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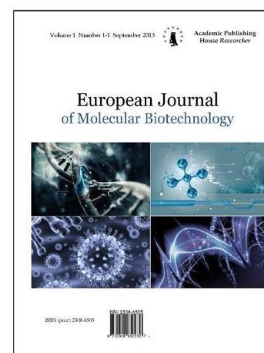

Extraction of Alkaloids from Three Nigerian Plants, Kola Acuminata (OJI IGBO) Vera Kola (OJI Hausa) and Garcinia Kola (BITTER KOLA)

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Abstract

The extraction of alkaloid from Garcinia Kola (bitter kola) Kola Accuminata (Oji Igbo) and Kola Vera (Oji Hausa) were carried out using 10 % enthanoic acid and 10 % methanol to separate the alkaloid from the residue. The alkaloid was extracted using ammonium hydroxide from the sample results obtained on alkaloid for the sample 9.16 % Oji Igbo, 6.20 % Oji Hausa and 8.20 % Bitter Kola. It was observed that the percentage of alkaloids was highest in Oji Igbo and the least for Oji Hausa. The extracts all exhibited antimicrobial activity against he tested organisms. These activities decreasing with a decrease in extract concentrations. The results suggest the usefulness of thee nuts in the treatment or management of microbial infections without the dangers of side effects from synthetic drugs.

Keywords: Extraction, Alkaloids, Kola Acuminata, Garcinia Kola.

1. Introduction

Kola nut is a plant that is grown in the costal rain forest in south western and south eastern part of Nigeria bitter Kola which is known as (Garcinia kola) is an African wonder nut (Wakeel et al., 2004; Leke, 2015). It comes from Garcinia Kola forest which belongs to the family of clusiaceae (Momoh Johnon, 2014; Leke, 2015).

Traditionally these nuts were chewed as a masticatory substance, to stimulate the flow of saliva (D.O. et al., 2014). But are widely consume as snack in West and Central Africa. The kernels of the nuts are widely traded and eaten as a stimulant (D.O. et al., 2014; Omwirhiren et al., 2016).

Medicinal plants are plants that are used to cure different types of disease e.g Garcinia Kola (Bitter kola) (Musa et al., 2011; Eltayeb et al., 2018). It cleans the digestive system without any side effect like abdominal problems alern when a lot of the nuts are eaten (Eleazu et al., 2012; Rajeshwar et al., 2016; Eltayeb et al., 2016).

Medicinal plants have been identified and used throughout human history. The plant exhibits very potent pharmacological activities such as antioxidants, antibacterial, antiviral, anti-fungal and anti-inflammatory properties (Akinpelu, 1999; Leke, 2015). The nuts are commonly used in Nigeria

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as well as other countries for the treatments of common microbial infections (Eleazu et al., 2012; Patil, Gaikwad, 2011; Qadir et al., 2015).

This work is mainly designed to extract alkaloids from kola nuts commonly used in Nigeria (Garcinia Kola)

2. Material and methods

Sample collection and preparation

Sample collection

Garcinia kola (bitter kola) and vera kola (Oji Hausa) were bought at wuntin-dada, market while Kola Accuminata (Oji Igbo) was bought at central market Bauchi in Bauchi State, Nigeria.

Sample preparation

The sample was peeled and sliced into very small sizes and dried at a room temperature for 5days to constant weight. It was then grinded into fine powder and stored in an air tight plastic rubber container ready for extraction

Extraction method

Methanol extraction:

10g of each of the sample was poured into a conical flask and 100 $cm^3$of methanol into the sample and covered and vigorously shake. It was then kept for 72 hours and filtered and ready for the analysis.

Extraction of Alkaloid

Ethanol, ammonium hydroxide and acetic acid were used. 5 g of the sample was weighed into a 250ml beaker and 100ml of 10 % acetic in ethanol was added and covered. This was vigorously shaken, venting the mounted pressure and allowed to partition into organic and aqueous layers for 4hours. This was filtered and the extract was concentrated on a water bath to one quarter of the volume.

Concentrated ammonium hydroxide was added drop wise to the extract until the precipitate was complete. The ammonium (NH_3) was added to neutralize the acid (to make alkaline), the whole solution was allowed to settle and the precipitated was collected, and washed with dilute (NH_4OH) ammonium hydroxide and the filtered residue was the alkaloid which was dried and weighed. The organic layer was tested for alkaloid with mayers and wagner's reagents respectively to ensure full extraction.

Phytochemical analysis

Test for tannins

In this case 1 g of the sample powder was boiled with 5.0ml of water, filtered and used for the test.

Test for saponins

20 ml of distilled water was added to 0.25 g of the sample powder in a test tube and boiled gently in a hot water bath for 20 minutes. The mixture was filtered hot and allowed to cool. The filtrate was used for the following tests.

1. **Frothing test:** – 5 ml of the filtrate was diluted with 20ml of distilled water and vigorously shaken and left to stand, stable from was observed in the filtrated which indicate the presence of saponins.
2. **Emulsion test:** – 2dropolive oil was added to the following solution and the content shaken vigorously. An emulsion was formed from the frothing solution which showed the presence of saponins
3. **Fehling's test:** – 5 ml of Fehling's solution A and B was added to 5 ml of the filtrate and the content was heated in a water bath. A reddish precipitate which turned brick red on further heating with added sulphuric acid which indicate the presence of saponins.

Anti-microbial activities

The agar-well diffusion method was used to determine the anti-microbial activity of the extracts. The bacterial isolate was first grown in nutrient broth for 18hrs. 0.2ml of the log phase culture was aseptically used to seed a molten nutrient agar which had been cooled to about 45°C,

mixed gently and poured into steric petri dishes and allowed to set. Extract was tested at 50mg/ml concentration this was delivered into walls (5mm in diameter) bored into surface of the already seeded nutrient agar plates.

Standard antibiotic concentration of ciprofloxacin 25mg/l, amoxicillin 25mg/l and fluconazole 5mg/l was assayed using the agar well diffusion techniques. The fungal isolate was treated in a sabouroud dextrose broth before were assayed using sabouroud dextrose agar. The bacterial plates were incubated at 37°C for 24hours, while the fungal plates were incubated at 25°C for 72hours. The zones of inhibition was measured in mm diameter and recorded.

Determination of minimum inhibitory concentration (MICs)

The ager dilution method was also used (Russell and Fur, 1977). The extract was incorporated into molten nutrient ager at concentrations of 40,30,20,15,10,7.5 and 5.0, 2.5mg/ml aseptically, mixed gentle in sterile petri dishes and then allowed to set. The surface of the agar plate was allowed to dry properly before inoculating with appropriate bacterial and fungal culture previously diluted to about 10^{6}cfu/ml. the plate was then incubated at 37°c for up to 72hours after about 30 minutes of inoculation. The lowest concentration preventing visible growth in each determination was taken as the maximum inhibitory concentration. The mean of the three replicate determinations was obtained.

3. Results and discussion

Table 1. Phytochemical analysis of Garcinia kola (Bitter kola)

Metabolite	Methanolic extract
Saponin	+
Tannin	+

Key:
+ = Present
- = Absent

Table 2. Phytochemical analysis of Kola accuminata (Oji Igbo)

Metabolite	Methanolic extract
Saponin	+
Tannin	+

Key:
+ = Present
- = Absent

Table 3. Phytochemical analysis of Kola vera (Oji Hausa)

Metabolite	Methanolic extract
Saponin	+
Tannin	+

Key:
+ = Present
- = Absent

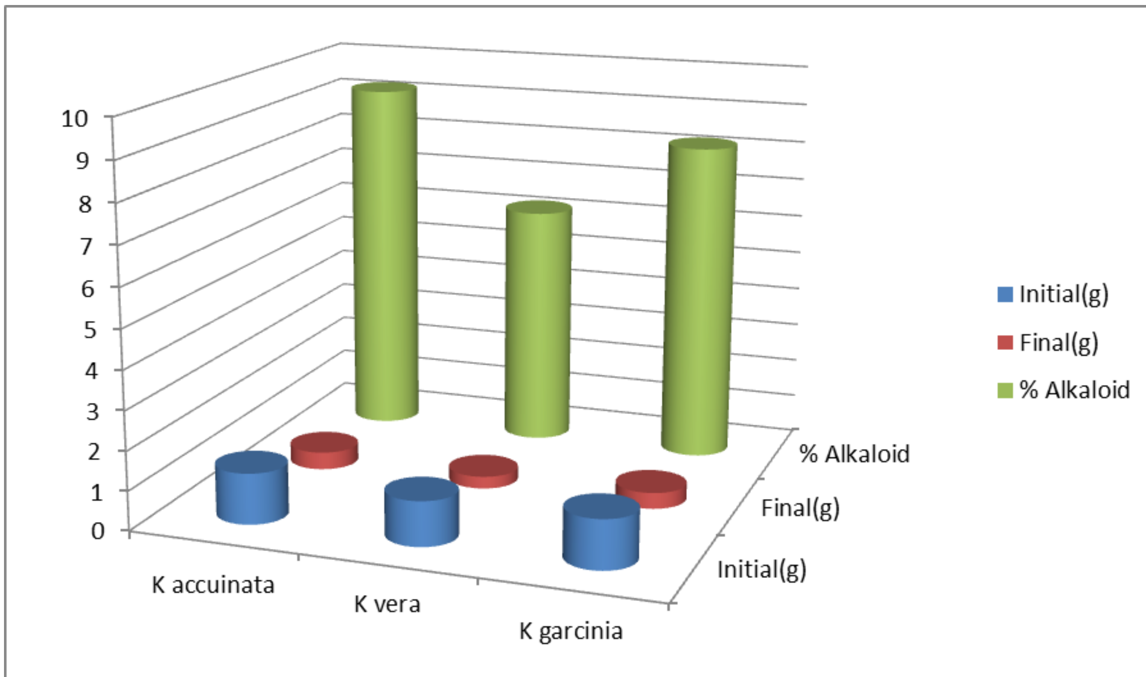

Fig. 1. Percentage Alkaloid in Kola nuts

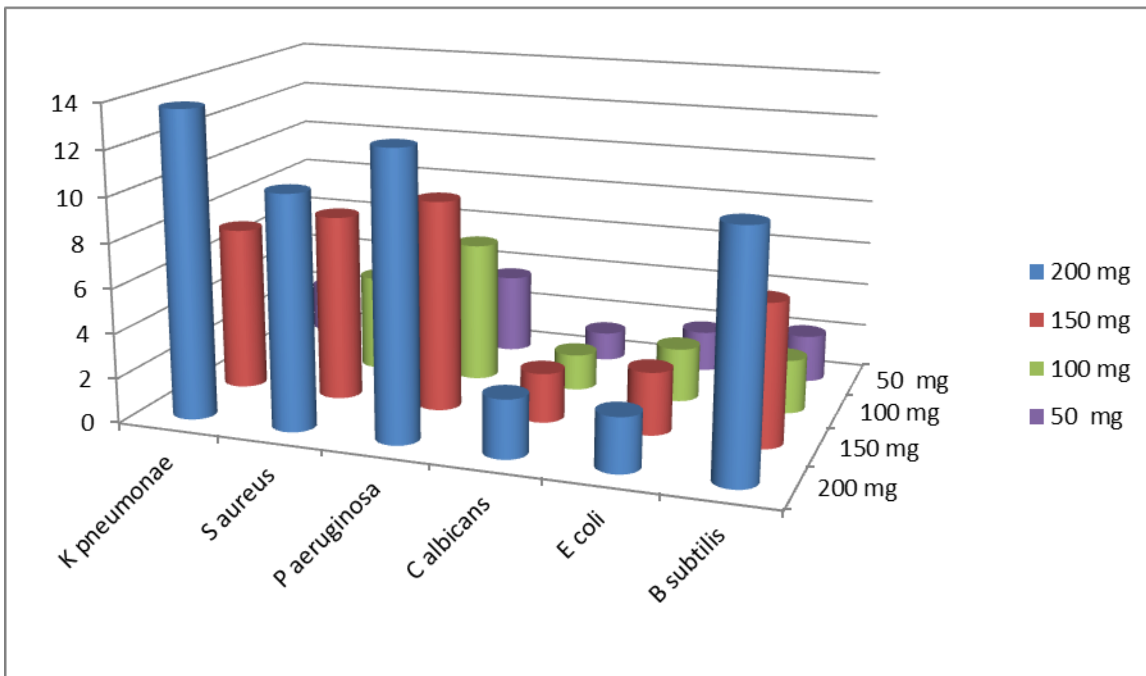

Fig. 2. Zone of inhibition (mm) of Garcinia Kola extract against the test organisms at different concentrations of extract

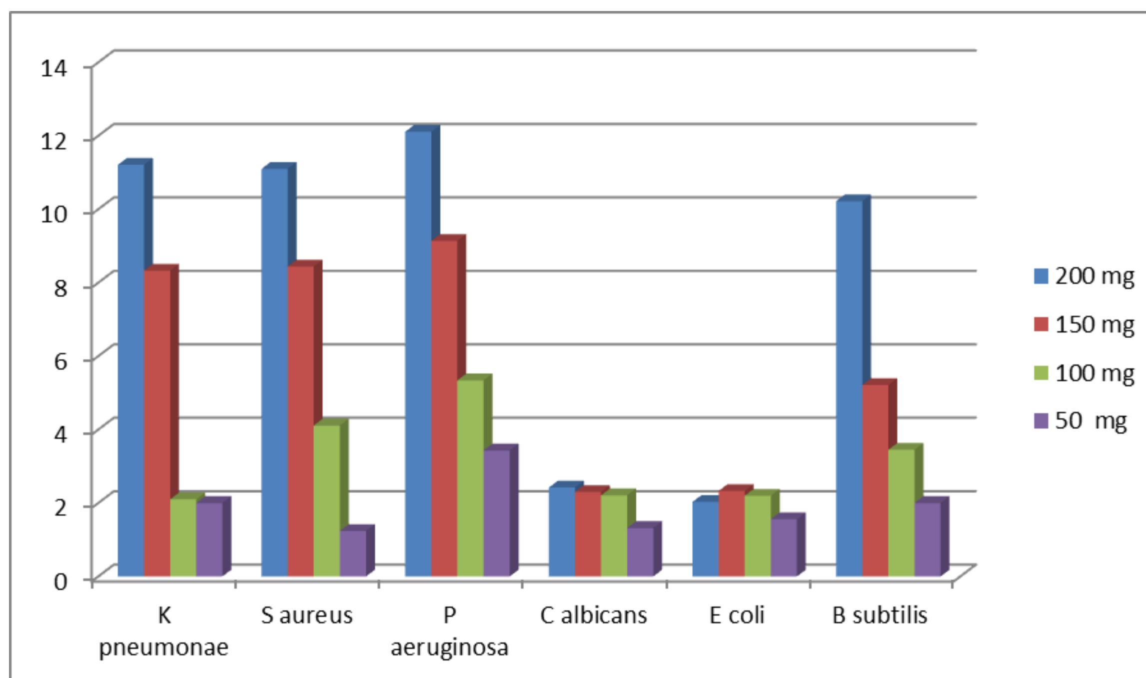

Fig. 3. Zone of inhibition (mm) of Vera Kola extract against the test organisms at different concentrations of extract

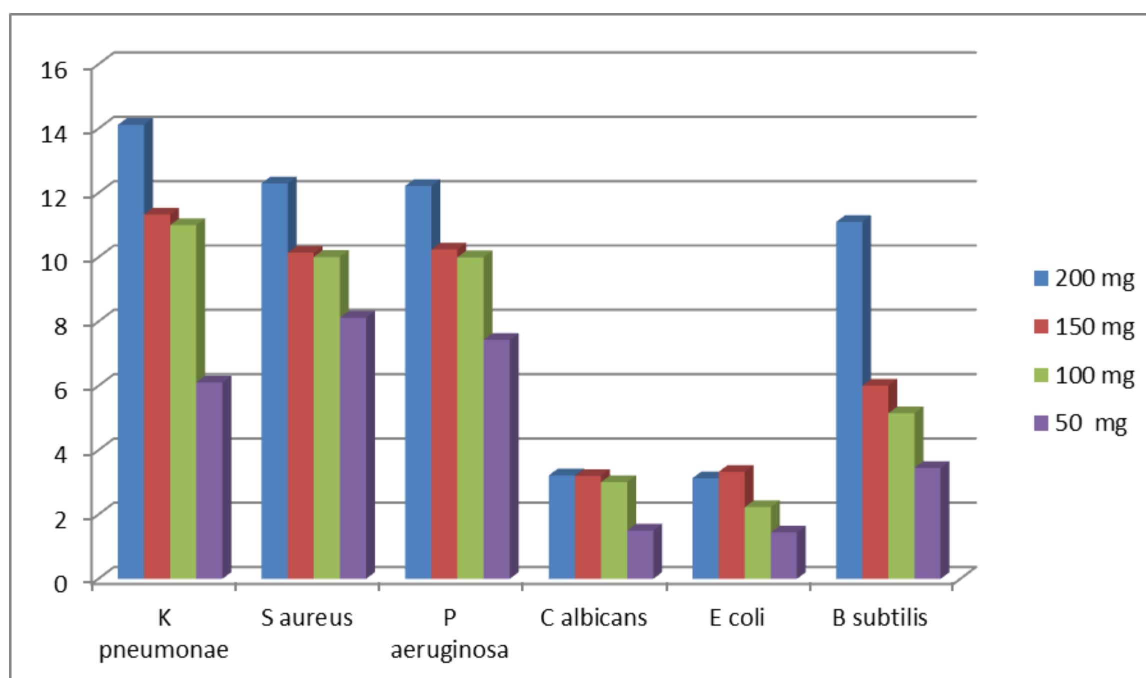

Fig. 4. Zone of inhibition (mm) of Kola acuminate extract against the test organisms at different concentrations of extract

3. Discussion

The phytochemical screening tests on the aqueous extract of Garcinia Kola, kola vera and kola accuminata seed showed that it had the presence of the secondary metabolites tested for (Table 1) which is also reported other researchers (Gumgumjee, Hajar, 2012; Bekele, 2015; Omwirhiren et al., 2016; Rajeshwar et al., 2016). Zones of inhibition of the tested organisms showed that the three tested nuts exhibited a myriad of zone of inhibitions on the tested organisms in response to varying degree of concentrations of the extracts (Fig. 2, 3 and 4). This expected when the concerntrations are altered (Wakeel et al., 2004; Eleazu et al., 2012; Leke, 2015) Diethyl ether

extract of Garcinia Kola, vera Kola and Kola acuminate seed are similarly strongly active against Pseudomonas aeruginosa; Bacillus subtilis and klebsiella pneumonia with similar zones of inhibition (Fig. 2, 3 and 4). The 50mg concentrations showed moderate activity against Klebsiella pneumonia and Bacillus subtilis respectively and poor inhibitory activity against Pseudomonas aeruginosa and Staphylococos aureus respectively.

All three concentrations of the diethyl ether extract of Garcinia Kola seed, Kola vera seed and kola accuminata showed poor inhibitory action against candida albicans in the order, the activity of 100mg>150mg>100mg >50mg with decreasing zones of inhibition respectively (Fig. 2, 3 and 4).

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