS

20 ml of sterile blood agar was poured aseptically into a sterile petri dish at cooling temperature of about $45^0$C and allowed to solidify. 5mm cork borer was used to bore a well in the solidify blood agar into which different concentration of the extracts was applied. The plates were inoculated at $37^0$C for 24 hours and the results were monitored (Reid *et al* 2005).

**Statistical Analysis**

The mean and the standard deviation of each treatment was calculated and analysis of variance was used to test for the significance of the result.

## DISCUSSION

Phytochemical screening of the extract revealed presence of Saponins, Carbohydrate, Resins, Alkaloids, Steroids, Tannin and Terpenes (Table 1). Various studies have shown that plants that are rich in alkaloids and tannin compounds possess antimicrobial activity against a number of microorganisms (McMahon *et al*., 1995) as well as intestinal infections associated with AIDS (McDevitt *et al*., 1996). While alkaloids have been found to have microbiocidal effects including against *Giardia* and *Entamoeba* species (Ghoshal *et al*., 1996), the major antidiarrheal effect is probably due to their effects on transit time in the small intestine. The presence of terpene explains the odours and flavour exhibited by the plant. The presence of the secondary metabolites (alkaloids, saponins and tannins) in *Cola millenii* both seed and pulp are also confirmed in the work of Mubo *et al.,* (2009) in which they were inferred as criteria in the classification of *Cola* species. This claim could be strengthened with a further evaluation of the active principles responsible for the antimicrobial activities observed in both the seed and pulp of the *Cola* species. Cardiac glycosides is confirmed positive in this present work but was recorded absent in the work of Mubo *et al.,* (2009) on the same species but different plant part (leaves). The difference in the result could be either due to unequal distribution of the chemicals in the plant or due to different environment. This factor has been documented to have effect on the distribution of phytochemicals on plant at different environment (Cybulskill *et al*., 2000).

Antimicrobial activities shown by the pulp and seed of *Cola millenii* are in line with previous antimicrobial works on the species of *Cola* (Reid *et al*., 2005) where *Cola* extracts were found to exhibit important inhibitory activities against the growth of certain bacteria and fungi. In the present investigation, the ethanolic extracts of seed and pulp of *Cola millenii* showed inhibitory activity against all the selected bacteria and mold isolates in which the diameter of zone of inhibition varied between 0.13±0.05$^+$ cm for *Pseudomonas aeruginosa* at 30mg/ml and 0.86±0.11$^+$ cm for *Salmonella typhii* at 100mg/ml (in pulp), and 0.17±0.12$^+$cm at 30mg/ml concentration and 1.73±0.06$^{++}$cm at concentration of 100mg/ml for *Aspergillus niger* (in seed) (Table 2).

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**Table 1 : Phytochemical constituents of crude extract of seed and pulp of *Cola millenii***

| SECONDARY METABOLITES | C. M. SEED | C. M. PULP |
|---|---|---|
| ALKALOID | ++ | + |
| SAPONINS | ++ | + |
| TANNINS | ++ | + |
| FLAVONOID | _ | _ |
| ANTHRAQUINONES | _ | _ |
| TARPENES | ++ | ++ |
| CARDIAC GLYCOSIDES | ++ | + |
| STEROLS | + | + |
| RESINS | + | + |
| ANTHRACYANINDES | _ | _ |
| CARBOHYDRATES | ++ | ++ |
| PHENOL | _ | _ |

**+ Present**
**- Absent**

**Table 2 : Antimicrobial activity of ethanolic extracts of *Cola millenii* seed and pulp on selected test isolates represented by zone of inhibition in centimetre at different concentration in (mg/ml) expressed in mean ± standard deviation.**

| Extract (mg/ml) *C. millenii* pulp | *Staph. aureus* | *Klebsiella Spp* | *E. coli* | *P. aeruginosa* | *Salmonella typhii* | *A. niger* | *A. flavus* |
|---|---|---|---|---|---|---|---|
| 100 | -- | -- | $0.56\pm0.12^{+}$ | $0.83\pm0.12^{+}$ | $0.86\pm0.11^{+}$ | $0.67\pm0.06^{+}$ | $0.77\pm0.12^{+}$ |
| 60 | -- | -- | $0.47\pm0.11^{+}$ | $0.53\pm0.32^{+}$ | $0.7\pm0.6^{+}$ | $0.2\pm0.1^{+}$ | $0.53\pm0.15^{+}$ |
| 30 | -- | -- | -- | $0.13\pm0.05^{+}$ | $0.26\pm0.6^{+}$ | -- | -- |
| 10 | -- | -- | -- | -- | -- | -- | -- |
| Extract (mg/ml) *C. millenii* seed | | | | | | | |
| 100 | $0.63\pm0.58^{++}$ | $0.43\pm0.12^{+}$ | $1.67\pm0.06^{+}$ | $1.6\pm0.06^{++}$ | $0.7\pm0.1^{++}$ | $1.73\pm0.06^{++}$ | $1.63\pm0.12^{++}$ |
| 60 | $0.33\pm0.06^{+}$ | $0.17\pm0.06^{+}$ | $1.07\pm0.12^{+}$ | $1.1\pm0.1^{++}$ | $0.57\pm0.12^{+}$ | $1.13\pm0.06^{+}$ | $1.07\pm0.06^{++}$ |
| 30 | -- | -- | $0.67\pm0.06^{+}$ | $0.46\pm0.06^{+}$ | $0.33\pm0.06^{+}$ | $0.17\pm0.12^{+}$ | -- |
| 10 | -- | -- | -- | -- | -- | -- | -- |
| Gentamycin | $2.0^{+++}$ | $2.4^{+++}$ | $1.8^{+++}$ | $1.4^{+++}$ | $2.0^{+++}$ | NA | NA |
| Ketoconazole | NA | NA | NA | NA | NA | $1.1^{+++}$ | $1.7^{+++}$ |
| Sodium metabisulphate | $0.6^{+}$ | $1.0^{++}$ | $0.7^{++}$ | $1.2^{+++}$ | $0.4^{+}$ | $0.7^{++}$ | $0.4^{+}$ |

The ethanolic extract from the seed showed higher effect in diameter of zone of inhibition than the pulp ethanolic extract. It has a strong effect against *Aspergillus niger* followed by *Escherichia coli* and *Aspergillus flavus*. These results are comparable to both the commercially available broad spectrum antibiotics (Gentamycin) and chemical preservatives (Sodium metabisulphite). The pulp and seed aqueous extract showed weaker effect even at its highest diameter of zone of inhibition $0.33\pm0.06^{+}$cm in diameter against *Salmonella typhii* followed by *Pseudomonas aeruginosa* with $0.93\pm0.06^{+}$cm.

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The minimum inhibitory activity was recorded against the seven selected organisms. The seed also showed a lower MIC against this selected isolates better than the pulp showing that they are more effective. According to the work of Mubo *et al.* (2009), *C. millenii* leaf ethanolic extract had weak inhibitory effects on the growth of all this selected microorganisms. The present study contradicts this assertion; this is probably due to the strong presence of alkaloids as reported by Adegoke *et al*. (1968) and other antimicrobial effective phytochemicals like tannin and saponin.

Our result suggests that water is less effective solvent for extracting the bioactive compounds from the seed and pulp of *Cola millenii*. However, traditional healers and herbalists reported water to be the most commonly used solvent to extract biologically active compounds due to its easy availability (Shale *et al*., 1999). Our findings of greater antimicrobial activity by the ethanolic extract of both the seed and pulp of *Cola millenii* contradict this assertion. The antibacterial activity of the crude plant extracts (Table 2 & 3) on the test organisms justifies the active principle or ingredient observed in herbal physician in their preference for the local gin "ogogoro" as extract in the preparation of crude drugs from medicinal plant materials. Local gin obtained from fermented palm wine distillation is known to contain a high concentration of alcohol.

**Table 3 : Antimicrobial activity of aqueous extracts of *Cola millenii* seed and pulp on selected test isolates represented by zone of inhibition in centimetre at different concentration in (mg/ml) express in mean ± standard deviation.**

| **Extract (mg/ml) *C. millenii* pulp** | ***Staph. aureus*** | ***Klebsiella Spp*** | ***E. coli*** | ***P. aeruginosa*** | ***Salmonella typhii*** | ***A. niger*** | ***A. flavus*** |
|---|---|---|---|---|---|---|---|
| 100 | -- | -- | $0.5\pm0.12^{+}$ | $0.93\pm0.06^{+}$ | $0.83\pm0.06^{+}$ | $0.33\pm0.06^{+}$ | $0.47\pm0.06^{+}$ |
| 60 | -- | -- | -- | -- | $0.33\pm0.06^{+}$ | -- | -- |
| 30 | -- | -- | -- | -- | -- | -- | -- |
| 10 | -- | -- | -- | -- | -- | -- | -- |
| Extract (mg/ml) *C. millenii* seed | | | | | | | |
| 100 | $1.13\pm0.12^{+}$ | $1.03\pm0.16^{+}$ | $0.73\pm0.12^{+}$ | $1.0\pm0.17^{++}$ | $0.87\pm0.12^{+}$ | $1.13\pm0.06^{+}$ | $1.03\pm0.06^{++}$ |
| 60 | $0.9\pm0.1^{+}$ | $0.67\pm0.12^{+}$ | $0.23\pm0.06^{+}$ | $0.37\pm0.06^{++}$ | $0.63\pm0.12^{+}$ | $0.26\pm0.15^{+}$ | $0.33\pm0.06^{+}$ |
| 30 | $0.37\pm0.06^{+}$ | -- | -- | -- | $0.37\pm0.06^{+}$ | -- | -- |
| 10 | -- | -- | -- | -- | -- | -- | -- |
| Gentamycin | $2.0^{+++}$ | $2.4^{+++}$ | $1.8^{+++}$ | $1.4^{+++}$ | $2.0^{+++}$ | NA | NA |
| Ketoconazole | NA | NA | NA | NA | NA | $1.1^{+++}$ | $1.7^{+++}$ |
| Sodium metabisulphate | $0.6^{+}$ | $1.0^{++}$ | $0.7^{++}$ | $1.2^{+++}$ | $0.4^{+}$ | $0.7^{++}$ | $0.4^{+}$ |

Key:
+ Weak effective
++ Moderate effective
+++ Strong effective
-- No growth
NA Not Applicable

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**Table 4 : Minimum inhibitory concentration (MIC) of pulp of *Cola millenii* ethanolic extract against Clinical isolates (Bacteria) and food associated mold on Nutrient agar medium using macro-dilution broth method.**

| Clinical isolated bacteria and Food-borne isolated fungi | Concentration of pulp of *Cola millenii* ethanolic extract (mg/ml) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1.25 | 2.5 | 5.0 | 10.0 | 20.0 | 40.0 | 80.0 | 160.0 | MIC | MBC/ MFC |
| *Escherichia coli* | + | + | + | + | + | + | + | - | 160.0 | ND |
| *Salmonella typhii* | + | + | + | + | + | + | - | - | 80.0 | 160.0 |
| *Pseudomonas aeruginosa* | + | + | + | + | + | + | + | - | 160.0 | ND |
| *Klebsiella pneumoniae* | + | + | + | + | + | + | + | - | 160.0 | ND |
| *Staphylococcus aureus* | + | + | + | + | + | + | + | + | ND | ND |
| *Aspergillus niger* | + | + | + | + | + | + | - | - | 80.0 | 160.0 |
| *Aspergillus flavus* | + | + | + | + | + | + | - | - | 80.0 | 160.0 |

**+ Growth; - No growth; ND Not Detected**

**Table 5 : Minimum inhibitory concentration (MIC) of seed of *Cola millenii* ethanolic extract against clinical isolated bacteria and food associated mold on Nutrient agar using macro-dilution broth method**.

| Clinical isolated bacteria and Food-borne isolated fungi | Concentration of seed of *Cola millenii* ethanolic extract (mg/ml) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1.25 | 2.5 | 5.0 | 10.0 | 20.0 | 40.0 | 80.0 | 160.0 | MIC | MBC/ MFC |
| *Escherichia coli* | + | + | + | + | + | - | - | - | 40.0 | 80.0 |
| *Salmonella typhii* | + | + | + | + | + | - | - | - | 40.0 | 160.0 |
| *Pseudomonas aeruginosa* | + | + | + | + | + | - | - | - | 40.0 | 80.0 |
| *Klebsiella Spp* | + | + | + | + | + | + | + | - | 160.0 | ND |
| *Staphylococcus aureus* | + | + | + | + | + | + | + | - | 160.0 | ND |
| *Aspergillus niger* | + | + | + | + | + | - | - | - | 40.0 | 160.0 |
| *Aspergillus flavus* | + | + | + | + | + | - | - | - | 40.0 | 160.0 |

**+ Growth; - No growth; ND Not Detected**

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When these solvents are used as herbal extracts, it may be possible that bioactive substances that are less soluble in water could dissolve in organic solvent (Oyagade *et al*., 1999). The cold extract of all these plant did not exert antibacterial effect on the organisms, due to the failure of the active ingredient to dissolve in it and all the sensitive extracts were more at higher concentrations than lower concentration. Failure of some of the extract to exert antibacterial effect on the test organisms is not enough to conclude that the leaf does not contain substances that can exert antibacterial activity against the test organism because the potency of extract depends on the method used to obtain the extract. Research has shown that the age of plant when harvested and the season of plant determine the amount of the active constituents and since the active ingredients of plants can vary in quality and quantity from season to season (Sofowora, 1982). The haemolytic assay carried out showed that the plant ethanolic extract have no significant haemolytic effect on the blood agar (Table 6).

**Table 6 : The haemolytic effect of the ethanolic extract of both the seed and pulp of *Cola millenii* on blood agar in centimetre**

| Extract (mg/ml) *C. millenii* pulp | Zone of inhibition on Blood agar in centimetre |
|---|---|
| 100 | 0.0 |
| 90 | 0.0 |
| 60 | 0.0 |
| 10 | 0.0 |
| Extract (mg/ml) *C. millenii* seed | |
| 100 | 0.0 |
| 90 | 0.0 |
| 60 | 0.0 |
| 10 | 0.0 |
| Gentamycin positive control | 0.0 |
| Sodium metabisulphite | 0.0 |

## CONCLUSION

In conclusion, this study was designed to analyze and compare the phytochemical constituent and antibacterial properties of seed and pulp of *Cola millenii* to confirm their ethno pharmaceutical claims. Therapeutically, the seed and pulp of *Cola millenii* has been found to possess the most active agent against these selected clinical and food borne pathogens which suggest good potentials for use as antimicrobial and medicinal plants. The ethanol crude extract of *Cola millenii* pulp and seed had the most important effect against both bacteria and fungi as they showed significant different $p<0.05$ using analysis of variance. Therefore, the plants seeds are justified in their ethnomedicinal uses in the treatment of certain diseases which may be caused by the various selected microorganisms (*Staphylococcus aureus*, *Klebsiella pnemoniae, E. coli, Salmonella typhii, Aspergillus niger nd Aspergillus flavus*), especially fungal diseases and as preservatives against food poisoning and food borne infection pathogens.

## RECOMMENDATIONS

Several plants are currently being investigated to know their antimicrobial and medicinal properties. The present study reveals that seed and pulp of *Cola millenii* have great potentials as antimicrobial and as medicinal plants due to the presence of Saponins, Tannins, Resins, Alkaloids, Steroids and Terpenes and their ability to inhibit the growth of all the selected organisms. I, therefore, recommend that:

1) Further studies on the *in vivo* activity, isolation and structural elucidation of the active component(s) and toxicological studies of the plant extract are recommended.
2) The potentially useful phytochemical structures present in these plants be synthesized chemically and used as antibiotics/chemical preservatives.

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