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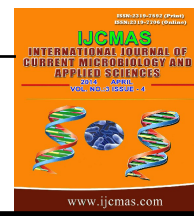


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## Original Research Article

### Preliminary phytochemical and *in vitro* control of selected pathogenic organisms by ethanolic extract of *Garcinia kola* seeds

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## A B S T R A C T

*Garcinia kola* is cultivated for its economic importance. The plant is mainly used as a medicinal herb in West Africa. Although it is bitter the plant is used as a snack and a stimulant due to the high content of caffeine in the seeds of the plant. The current study was done to investigate the antibacterial activity of the plant seeds and to analyse the presence of important pharmaceutical compounds. From the study the plant seeds were found to contain tannins, saponins, flavonoids, terpenoids, glycosides and alkaloids but phenols and steroids were found to be absent in the plant seeds. Among all the organisms tested with the ethanol extract of *G. kola*, only *B. cereus* with an inhibition zone of  $10.17 \pm 0.477$  and *E. coli* with an inhibition zone of  $12.83 \pm 0.833$  were inhibited. All other organisms were not inhibited. The penicillin control showed large zones of inhibition and DMSO did not show any zones of inhibition. The data obtained in this research is a scientific justification of the plants traditional use in the treatment of various stomach problems. From this research it is worthy to recommend the plant seeds for the treatment of diarrhea caused by *E.coli* and all the ailments caused by *B. cereus*, however further research needs to be done to isolate the pharmaceutical compounds, investigate their mode of action and the effect of the same in the *in vivo* environment.

## Keywords

Antibacterial activity;  
Phytochemical;  
Kola;  
Medicinal plants

## Introduction

Traditionally plants are used as substitute drugs for various ailments affecting humankind. The information on medicinal value of plants conventionally was passed from generation to generation. This passing of information some how has led

to preservation of the knowledge, however the trend is changing with many communities abandoning their cultural practices, this therefore creates the need for the documentation of the information on traditional medicine in both the

traditional way and also provide scientific rationalization in order to increase the confidence on the use of plants as alternative means of treatment. Since time immemorial plants have been used as novel source and reservoir of chemical agents with great restorative activities (Gobalakrishnan et al., 2013, Mensah et al., 2009, Sindigawad, 2010). According to Panda et al., (2012) nature is a paradise which offers medicinal principles to humanity through plants.

Plants consist of a wide spectrum of compounds which human beings can use in dealing with the ailments which affect them. When God created man, He put him in the Garden of Eden and then He planted all kinds of beautiful trees. This marked the beginning of the human kind to use plants as food. The creator had a purpose for providing human beings with plants originally. Christian believe that it is sin which brought sicknesses upon the world and also hold to the believe that it is the continued disobedience to the creator which continues to increase the ailments affecting humans and animals today. After man disobedient, God did not provide alternative source of food to man and this could impress that the first provision God had given to the human kind was enough to sustain man.

Nature provides us with great source of cheap and safe alternative drugs. According Ellen G. White (1897), the Creator of the universe has given some simple herbs of the field that at times are beneficial and if every society was educated on the use of these medicinal herbs in case of sickness, such suffering might be prevented, and no doctor would be required. These old-fashioned, simple herbs, used intelligently, would have recovered many sick people, who have died under drug medication.

A number of studies have been done to validate the use of traditional medicine in the treatment against microorganisms causing diseases. Studies have shown drug reaction and the side effects caused by synthetic drugs have increased the risk of malignancy with fake and adulterated drugs increasing the problem of antibiotic resistance which on the other hand has imposed both biological and economic costs (Chabot et al., 1992., Chessin et al., 1995 and Green, 2007). The failure of synthetic drugs to treat against various diseases has led to increased hospitalization and mortality which have been associated with methicillin resistance *Stapylococcus aureus* (MRSA) infections (Arekemase et al., 2012).

Medicinal plants have been tested extensively and found to have great pharmacological uses such as, anti-inflammatory activity, antibacterial activity, anti-diabetic activity, anti-fungal activity, anti cancer activity, antioxidant activity, hepatoprotective activity, haemolytic activity, larvicidal activity, anthelmintic activity, pain relief activity, central nervous system activity, sexual impotence and erectile dysfunction and hypolipidemic activity (Hosahally, et al, 2012, Anthoney et al., 2013., Farook et al., 2011., Kisangau et al., 2007., Kamatenesi-Mugisha et al., 2005., Adu et al., 2011., Deep et al., 2007., Joshi et al., 2011., Arivoli et al., 2012., Ngule et al., 2014., Anthoney et al., 2014).

*Garcinia kola* tree is cultivated for its economic importance. The plant is used as a medicinal plant in West Africa. Although it is bitter the plant is used as a snack and a stimulant due to the high content of caffeine in the seeds of the plant. The plant is used in folk medicine to treat against gram positive bacteria, Ebola

virus infections, Flu, dysentery and diarrhea (Iwu, 1993). According to Chinyere et al. (2013), the plants bark, seeds and stem are traditionally used in the treatment of throat infections, acute fever and inflammation of the respiratory tract. The leaves are also used to treat against stomach problems and also a remedy for anthelmintic and typhoid (Irrine, 1981., and Gill, 1992). The current study was done to investigate the antibacterial activity of the plant seeds and to analyse the presence of important pharmaceutical compounds.

## **Materials and Methods**

### **Sample collection and preparation**

The herb *Garcinia kola* was bought from Monrovia, Liberia in the West Africa region. The samples were identified by a taxonomist in the University of Eastern Africa, Baraton. The samples were cut in to small pieces and spread to dry at room temperature in the chemistry laboratory for about three weeks. They were then ground into fine powder and put in transparent polythene bags.

### **Extraction procedure**

Using electric analytical beam balance fifty grams of the powdered seeds of the *Garcinia kola* were placed in 500 ml conical flask, ethanol was added until the samples were completely submerged in the solvent. The mixture was then agitated for thorough mixing. The mixture was kept for 24 hours on a shaker for effective extraction of the plant components. The extract was filtered using Butchner funnel; Whatman number 1 filter paper and a vacuum-pressure pump. The filtrate was re-filtered again using the same apparatus. The solvent was evaporated using rotary

vacuum evaporator (R -11) with a water bath at 40°C. The extract was brought to dryness using vacuum and pressure pump at room temperature. The residue was then obtained and used for the experiment.

## **Qualitative phytochemical analysis**

The phytoconstituents analysis of extracts for identification of bioactive chemicals was done using standard procedures (Trease and Evans, 1989., Harbone, 1973 and Sofowara, 1993).

### **1. Tannins**

About 0.5 g of the sample was put in a test tube and 20 ml of distilled water was added and heated to boiling. The mixture was then filtered and 1 % of FeCl<sub>3</sub> was added to the filtrate and observations made. A brown green color or a blue black coloration indicates the presence of tannins.

### **2. Saponins**

The crude solvent extract was mixed with 5 ml of water and vigorously shaken. The formation of stable foam indicates the presence of saponins.

### **3. Flavonoids**

A portion of the aqueous extract was added in a test tube. To this, 5 ml of dilute ammonia and 2 ml of concentrated sulphuric acid were added. The appearance of a yellow color indicated the presence of flavonoids.

### **4. Terpenoids**

The extracts of the plant material was taken in to a clean test tube 2 ml of chloroform was added and vigorously

shaken and then evaporated to dryness. To the residue, 2 ml of concentrated sulphuric acid was added and heated for about 2 minutes. A grey color indicates the presence of terpenoids.

## 5. Glycosides

### Salkowsks' test

The extract of the plant material was mixed with 2 ml of chloroform and 2 ml of concentrated sulphuric acid was carefully added and shaken gently, then the observations were made. A red brown color indicate the presence of steroidal ring (glycone portion of glycoside).

## 6. Alkaloids

The crude extract was mixed with 1% of HCl in a test tube. The test tube was then heated gently and filtered. To the filtrate a few drops of Mayer's and Wagner's reagents were added by the side of the test tube. A resulting precipitate indicates the presence of alkaloids.

## 7. Steroids

### Libermann Burchard reaction

About 2 g of the extract was put in a test tube and 10 ml of chloroform added and filtered. Then 2 ml of the filtrate was mixed with 2 ml of a mixture of acetic acid and concentrated sulphuric acid. Blue green ring indicate the presence of steroids.

## 8. Phenols

The plants extract was put in a test tube and treated with a few drops of 2% of  $\text{FeCl}_3$  blue green or black coloration indicate the presence of phenols.

## Bioassay study

### Preparation of the Bacterial Suspension

The turbidity of each of the bacterial suspension was prepared to match to a 0.5 McFarland standard. The McFarland standard was prepared by dissolving 0.05 g of  $\text{BaCl}_2$  in 50 ml of water to obtain a 1% solution of Barium chloride (w/v). This was mixed with 99.5 ml of 1% sulfuric acid solution. Three – five identical colonies of each bacterium was taken from a blood agar plate (Himedia) culture and dropped in Mueller Hinton broth (Himedia). The broth culture was incubated at  $37^\circ\text{C}$  for 2 - 6 hours until it achieved turbidity similar to the 0.5 McFarland standards. The culture that exceeded the 0.5 McFarland standard were each adjusted with the aid of a UV spectrophotometer to  $0.132A^0$  at a wavelength of 600 nm in order to obtain an approximate cell density of  $1 \times 10^8$  CFU/ml.

### Preparation of the Extract Concentrations and Antibiotic

Stock solutions for the extract were prepared by dissolving 500 mg in 1 ml of dimethylsulfoxide (DMSO). An antibiotic control was made by dissolving  $1\mu\text{g}$  of penicillin in 1 ml of sterile distilled water. DMSO served as a negative control.

### Determination of bioactivity of the Extract

Mueller Hinton agar plates were prepared as per the manufacturer's instruction. The plate containing the medium were inoculated and sterile paper disc (whatmann no. 1, 5mm diameter) were

impregnated with the 500mg/ml solution of the extract (100µl) and allowed to dry. Three discs containing the plant extract and two discs one containing penicillin as the positive control and the other containing DMSO as the negative were then spaced on the surface of the sterile petri dishes using sterile pair of forceps. The discs were labeled on the underside of the plate. The plates were incubated at 37°C for between 24 to 48 hours and the zones of inhibition were measured in millimeters with the aid of a transparent ruler.

## Results and Discussion

From the study the extract of *Garcinia kola* was found to contain tannins, saponins, flavonoids, terpenoids, glycosides, alkaloids but steroids and phenols were found to be absent (Table. 1). Tannins are secondary metabolites which were found to be present in the plant seeds. They are glycosides of gallic or protocatechvic acids. Their astringent property makes them useful in preventing diarrhea and controlling hemorrhage due to their ability to precipitate proteins, mucus and constrict blood vessels (Kokwaro, 2009). This is the reason why traditional healers use plants rich in tannins to treat wounds and burns since they are able to cause blood clotting. Some tannins have been reported to inhibit HIV replication selectively besides the use of diuretics (Argal and Pathak, 2006). This shows how traditional plants rich in tannins can be used to control this dangerous disease. Tannins have also shown antiparasitic effects (Akiyama et al., 2001). According to Bajal (1988), tannins can also be used to protect the kidney since when taken the poliovirus, herpes complex virus and various enteric viruses are inactivated. Foods rich in

tannins can be used to treat hereditary hemochromatosis which is a hereditary disease characterized by excessive absorption of dietary iron. According to Chung et al. (1998), many tannin molecules have shown the ability to reduce the mutagenic activity of a number of mutagens. The anti-carcinogenic and anti-mutagenic potentials of tannins may be related to their antioxidative property which is important in protecting cellular oxidative damage including lipid peroxidation. The growths of many fungi, yeast, bacteria and viruses have been proven to be inhibited by tannins. Tannins have also been reported to exert physiological effects, such as to accelerate blood pressure, decrease the serum lipid level, and produce liver necrosis and modulate immune responses. The dosage and kind of tannins are critical to these effects (Chung et al., 1998).

The presence of Saponins shows the potential of the plants to be used to produce mild detergents and intracellular histochemistry staining to allow antibody access to intercellular proteins (Maobe et al., 2013). They have been found to treat hypercholesterolemia, hyperglycemia, antioxidant, anti-inflammatory, central nervous system activities, anticancer and weight loss (Maobe et al., 2013). They are used to stop bleeding, treating wounds and ulcers as it helps red blood cells to precipitate and coagulate (Just et al., 1998). This can be attributed to the ability of saponins to bind with glucose and cholesterol molecules. Saponins have also been associated with inhibitory effect on inflammation (Just et al., 1998). Saponins are used by the folkloric remedies of Kashmir (India) in treating wounds (Foster and Duke, 1990) this is because of their ability to cause red blood cells coagulation and therefore help in blood clotting,

treating wounds and enteric ulcers problems (Chiej, 1984). Saponins have also been used to prevent hypercholesterolemia, antibiotic activity, anti-inflammatory and anti-diabetic.

Flavonoids are secondary metabolites with polyphenolic structure and synthesized in plants, through polypropanoid pathway (Ghasemzadeh et al., 2011). Flavonoids have been classified in to six sub-groups which include flavones, flavanol, flavanone, flava-3-ols, isoflavone and anthocynidin. Flavonoids are known to contain specific compounds called antioxidants which protect human, animal and plant cells against the damaging effects of free radicals. Imbalance between free radicals and antioxidants leads to oxidative stress which has been associated with inflammation, autoimmune diseases, cataract, cancer, Parkinson's disease, aging and arteriosclerosis. It also plays a role in heart diseases and neurodegenerative diseases. Flavonoids are used as antioxidants because of their ability to scavenge free radicals such as peroxide and hydroperoxide of lipid hydroxyl hence inhibiting oxidation that lead to degenerative diseases (Samatha et al., 2012). They can be used as anti-diabetic. According to Namki (1990), flavonoids can be used to prevent synthesis of flavors that are caused by fat oxidation. Flavonoids have been found to have antibacterial activity due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall (Marjorie, 1999). Flavonoids are produced by plant in response to microbial infection and studies have shown that they have antibacterial activity against a wide range of microorganisms (Yadav and Agarwala, 2011). Flavonoids have also vasodilator activity a

property which is useful in improving blood circulation in brain and in Alzheimer disease (Sharma, 2006). Leaf extract of *Ginkgo biloba* which contains flavonoids was used for improving blood circulation in brain varix. Several isoflavone can be used to improve blood circulation. Free radicals including the hydroxyl, hydrogen peroxide, superoxide and lipid peroxide have been associated with a number of diseases such as cardiovascular disease, cataracts, diabetes, gastrointestinal inflammatory diseases, cancer, asthma, liver disease, macular degeneration, periodontal disease and other inflammatory processes. These oxidants are produced during normal body chemical processes. They can be damaged through free-radical damage.

Terpenoids have medicinal value such as ant-carcinogenic, antimalarial, antimicrobial and diuretics activity (Deganhardt, 2003 & Pichersky and Gershezon, 2002). Evaluation of the anti-inflammatory activity of three different Copaiba oleoresins showed that the crude extract of the plant and its' fractions of hexane, dichloromethane and methanolic extracts of other plant such as *C. cearensis*, *C. reticulata* and *C. multijuga* have anti-inflammatory potential (Viega et al., 2007). Terpenoids have also shown a great potential in treatment against disease causing microorganisms. Terpenoids have exhibited antibacterial activity against *E. coli*, *Staphylococcus*, *Pseudomonas aeruginosa* (Piera et al., 2011), *Proteus mirabilis* (Piera et al 2011), *Klebsiella pneumoniae* (Piera et al., 2011 & Santo et al., 2008), methillin-resistant *S. aureus*, *Staphylococcus epidermidis* (Santo et al., 2008), *Listeria monocytogenes* (Nero et al., 2010), *Enterobacter cloacae*, yeast; *Candida albicans* and fungi, *Aspergillus flavus* (Leandro et al., 2012). Terpenoids

**Table.1** Phytochemical analysis results

Phytochemical	Observation	Inference
Tannins	Brown black color	Present
Saponins	Stable foam	Present
Flavonoids	Yellow color	Present
Terpenoids	Black-grey	Present
Glycosides	Green brown	Present
Alkaloids	Precipitate	Present
Steroids	No blue green ring	Absent
Phenols	No black coloration	Absent

**Table.2** Antimicrobial Activity of *Garcinia kola* (bitter kola) against selected pathogenic microorganisms

Microorganisms	Extract mean $\pm$ S.E (mm)	Penicillin mean $\pm$ S.E (mm)	DMSO mean $\pm$ S.E. (mm)
<i>Serratia marcescens</i>	0.00 $\pm$ 0.000	32.33 $\pm$ 0.760	0.00 $\pm$ 0.000
<i>Bacillus cereus</i>	10.17 $\pm$ 0.477	29.00 $\pm$ 0.516	0.00 $\pm$ 0.000
<i>Escherichia coli</i>	12.83 $\pm$ 0.833	21.50 $\pm$ 0.563	0.00 $\pm$ 0.000
<i>Proteus vulgaris</i>	0.00 $\pm$ 0.000	25.83 $\pm$ 0.307	0.00 $\pm$ 0.000
Salmonella sp	0.000 $\pm$ 0.000	28.83 $\pm$ 0.703	0.00 $\pm$ 0.000

Key: S.E. = Standard error

**Table.3** Turkey's honestly significant Difference among micro - organisms using 500mg/ml of *Garcinia kola* (bitter kola) extract

Comparison	P-value	Significance
<i>S. marcescens</i> vs <i>B. cereus</i>	0.000	S
<i>S. marcescens</i> vs <i>E.coli</i>	0.000	S
<i>S. marcescens</i> vs <i>P. Vulgaris</i>	1.000	NS
<i>S. marcescens</i> vs Salmonella s.p	1.000	NS
<i>S. marcescens</i> vs <i>S. marcescens control</i>	0.000	S
<i>B. cereus</i> vs <i>E. coli</i>	0.020	S
<i>B. cereus</i> vs <i>P. vulgaris</i>	0.000	S
<i>B. cereus</i> vs Salmonella s.p	0.000	S
Salmonella s.p. vs <i>P. vulgaris</i>	0.000	S
<i>B. cereus</i> vs <i>B. cereus control</i>	0.000	S
<i>E. coli</i> vs <i>P. vulgaris</i>	0.000	S
<i>E. coli</i> vs Salmonella s.p.	0.000	S
<i>E. coli</i> vs <i>p. Vulgaris</i>	0.000	S
<i>E.coli</i> vs <i>E.coli control</i>	0.000	S
<i>P. Vulgaris</i> vs Salmonella s.p.	1.000	NS
<i>P. Vulgaris</i> vs <i>P. Vulgaris control</i>	0.000	S
Salmonella s.p. vs Salmonella s.p. control	0.000	S



have also been found to reduce the growth of melanoma cells on mice after oral administration (Lima et al., 2003). Terpenoids have been proved scientifically to kill mosquito larvae. Terpenoids extracted from *C.reticulata* species have shown potential in killing *A. aegypti* (Geris et al., 2003).

Glycosides are secondary metabolites from plants or animal sources in which a sugar is bound to a non-carbohydrate moiety. The term Glycoside is a collective term used for compounds formed with a glycosidic bonding between a sugar and another compound other than sugar. Cardiac glycosides have been used traditionally as arrow poisons or as heart drugs. They are used to strengthen the heart and make it function properly under controlled therapeutic dose. Cardiac glycosides bind to and inhibit  $\text{Na}^+/\text{K}^+$ -ATPase, inhibition of  $\text{Na}^+/\text{K}^+$ -ATPase raises the level of sodium ions in cardiac myocytes, which leads to an increase in the level of calcium ions and an increase in cardiac contraction force (Schatzmann and Rass., 1965). The unexpected results relating glycosides with anticancer properties have created a great interest in this secondary metabolite. This has led to clinical trial of glycosides based drugs in clinics (Newman et al., 2008).

Alkaloids which are secondary metabolites, they can be defined as a cyclic compounds which have nitrogen in a negative oxidation state. They affect the chemical transmitters' action of the nervous system. They also have other pharmacological activities such as analgesic, antispasmodic, antihypertensive effects and anti-arrhythmic effects and antibacterial effect. Cryptolepine a major alkaloid in the plant *S.acuta* was found to be an antimalarial agent (Banzouzi et al.,

2004). Cryptolepine has also been used clinically to treat malaria, colic and stomach ulcers (Boye and Ampufo, 1983), and also used in anticancer drugs. According to Karou et al. (2006), much study has been done on pharmacological properties of alkaloids and proved to have antiprotozoal, cytotoxic and anti-inflammatory properties.

The presence of alkaloids in the plant justifies its' medicinal value. Alkaloids have been isolated from different plants and their medicinal values tested. The most important use of alkaloids already known with its originality from plants is the use of alkaloids compounds in the treatment of malaria. According to Ameyawn and Duker-Eshon (2009), many of the antimalarial drugs used today are quinoline derivatives manipulated from cinchona species bark (Garnham, 1966). Alkaloids have been identified for their functions which include analgesic, antiplasmodic and antibacterial activity (Okwu and Josiah, 2006). According to Ayitey and Addae (1977), bitter leaves containing alkaloids are capable of reducing headache associated with hypertension.

Among all the organisms tested with the methanol extract of *G. kola*, only *B. cereus* and *E. coli* were inhibited (Table 2). All other organisms were not inhibited. The penicillin control showed large zones of inhibition and DMSO did not show any zones of inhibition. Analysis of variance showed there was significant differences in the zones of inhibition among the bacterial organisms tested ( $p < 0.001$ ). A multiple comparison with the Tukey's test (Table.3) showed that the zones of inhibition of *S. marcescens* was significantly lower than that of *B. cereus*, lower than *E. coli* and *S. marcescens*

control ( $p < 0.001$ ). There was no significant difference between *S. marcescens* and *P. vulgaris* and *S. marcescens* and *Salmonella* sp. ( $p > 0.05$ ). *B. cereus* had significantly higher zones of inhibition than all the organisms ( $p < 0.001$ ) but had significantly lower zones of inhibition than *E. coli* ( $p < 0.05$ ). *E. coli* also had significantly higher zones of inhibition than *P. vulgaris* and *Salmonella* sp. ( $p < 0.001$ ). There was no significant difference in the zones of inhibition of *P. vulgaris* and *Salmonella* sp. ( $p > 0.05$ ). All the penicillin controls zones of inhibition against each organism were significantly higher than those for the treatment with the extracts against the organisms.

The study is in conformity with other studies in which Polyisoprenyl benzophenone, kolanone in the petroleum ether and hydroxyl biflavonols in the ethyl acetate fraction of *G. kola* showed activity against gram positive and gram negative bacteria (bacteriostatic) and against *Candida albicans* and *Aspergillus flavus* (fungistatic). MIC of the GB 1 fraction was  $3.1 \times 10^{-7}$  mg/ml and  $3.0 \times 10^{-3}$  mg/ml for *E. coli* (Madubunyi, 1995). In an agar well diffusion assay, *G. Kola* was tested against several microorganisms and showed that the ethanol extracts exhibited zones of inhibition ranging from 17 to 23 mm while the aqueous hot water extract showed zones ranging from 20 to 27 mm. Antifungal activity was shown against *Staphylococcus aureus* at 0.008 mg/ml. Phytochemical compounds such as flavonoids, tannins saponins, steroids, cardiac glycosides and reducing sugar were found to be present (Arekemaje et al., 2012). The acetic acid extract of *Garcinia kola* has also shown zones of inhibition as high as 17.5 mm for *S. aureus*, 18.5 mm for *E. coli*, 35.0 mm for

*Streptococcus pyogenes* and 37.0 mm for *Salmonella typhi*, (Ezeanya and Daniel, 2013). Between 5 mg/ml to 25 mg/ml of *Garcinia kola* and *Cola nitida* showed zones of inhibition against *E. coli*, *S. aureus*, *S. typhi* and *K. pneumoniae*. The methanol soluble extract of *G. kola* showed zones of inhibition of 20 mm, 25 mm, 24 mm, 9 mm and 20 mm against *E. coli*, *S. aureus*, *S. typhi* and *K. pneumoniae* respectively. That of *Cola nitida* showed 23 mm, 9 mm, 18.5 mm, 12.5 mm against *E. coli*, *S. aureus*, *S. typhi*, *K. pneumoniae* respectively. This study showed that *Cola nitida* is a better antimicrobial against *E. coli* while *G. kola* is good for *S. aureus*, *S. typhi* and *K. pneumoniae*, (Indabawa and Arzai, 2011).

From the data obtained in this study it is therefore worthy to mention that the plant can be used to treat against all the infections caused by *Bacillus cereus* and even other species of *Bacillus* viz self-limited gastroenteritis, post traumatic wounds, surgical wounds infections, burns, ocular infections such as endophthalmitis, corneal abscess and panophthalmitis (Sankararaman and Velayuthan, 2013 & Garcia-Arribas et al., 1988). The plant compounds can also be used to treat immunologically compromised patients including AIDS and malignant disease victims (Cotton et al., 1987 & Tuazon et al., 1979). The plant's capability to inhibit the growth of *E. coli* is a scientific prove that the plant can be used to a great extent to treat against enteric infections caused by the bacteria.

The presence of the important phytochemicals is in conformity with the results obtained in previous studies in which the plant seeds medicinal value was attributed to the presence of flavonoids, tannins, saponins, triterpenes,

benzophenones and xanthenes (Iwu, 1982 and Geiger, 1988). The medicinal value of the plant could directly be attributed to one or a combination of two or more phytochemicals found in the plant to give a synergistic effect on the ailment been treated against. The antibacterial activity observed in this study could be directly attributed to the presence of these important phytochemicals as indicated in previous studies (Ngule et al., 2013 and Anthony et al., 2013).

The data obtained in this research is a scientific justification the plants traditional use in the treatment of various stomach problems. From this research it is worthy to recommend the plant seeds for the treatment of diarrhea caused by *E.coli* and all the ailments caused by *B. cereus*, however further research needs to be done to isolate the pharmaceutical compounds, investigate their mode of action and the effect of the same in the *in vivo* environment.

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### References

Adu, F., Gbedema, S.Y., Akanwariwiak, W.G., Annan, K., and Boamah, V.E. 2011. The effects of *Acanthospermum hispidum* extract on the antibacterial activity of amoxillin and ciprofloxacin, *Journal for drugs and Medicines*, 3(1): 58-63.

Akiyama, H., Fujii, K., Yamasaki, O.,

Oono, T., Iwatsuki, K. 2001. Antibacterial action of several tannins against *Staphylococcus aureus*. *J. Antimicrobe*.

Ameyaw, Y. and Duker-Eshun, G. 2009. The alkaloid content of the ethano-plant organs of three antimalarial medicinal plants species in the eastern region of Ghana. *Int.J.Chem*, 7(1):48-58.

Anthony, S.T., & Ngule, C.M. 2013. Chemical constituents of infused *Plectranthus argentatus* leaves. *World Journal of Publisher*, 1(3): 151-160.

Anthony, S.T., Jackie, O., Ngule, C.M. 2013. *In vitro* control of selected pathogenic organisms by *Vernonia adoensis* roots. *International Journal of Pharmacy and Life Sciences*, 4(8): 2855-2859.

Anthony, S.T., Ngule, C.M. & Obey, J. 2013. *In vitro* control of selected pathogenic organisms by *Vernonia adoensis*. *Int. J. of Pharm. & Sci*, 4(8): 2855-2859.

Anthony, S.T., Ngule, C.M. and Machoka, R. 2014. Histopathological effects of *Vernonia adoensis* roots extract on the larvae of the mosquito *Culex quinquefasciatus*. *Journal of Pharmaceutical Biology*, 4(1): 1-3.

Anthony, S.T., Ngule, C.M. and Obey, J. 2013. Phytochemical analysis of *Vernonia adoensis* leaves and roots used as a traditional medicinal plant in Kenya. *International Journal of Pharmacy and Biological Sciences*, 3(3): 46-52.

Anthony, S.T., Ngule, C.M. and Obey.2013. Phytopharmacological analysis of methanolic –aqua extract (fractions) of *Senna didymobotrya* roots. *International Journal of Bioassays*, 02(11): 1473-1479.

Arekemaje, M.O., Aliyu., M.B., Kayode, R.M.O., Ajiboye A. E., Ajijolakewu

- A.K. 2012. Antimicrobial effects of *Garcinia kola* (Bitter kola) on some selected pathogens from University of Ilorin Teaching hospital, Ilorin, Nigeria. *Journal of Asian scientific research*; 2 (4): 159-169.
- Arekemase, M.O., Badandoko, A.M., Ojo, K.R.M., Elizabeth, A.A. and Kamoldeen, A.A. 2012. Antimicrobial effects of *Garcinia kola* (bitter kola) on some selected pathogens from University of Ilorin teaching hospital Ilorin, Nigeria. *Asian Economic and Social Society*, 2(4): 157-169.
- Argal, A., and Pathak, A. K. 2006. CNS Activity of *Calotropis gigantea* roots. *Journal of Ethnopharmacology*, 19:425-428.
- Arivoli, S. and Tennyson, S. 2012. Larvicidal efficacy of *Strychnos nuxvomica* Linn.(Loganiaceae) leaf extracts against the filarial vector *Culex quinquefasciatus* Say (Diptera : Culicidae). *World Journal of Zoology*, 7(1): 06-11.
- Ayitey, S.E., and Addae, M.L. 1977. Phytochemical nutritional and medical properties of some leafy vegetables consumed by Edo people of Nigeria. *J.Pharmacol.Drug Res*, 4:7-8.
- Bajal, Y.P.S. 1988. *Medicinal and aromatic plants*. Biotechnology in agriculture and forestry. Berlin: Springer-Verlag.Vol.24.
- Banzouzi, J.T., Prado, R., Menan , H., Valentin, A., Roumestan, C., Mallie, M., Pelissier, Y., and Blanche, Y. 2004. Studies on medicinal plants of Ivory Coast: *Investigation of an active constituent phytomed*, 11:338-341.
- Boye, G.I., and Ampufo, O. 1983. Proceedings' on the first international seminar on cryptolepic (ends Boakye Yiadom k Bamgbose, S.O.A) (University of Kumasi, Ghana)
- Chabot, S., Bel-Rhlid, R., Chenevert, R. and Piche, Y. 1992. Hyphal growth promotion in vitro of the VA Mycorrhizal fungus, *Gigaspora margarita* Becker and Hall, by the activity of structurally specific flavonoid compounds under CO<sub>2</sub> – enriched conditions. *New Phytology*, 12: 461-467.
- Chessin, M., Deborde, D. and Zip, F.A. 1995. *Antiviral proteins in higher plants*. CRC Pres, Inc., BocaRaton, Fla.
- Chiej, R.1984. *Encyclopedia of medicinal plants*. MacDonald publishers:London.
- Chinyere, C.E., and Ebakota, O.D. Antibacterial activity of *Garcinia kola* seed and leaf extract on some selected clinical isolates. *Science Journal of Microbiology*, 2013;9:1-8.
- Chung, K.T., Wong, Y.T., Wei, C.I., Huang, Y.W. and Lin, Y. 1998.Tannins and human health. *Critical Reviews in Food Science and Nutrition*, 38(6):421-464.
- Cotton, D.J., Gill, V.J., Marshall, D.J., Gress, M., Thaler, M. and Pizzo, P. 1987. Clinical features and therapeutic interventions in 17 cases of *Bacillus bacteremia* in an immunosuppressed patient population .*J. Clin. Microbiol*, 25: 672-674.
- Deeepa, N. and Rajendran, N.N. 2007. Antibacterial and anti-fungal activities of various extracts of *Acanthospermum hispidum* DC. *Journal of Natural Remedies*, 7(2): 225-228.
- Deganhardt, J. 2003. Attracting friends to feast on foes: Engineering terpene emission to make crop plant more attractive to herbivore enemies. *Curr.Opin.Biotechnol*, 14:169-176.
- Ezeanya, C.C., Daniel, E. O. 2013. Antibacterial activity of *Garcinia kola* seed and leaf extracts on some selected clinical isolates. *Science Journal of Microbiology*, no Vol, 1-9.

- Farook, S.M., and Atlee, W.C.2011. Antidiabetic and hypolipidemic potential of *Tragia involucrata* Linn. In streptozotocin-nicotinamide induced type II diabetic rats. *International Journal of Pharmacy and Pharmaceutical Sciences*, 3(4): 103-109.
- Foster, S., and Duke, J.A. 1990. *A field guide to medicinal plants*. Houghton mifflin Co, Boston.
- Garcia-Arribas, M.L., Plaza, C.J., De La Rose, M.C. and Mosso, M.A. 1988. Characterisation of *Bacillus cereus* strains isolated from drugs and evaluation of their toxins. *J.Appl. Bacteriol*, 64: 257-264.
- Garnham, P.C.C. 1966. *Malaria parasites and other haemosporidia*. Bookwell Scientific Publications, Oxford.
- Geris, R., Silva, I.G., Silva, H.H.G., Barrison, A.G. 2003. Diterpenoids from *Capaifera reticulata* ducke with larvicidal activity against *Aedes aegypti* (L): Diptera (Culicidae). *Rev. Inst.Med. Trop.S.Paulo*, 50:25-28.
- Ghasemzadeh, A. and Ghasemzadeh, N. 2011. Flavonoids and phenolic acids: Role and biochemical activity in plants and human. *Journal of medicinal plants research*, 5(31):6697-6703.
- Gill L, S. 1992. *Ethno medicinal use of plants in Nigeria*.1992. University of Benin Press Benin City, Nigeria, 276.
- Gobalakrishnan, R., Kulandaivalu, M., Bhuvanewari, R., Kandavel, D. and Kannan, L. 2013. Screening of wild plant species for antibacterial activity and phytochemical analysis of *Tragia involucrata* L. *Journal of Pharmaceutical Analysis*, 07(1): 01-06.
- Green, B.O. 2007. Significance and efficacy of medicinal plants in the Niger Delta. *Continental Journal of Pharmaceutical Sciences*, 1(3); 23-29.
- Harbone, J. B. 1973. *Phytochemical methods Chapman and hall ltd*, London, pp 149-188
- Hosahally, R.V., Sero, G., Sutar, P.S., Joshi, V.G., Sutar, K.P. and Karigar, A.A. 2012. Phytochemical and pharmacological evaluation of *Tragia cannabina* for anti-inflammatory activity. *International current Pharmaceutical Journal*, 1(8):213-216.
- White, E.G. Medicinal herbs. [http://csda-adventistchurch.to/EGW\\_Messenger.html](http://csda-adventistchurch.to/EGW_Messenger.html)
- Indabawa, I.I. and Arzai, A. H. 2011. Antibacterial activity of *Garcinia kola* and *Cola nitida* seed extract. *Bayero Journal of pure and applied sciences*, 4(1): 52 -55.
- Irrine, F. 1981. *Woody plants of Ghana*. Oxford University Press, London, 300.
- Iwu, M. 1993. *A hand book of Africa medicinal plants*. CRC Press, Florida, 230.
- Joshi, C.G., Gopal, M. and Byregowda, S.M. 2011. Cytotoxic activity of *Tragia involucrata* Linn. extracts. *American-Eurasian Journal of Toxicology Sciences*, 3(2): 67-69.
- Just, M.J., Recio, M.C., Giner, R.M., Cueller, M.U., Manez, S., Billia, A.R., Rios, J.L. 1998. Antinflammatory activity of unusual lupine saponins from *Bupleurum fruticosens*. *Thieme-E Journals*, 64:404-407.
- Kamatenesi-Mugisha, M. and Oryem-Origa, H. 2005. Traditional herbal remedies used in the management of sexual impotence and erectile dysfunction in western Uganda. *African Health Sciences*, 5(1): 40-49.
- Karou, D., Savadogo, A., Canini, A., Yameogo, S., Montesano, C., Simpre, J., Colizzi, V and Traore, A.S. 2006. Antibacterial activity of alkaloids from *S.acuta*. *African journal of*

- biotechnology*, 5(2):195-200.
- Kisangau, D.P., Hosea, K.M., Joseph, C.C. and Lyaruu, H.V.M. 2007. *In vitro* antimicrobial assay of plants used in traditional medicine in Bukoba rural district, Tanzania. *Afr. J. Traditional, Complementary and Alternative Medicines*, 4(4): 510-523.
- Kokwaro, J.O. 2009. *Medicinal plants of east Africa*. Nairobi: University Press.
- Leandro, L.M., Vargas, F.S. 2012. Chemistry and Biological activities of terpenoids from Copaiba (Coparfera SPP). *Oleoresins*, 17:3866-3889
- Lima, S.R.M., Veiga, V.F. Jr., Christo, H.B., Pinto, A.C., Fernades, P.D. 2003. *In vivo* studies on the anticancer activities of the *Capeifera miltifuga* (Hayne) and its fractions. *Phytother. Res*, 17:1048-1052.
- Madubunyi, I.I. 1995. Antibacterial Activities of the constituents of *Garcinia kola* seeds. *Pharmaceutical Biology*; 33(3), pp 232 – 237.
- Maobe, M.A.G., Gatebe, E., Gitu, L. and Rotich, H. 2013. Preliminary phytochemical screening of eight selected medicinal herbs used for the treatment of diabetes, malaria and pneumonia in Kisii region, southwest Kenya, *European journal of applied sciences*, 5(10) : 01-06.
- Marjorie, M.C. 1999. Plant Products as Antimicrobial Agent. *Clin.Microbiol. Rev.* pp. 564-582.
- Mensah, J.K., Okoli, R.I., et al. Phytochemical analysis of medicinal plants used for the management of hypertension by Esan people of Edo state, Nigeria. *Ethnobot. Leaflets*, 2009; 13: 1273-1287.
- Namki, M. 1990. Antioxidants and antimutagens in food. *Crit.Rev. Food Sci.Nutri*, 29:273-300.
- Nero, L.A., Moreira, M.A.S. 2010. Antimicrobial activity of autoclaved and non-autoclaved Capaiba oil on *Lutera monocytogenes*. *Cienc. Rural*, 40:1797-1801.
- Newman, R.A., Yang, P., Pawlus, A.D. and Block, K.I. 2008. *Cardiac glycosides as novel cancer therapeutic agents*, 8:36-49
- Ngule, C.M. and Anthoney, S.T. Phytochemistry of infused *Tragia brevipes* stem. *International Journal of Medical and Pharmaceutical Sciences Research and Review*, 1(3): 1-15.
- Ngule, C.M., Anthoney, S.T., and Machoka, R. Evaluation of larvicidal activity of *Vernonia adoensis* leaves against *Culex quinquefanciatus*. *International Journal of Phytotherapy*, 2014; 4(1): 11-13.
- Ngule, C.M., Anthoney, S.T., Obey, J. 2013. In Vitro control of selected pathogenic microorganisms by *Vernonia adoensis* leaves. *International journal of Bioassays*, 02(08): 1113-1117.
- Ngule, C.M., Anthoney, S.T., Obey, J. 2013. Phytochemical and bacterial evaluation of *Senna didymobotrya* fresen Irwin used by the Nandi community in Kenya. *International Journal of Bioassays*, 02(07): 1037-1043.
- Okwu, D.E. and Josiah, C. 2006. Evaluation of the chemical composition of two Nigerian medicinal plants Africa. *J.Biotechnology*, 5:357-361
- Panda, D., Dash, K.S. and Dash, G.K. 2012. Phytochemical examination and antibacterial activity of various solvent extracts and the selected isolated compounds from roots of *Tragia involucrata* Linn. *International Journal of Pharmaceutical Sciences and Drug Research*, 4(1): 44-48.
- Pichersky, E. and Gershezov, J. 2002. The formation and function of plant

- volatiles, perfumes for pollinator attraction and defence .*Curr.Opin.Plant Biol.*5:237-243.
- Piera, F.A., Souza, C.F., Costa, J.C.M., Barreto, M.A.O., Espescheit, I.F., Silva, V.O., Moreira, M.A.S. 2011. Inhibition of *E.coli* from mastitic milk by Agrarias, 32: 1929-1934
- Samatha, T., Shyamsundarachary, R., Srinivas, P., and Swamy, R.S. 2012. Quantification of total phenolic and total flavonoids contents in extracts of *Oroxylum Indicum* L.Kurz, *Asian Journal of Pharmaceutical and Clinical Research*, 5(4):177-179.
- Sandigawad, A.M. 2010. *In vitro* evaluation of antibacterial activity of bark and flower extracts of *Pimenta officinalis* Lindl. *Adv. Bio Res*, 1(2): 61-68.
- Sankararaman, S. and Velayuthan, S. 2013. *Bacillus cereus*. *Pediatrics in Review*, 34: 196.
- Santo, A.O., Ueda-Nakumura, T., Dias-Fiho, B.P., Veiga, V.F. Jr., Pinto, A.C., Nakamwa, C.V. 2008. Antimicrobial activity of *Brazilian Copaiba* oils obtained from different species of the Copalifera genes. *Mem. Inst. Oswaldo Cruz*, 103:277-281.
- Schatzmann, H.J. and Rass, B. 1965. Inhibition of the active Na-K-transport and Na-K-activated membrane ATPase of erythrocytes stroma by Ovacain. *Helv. Physiol. Pharmacol*, 65:47-49.
- Sharma, D.K. 2006. Pharmacological properties of flavonoids including flavonolignans-integration of petrocrops with drug development from plants. *Journal of scientific and industrial research*, 65:477-484.
- Sofowara, A. 1993. *Medicinal plants and traditional medicine in Africa*. Spectrum books ltd, Ibadan Nigeria, pp 191-289
- Trease, G. E. & Evans, W. C. 1989. *Pharmacognosy*, 11th end, brailliere tindall, London, pp 45-50.
- Tuazon, C.U., Murray, H.W., Levy, C., Solny, M.N., Curtin, J.A. and Shegren, J.N. 1979. Serious infections from *Bacillus* species. *JAMA*, 241:1137-1140.
- Viega, V.F., Jr., Rosas, E.C., Garvalho, M.K., Henriques, M.G.M.O., Pinto, A.C. 2007. Chemical composition activity of *Brazilian Copaiba* oils from *Copaifera reticulate* (Ducke) and *Copaifera multifuga* (Hayne). A Comparative study. *J Ethnopharmacol*, 112: 248-254.
- Yadav, R.N.S., and Agarwala, M. 2011. Phytochemical analysis of some medicinal plants. *Journal of phytology*, 3(12):10-14.