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Phytochemical and Antimicrobial Screening of *Cola gigantea* Leaves, Stem and Bark

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Abstract Phytochemical and antimicrobial analysis were carried out on the leaves, stem and bark extracts of *Cola gigantea*. The Phytochemicals present in the plant were Flavonoid, Tannins, Alkaloid, Cardiac glycoside, Steroids, Terpenoids and phenol. Flavonoid, Alkaloid and steroids were present in high amount in the plant than the other phytochemicals. For antimicrobial screening, the well-in-agar diffusion method was employed and the various zones of inhibition produced by the extract of the various plant parts on *Escherichia coli, Bacillus subtilis, Staphylococcus aureus and Candida albicans* were observed and recorded in millimeters (mm). The leaves of the plant had highest antimicrobial activities on *Escherichia coli, Bacillus subtilis and Staphylococcus aureus,* while the stem had highest antimicrobial activity on *Candida albicans*. The observed antimicrobial activity of the extract suggests its potential use in the treatment of diseases produced by the various test pathogenic microorganisms.

Keywords Phytochemistry, Antimicrobial Screening, *Cola gigantea*, Leaf, Stem, Bark

1. Introduction

The use of traditional remedies in West-Africa in the treatment of diseases has witnessed a flourishing patronage. This has been influenced both by the development of resistance by pathogenic microorganisms [Tenover, 2006] and by the ready availability of potential plants in the West-African forests. Plants contain a plethora of bioactive substances that could be antispasmodic, emetic, anti-cancer and antimicrobial in nature [Frank and Kingsley, 2014]; [Okigbo et al., 2015]. A large number of the plants are claimed to possess the antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide. It is now believed that nature has given the cure of every disease in one way or another [Tiwari et al., 2011]. Seeking a better and more cost effective means in the treatment and management of diseases, man has continually utilized the resources in his immediate environment.

Cola gigantea belongs to *Sterculiaceae* family and grows as a large forest tree. It is found both in the relatively dry and wet parts of the rain forest. It is an ever green moderately sized tree often growing to a height of 20-25metres with an erect trunk of about 1.5metres of diameter and possessing a vertically fissured grey or brown bark. It is erect, non-cylindrical and bearing a dense spreading crown with glossy ovoid leaves up to 20cm [Irvine, 1961]. This specie is native to Western Tropical Africa where it is predominantly found in the Savannahs and semi-deciduous forests.

Cola gigantea is one of the plants utilized in traditional healing remedies [Sonibare et al., 2009].The characteristic bitter taste of its fruit makes it a favorite amongst the elderly as it is widely used in social and cultural events to welcome guests and say traditional prayers. It is also known to reduce hunger pangs. Several other parts of the plant such as the leaves, bark and fruits are utilized in traditional healing remedies. The fruits when ingested acts as a stimulant, creating an ecstatic and euphoric state [Benjamin et al., 1991]. It is also used traditionally to treat whooping cough, malaria, depression, anxiety and sea-sickness [Odugbem, 2006], [Muthu et al., 2006]. A study by [Christian et al., 2012] shows that the ethanolic extracts of the stem bark and leaves of *cola gigantea* exhibit anti-inflammatory properties in carrageenan induced arthritis in seven-day old chicks and anti-microbial properties against strains of *E.coli, P. aeruginosa, S. aureus* and *B. subtilis.*

This work was aimed at determining the phytochemicals present in the leaf, stem and bark of cola gigantean and the anti-microbial properties of various extracts of the plant parts on the micro-organisms *Candida albicans, Bacillus subtilis, Staphylococcus aureus,* and *Escherichia coli.*

2. Materials and Methods

Plant Collection and Identification

Cola gigantea leaves, stem and bark were collected from an uncultivated farmland located at Agwa in Oguta Local Government Area of Imo State. The plant was identified at the Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

Sample Preparation

The leaves, stem and bark of the plant were washed with tap water to remove debris and air dried for two weeks. Grinding of the plant parts was carried out using a manual grinder and then an electric blender to obtain a fine powder.

Preparation of Aqueous Extracts: To 50g each of the blended plant parts, 1000ml of distilled water was added. The samples were kept for 72hours at room temperature with stirring at regular intervals. After the 72 hour incubation, the samples were filtered using Whatmann No. 1 filter paper and the filtrate was kept for analysis. (Tasmin et al., 2015).

Preparation of Ethanol and Hexane Extracts: 2kg each of the blended plant parts were dissolved in absolute ethanol (98%) and another 2kg each of the blended parts were dissolved in hexane (96%). They were incubated for 72 hours and filtered at the end of the incubation period using Whatmann No. 1 filter paper. The filtrate was kept for analysis. (Sayed et al., 2012).

Phytochemical Screening Tests

Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

3. Qualitative Analysis

Test for Flavonoids

5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

Test for Tannins

About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for Saponin

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion. (Harborne 1973).

Test for Alkaloids

To the hexane extract of the plant sample 5ml of 2% HCl was added. The mixture was heated, filtered and a few drops of picric acid were poured into it. Formation of yellow colour precipitate indicates the presence of alkaloids.

Test for Terpenoids

5ml of plant extract is mixed in 2ml of chloroform and 3ml of concentrated H_2SO_4 is carefully added to form a layer. Reddish brown interface formed shows presence of terpenoids.

Test for Steroids

2ml of acetic anhydride is added to 0.5g of ethanolic extract of each sample with 2ml of H_2SO_4.Colour change from violet to blue or green indicates presence of steroids.

Test for cardiac glycosides (Keller-Killani test): Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

4. Quantitative Analysis

Alkaloid determination using Harborne (1973) method: 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a waterbath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

Flavonoid determination by the method of Bohm and Kocipai - Abyazan (1994): 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

Cardiac Glycoside Determination

To 1ml of the water extract of each sample was added 1ml of 2% solution of 3, 5-DNS (Di-nitro Salicylic acid) in

methanol and 1 ml of 5% aqueous NaOH. It was boiled for 2mins (until brick-red precipitate was observed) and the boiled sample was filtered. The weight of the filter paper was taken before filtration. The filter paper with the absorbed residue was dried in an oven at 50 ^{0}C till dryness and weighed.

Tannin Determination

20g of powdered sample was put in a conical flask and 100ml of petroleum ether was added and covered for 24hours. The sample was then filtered and allowed to stand for 15 minutes allowing petroleum ether to evaporate. It was then re-extracted by soaking in 100ml of 10% acetic acid in ethanol for 4 hours. The sample was then filtered and the filtrate collected. 25ml of NH_4OH was added to the filtrate to precipitate the alkaloids. The alkaloids were heated with electric plate to remove some of the NH_4OH still in solution. The remaining volume was measured to be 33ml. [Pearson, 1974].

5ml of this was taken and 20ml of ethanol was added to it. It was titrated with 0.1MNaOH using phenolphthalein as indicator until a pink end point is reached.

Determination of Total Phenols by Spectrophotometric Method

The quantity of phenol is determined using the spectrometer method. The plant sample is boiled with50ml of butan-1-ol for 15minutes. 5ml of the boiled sample is pipetted into a 50ml flask, and 10ml of distilled water is added. After the addition of distilled water, 2ml of NH_4OH solution and 5ml of concentrated propanol is added to the mixture. The sample is made up to the mark and left for 30minutes to react for colour development and measured at 505nm wavelength using spectrometer.

Determination of Steroids

1.0g of powdered sample was weighed and mixed in 100ml of distilled water in a conical flask. The mixture was filtered and the filtrate eluted with 0.1N ammonium hydroxide solution. 2ml of the eluent was put in a test tube and mixed with 2ml of chloroform. 3ml of ice cold acetic anhydride was added to the mixture in test tube.

Determination of Terpenoid Content

1.0g of powdered sample was weighed and mixed in 100ml of distilled water in a conical flask. The mixture was filtered and the filtrate eluted with 0.1N ammonium hydroxide solution. 2ml of the eluent was put in a test tube and mixed with 2ml of chloroform. 3ml of ice cold acetic anhydride was added to the mixture in test tube.

Test Organisms

A total of three pathogenic bacteria namely: *Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and one fungus, *Candida albicans* were obtained from the Medical Laboratory section of Nnamdi Azikiwe University Teaching Hospital, Anambra State, Nigeria.

Antimicrobial Susceptibility Test

Agar well diffusion method was used to assay the effect of the extract on the various microorganisms. Mueller-Hinton agar was used for the bacteria and Sabouraud's Dextrose Agar (SDA) for the fungus. Twenty four hour broth cultures of the test organisms were serially diluted, then 10^{-3} and 10^{-4} dilution of each microorganism was uniformly spread using a sterile glass spreader on the surface of the media, and two wells were borne on each petri dish. Thereafter, 0.1 ml of n-hexane and water extracts of each sample was added in the well on the petri dish. Incubation was done at 37 ^{o}C for 24 hours and clear zones of inhibition around the wells indicated antimicrobial activities of the extracts against the test organisms. The diameter of the zone of inhibitions were measured and recorded in millimeter. All experiments were done in duplicates.

5. Results of the Phytochemical Screening

Qualitative Analysis

Leaves

Table 1. Result of qualitative phytochemical screening on the leaves of *Cola gigantea.*

TEST	OBSERVATION	INFERENCE
Alkaloid	Precipitation present	+
Saponins	Froth absent	-
Flavonoid	Yellow colouration present	++
Tannins	Brownish green colouration	+
Steroids	Colour change from violet to blue	+
Terpenoids	Presence of Reddish–brown layer.	+

Stem

Table 2. Result of qualitative phytochemical screening on the stem of *Cola gigantea.*

TEST	OBSERVATION	INFERENCE
Alkaloid	Precipitation present	+
Saponins	No froth formed	-
Flavonoid	Yellow colouration present	++
Tannins	No colouration	-
Steroids	Colour change from violet to green	+
Terpenoids	Absence of Reddish-brown layer.	-

Bark

Table 3. Result of qualitative phytochemical screening on the Bark of *Cola gigantea*.

TEST	OBSERVATION	INFERENCE
Alkaloid	Precipitation present	+
Saponins	Froth present	++
Flavonoid	Yellow colouration present	++
Tannins	No colouration	-
Steroids	Colour change from violet to green	+
Terpenoids	Absence of Reddish-brown layer.	-

Key

S/No	Symbol	Meaning
1.	+	Indicated
2.	++	Strongly Indicated
3.	-	Not Indicated

Quantitative Analysis

Leaves

Table 4. Results of quantitative phytochemical screening of *Cola gigantea*.

TEST	QUANTITY g/100g
Alkaloid	5.8
Flavonoid	12.6
Tannins	9.08
Steroids	17.26
Terpenoids	5.209
Phenol	27.98
Cardiac glycoside	2.2

Stem

Table 5. Result of quantitative phytochemical screening on the stem of *Cola gigantea.*

TEST	QUANTITY g/100g
Alkaloid	7.3
Flavonoid	10.4
Tannins	0.15
Steroids	8.14
Terpenoids	1.01
Phenol	12.21
Cardiac glycoside	1.4

Bark

Table 6. Result of qualitative phytochemical screening on the bark of *Cola gigantea*.

TEST	QUANTITY g/100g
Alkaloid	3
Flavonoid	5.4
Tannins	0.106
Steroids	6.34
Terpenoids	1.02
Phenol	10.88
Cardiac glycoside	2.3

Table 7. Mean Viable Microbial Count (CFU/ml)

Microorganisms	Mean Total microbial count cfu/ml
Bacillus subtils	2.54×10^7
Staphylococcus aureus	2.66×10^7
Escherichia coli	2.48×10^7
Candida albicans	2.60×10^8

Table 8. Microorganism's Mean Zone of Inhibition

	Zones of inhibition in mm (mean ± SD)		
Microorganisms	***Cola gigantea* leaves**	***Cola giganteas*tem**	***Cola gigantea* bark**
Bacillus subtils	18.00 ± 1.67	4.50 ± 1.45	4.50 ± 1.45
Staphylococcus aureus	23.50 ± 1.45	20.50 ± 0.84	23.00 ± 1.18
Escherichia coli	18.00 ± 2.05	14.00 ± 1.18	11.00 ± 1.18
Candida albicans	10.00 ± 1.67	11.00 ± 1.18	8.50 ± 0.83

6. Discussion

The results of the phytochemical screening of the ethanolic extracts of the leaves, stem and bark of Cola gigantean showed very high levels of phenol in all samples. The leaves recorded the highest phenol levels of 27.98g/100g with lowest levels of phytochemicals observed in cardiac glycosides with a value of 2.2g/100g. Phenol levels of 12.21g/100g and 10.88g/100g were observed for the stem and bark respectively. Both the stem and bark recorded the lowest levels of the phytochemical tannin with values of 0.15g/100g and 0.106g/100g respectively. This is in line with the work by [Christian et al., 2012] which revealed the presence of tannins, saponins, alkaloids and steroids in the leaves and stem bark of *Cola gigantea*.

The plant was tested for antimicrobial activities against a number of selected microorganisms *viz*. *Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Candida albicans*. The aqueous extracts of the plant had no activity on the microorganisms used because water is polar in nature. The n-hexane extracts had some degree of activity on the selected test microorganisms probably because it is a non-polar solvent. This was shown by the various zones of inhibition observed on the agar plates. *Bacillus subtilis*, leaf extract was 18.00±2.82mm the stem extract was 4.50±2.21mm and the bark extract was 4.50±2.12mm. *Escherichia coli*, leaf extract 18.00±4.24mm; stem extract 14.00±1.4mm and bark extract 11.00±1.41mm. *Staphylococcus aureus* leaf extract, 23.50±2.12mm; stem extract 20.50±0.71mm and bark extract 23.00±1.41. *Candida albicans,* leaf extract 10.00±2.82 stem extract 11.00±1.41 and bark extract 8.50±0.70. The zone of inhibition was used to determine the effectiveness of the plant extract in inhibiting the microorganism. The leaf extract showed the highest activity against the test micro organisms, but the the stem and bark extracts also had some some degree of activity. This finding is in consonance with that of [Harbon, 1998], who showed that *Cola gigantea* contains a variety of phytochemicals including alkaloids, flavonoids and tannins. Moreover, [Onyema and Ajiwe, 2014], reported that the results of the phytochemical analysis of *Colagigantea* leaves showed the presence of some secondary metabolites such as Alkaloids, Carbohydrates, Cardiac glycosides, flavonoids, steroids, tannin, terpenoids in various concentrations while cyanogenic glycosides and saponin were conspicuously absent. The values of Mineral elements; Cd (0.40mg/g), As (0.03mg/g), Cr (0.90mg/g), Co (0.43mg/g), Fe (0.04mg/g) etc in the leaves all fell below the WHO recommendations thus showing its overall safety for therapeutic purposes. [Onyema and Ajiwe, 2014 also studied the antimicrobial analyses on eleven bacterial and three fungal species using the Punched Agar diffusion method with two isolated fractions with R_f values of 0.4467 and 0.7067 for leaf fractions and compared with a standard drug cipromax fort (a broad spectrum antibiotic) and recorded average diameter zones of inhibition which ranged between 10mm and 28mm.

The presence of high concentrations of flavonoid and steroids in the leaves confirms its medicinal value. In the stem and leaves, flavonoids, steroids and alkaloids are found in the highest concentration. The major constituent in the plant that shows most of the therapeutic properties is the flavonoids [Onyema and Ajiwe, 2014].

The activity of the n-hexane extracts may be due to the fact that the active substances in the leaf, stem and bark of the *Colagigantea* were non-polar in nature. This report is in contrast with the findings of[Agu et al., 2013], who showed that the ethanolic solutions (polar) of the tangerine seed extracts displayed broad spectrum activity with zones of inhibitions of 20 mm for *Staphylococcus aureus*, 15 mm for *Escherichia coli*. *Bacillus subtilis* showed the lowest sensitivity, this may be as a result of the fact that it is a spore-former, whereas *Staphylococcus aureus* and *Candida albicans* showed a great deal of sensitivity to the extracts.

7. Conclusions

The high amount of phytochemicals present in *Cola gigantea* makes it a suitable active ingredient for drug formulation and use for the treatment of diseases caused by the selected microorganisms used for this work. Since most microorganisms now develop resistance to most of the drugs presently used for treatment of infections they cause, *Cola gigantea* can now serve as a new remedy against them. More research work should be done to determine the active ingredients that possess these antimicrobial activities. The ingredient can then be extracted and used in producing new antimicrobial drugs. The plant should also be tested on other disease causing microorganisms for its potency on them. This would encourage its used in orthodox medicine.

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