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Full Length Research Paper

Evaluation of the antibacterial properties and synergistic effect of *Garcinia kola* Heckel (Family: Guttiferae) seed extract and honey on some bacteria

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The antibacterial activity of aqueous extract of *Garcinia kola*, honey and mixture of aqueous *G. kola* extract and honey was investigated against *Escherichia coli, Klebsiella pneumonia*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella* sp. and *Pseudomonas aeruginosa* using agar well diffusion method. Sensitivity pattern of these bacteria was varied and extract concentration-dependent. It was observed that the synergistic use of aqueous *G. kola* extract and honey mixture was more effective in inhibiting bacterial growth than the separate use of aqueous *G. kola* extract and honey. The susceptibility of the test bacteria to the aqueous *G. kola* extract and honey mixture was in descending order as follows: *P. aeruginosa* > *E. coli* > *S. aureus* > *Salmonella* > *K. pneumonia* > *B subtilis* (> = more susceptible). Aqueous *G. kola* extract was most active against *P. aeruginosa* with zones of inhibition ranging from 16.7 ± 0.21 to 18.00 ± 1.00 mm and least active against *B. subtilis* with zone of inhibition of 0.00 - 6.00 ± 1.00 mm. Honey at 100% concentration inhibited growth of all the test organisms except *B. subtilis.* Statistical analysis showed that *E. coli*, *S. aureus and P. aeruginosa* at P < 0.05, exhibited significant difference in their susceptibility to the synergistic mixture and separate use of aqueous *G. kola* extract and honey. Synergistic use of aqueous *G. kola* extract and honey is therefore recommended as a better option than their separate use in the treatment of infections caused by the test bacteria.

Key words: *Garcinia kola,* honey, bacteria, antimicrobial activity, synergy.

INTRODUCTION

Plants act generally to stimulate and supplement the body's healing forces, they are the natural food for human beings (Fabiola et al., 2003). Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Infectious diseases are the number one causes of death accounting for approximately one half of all deaths in tropical countries (Iwu et al., 1999). Today plant materials continue to play a major role in primary healthcare as therapeutic remedies in many developing countries (Jonathan et al., 2007; Jonathan and Fasidi, 2005; Jonathan and Fasidi, 2003).

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The different parts of plants used include root, stem, flower, fruit, twig, exudates and modified plant organs (Mahesh and Satish, 2008; Uniyal et al., 2006). Nearly all cultures and civilizations from ancient times to the present day have used herbal medicines (Lino and Deogracious, 2006; El-Mahmood et al., 2008) to cure infections. Plants still continue to be almost the exclusive source of drugs for the majority of the world's population (Fabiola et al., 2003; Jonathan and Fasidi, 2005; Jonathan and Fasidi, 2003; Ajayi et al., 2008).

Some plants have been discovered to be rich in secondary metabolite, such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, and volatile oil. These compounds are responsible for their therapeutic activities (Cowan, 1999; Rabe and Vanstoden, 2000). Also, since times past, some plant parts have been used

as antimicrobial agents, especially their extracts, either as decoctions, infusions or oral administration (Okemo et al., 2001). Benefits derived from using medicine obtained from plants are relatively safer than synthetic alternative by offering profound therapeutic benefits and give more affordable treatment (Iwu et al., 1999; Idu et al., 2007).

Honey, whose medicinal uses dates from ancient times, has lately been rediscovered as a therapy for wounds (Vishnu et al., 2012). Many publications attest to honey's antimicrobial properties. Strong solutions of honey or sugar and sugar pastes, inhibit microbial growth because of their high osmolarity (Deshpande and Kulkarni, 2010), but when used as dressings they become diluted to the point where this action ceases, especially in the case of *Staphylococcus aureus* (Cooper et al., 1999). Recently, the potent activity of honey against antibiotic-resistant bacteria has further increased the interest for application of honey, but incomplete knowledge of the antibacterial activity is a major obstacle for clinical applicability (Cooper et al., 2002a; Cooper et al., 2002b; Levy and Marshall, 2004; Walsh, 2003). Traditional preparation and medicinal plants with antimicrobial activities have been extensively used in the West African regions (Adesuyi et al., 2012; Dunford, et al., 2000; Mboto et al., 2009; Mythilypriya et al., 2007).

Garcinia kola, commonly called bitter kola belongs to the family *Guttiferae* (Adesuyi et al., 2012). The parts used in this plant are the stem bark, seeds and root. The active ingredients are flavonoids, apigenin, kolaviron, biflavonoid-ametoflavone, saponins, tannins, resin (Gill, 1992; Okunji et al., 2002). Medicinally, the stem bark and seeds are used for acute fever, cough, liver disorders and as an anti-vomiting agent (Odugbemi, 2006; Gill, 1992). It is also used as a remedy for inflammations of respiratory tract, bronchitis, throat troubles, stomach ache and gastritis (Adegboye et al., 2008; Ajebesone and Aina, 2004). The seed extract is very efficacious for hepatitis, antiseptic and is active against Gram-positive and negative bacteria (Gill, 1992). The decoction of the root is used as aphrodisiac, evacuant, anticancer and is also recommended for dysentery, headache, malignant tumours and respiratory ailments (Odugbemi, 2006). The root is chewed for cleaning teeth and toothache (Gill, 1992).

This study was carried out to assess and compare the antimicrobial efficacy of the synergy of honey and *G. kola* and their use separately on some pathogenic bacteria.

MATERIALS AND METHODS

Sample collection

The seeds of *G. kola* were purchased from Oba market in Benin City, Nigeria and identification confirmed by Odugbemi (2006). Freshly harvested honey was purchased from local supermarket in Ugbowo, Benin City, Nigeria. It was diluted with distilled water to different concentrations of 25, 50, 75 and 100%. These samples were obtained during the dry season.

Test organisms

Bacterial cultures of *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Bacillus subtillis, Salmonella* sp. *and Pseudomonas aeruginosa* obtained from the Department of Medical Microbiology, University of Benin teaching hospital, Benin City, Nigeria, were used as antimicrobial test organisms. Their identity was confirmed using cultural, morphological and biochemical test as previously described (Akinnibosun et al., 2009; Cheesebrough et al., 2002). The bacterial isolates were maintained on nutrient agar slants at 4°C. The test bacteria have been previously described (Prescott et al., 2005; Akinnibosun et al., 2008a, 2008b).

Preparation of aqueous extract

Ten grams of dried grinded seed powder was dissolved in 100 ml of distilled water for 24 h. The mixture was filtered using Whatman's filter paper No. 1 to obtain solution free of solids. The filtrate was concentrated by drying at 37°C and stored at 4°C.

Preparation of ethanolic extract

Ten grams of dried grinded seed powder was dissolved in 100 ml of 95% ethanol for 24 h. The mixture was filtered using Whatman's filter paper No. 1 to obtain solution free of solids. The filtrate was placed into evaporator to drive-off the solvent and stored at 4°C.

Preparation of honey and *G. kola* mixture

Various concentrations were prepared from the grinded seed and the honey, 0.05, 0.10, 0.15 and 0.20 g of the powdered seed was added to 10 ml of 100% honey to obtain 5.0, 10.0, 15.0 and 20.0 mg/ml, respectively.

Determination of antibacterial activity

The zone of inhibition was measured by the agar-well diffusion method (Stoke, 1975). Sterilised nutrient agar was poured into Petri-dishes to the level of obtaining a standard well and allowed to set. Nutrient broth inoculated with the test bacteria was poured into the already set Petri-dishes and uniform distribution was ensured. Sterile cork borer was used to bore 10 mm wells (holes) in the agar. Each of the holes in the Petri-dish was filled with the samples and incubated for 24 h at 37°C. Standard antibiotic, gentamicin was used as positive control and water was used as negative control. The active extracts had zones of inhibition which were measured to indicate the degree of sensitivity.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC of the extracts against the test organisms was determined using the broth dilution method (Sahm and Washington, 1990). Briefly, 1 ml of the extracts was added to 1 ml of nutrient broth and subsequently transferred. From the first test-tube, 1 ml was transferred to the next for up to the seventh test-tube. After which 1 ml of 24 h culture of test organisms was inoculated into each test-tube and mixed thoroughly by vortexing. The test tubes were then inoculated at 37°C for 24 h. This procedure was carried out for all the different concentrations of extract. The test-tube with the lowest dilution with no turbidity was considered as the MIC. Subsequently, those tubes that showed no turbidity were plated out on nutrient

Table 1. Antibacterial activity *(zone of inhibition in mm) of honey against the test bacteria

Test organism	25%	50%	75%	100%
E. coli	3.33 ± 0.58	8.00 ± 1.00	14.33 ± 0.58	20.00 ±1.00
K. penumoniae	2.33 ± 0.58	4.33 ± 0.58	11.00 ± 1.00	15.00 ± 1.00
S. aureus	0.00	0.00	0.00	9.00 ± 1.00
B. subtilis	0.00	0.00	0.00	0.00
Salmonella sp.	2.33 ± 0.58	4.33 ± 0.58	13.00 ± 1.00	18.00 ± 1.00
P. aeruginosa	2.33 ± 0.58	5.67± 0.58	9.00 ± 1.00	14.67 ± 0.58

*Mean **±** standard deviation.

Table 2. Antibacterial activity* (zone of inhibition in mm) of aqueous extract of *G. kola*

Test organism	5.0 mg/ml	10.0 mg/ml	15.0 mg/ml	20.0 mg/ml
E. coli	3.33 ±0.58	8.00 ±1.00	11.00 ±1.00	15.33 ±0.58
K. pneumoniae	0.00	2.33±0.58	5.67 ± 1.16	13.33 ± 0.58
S. aureus	0.00	5.33± 0.58	13.00± 1.00	15.33 ± 0.58
B. subtilis	0.00	0.00	1.33 ± 0.58	6.00 ± 1.00
Salmonella sp.	0.00	5.33 ± 0.58	8.67 ±0.58	14.00 ± 1.00
P. aeruginosa	16.70±0.21	9.33± 0.58	13.33±0.58	18.00 ± 1.00

*Mean **±** standard deviation.

Table 3. Synergistic effect* (zone of inhibition in mm) of aqueous extract of *G. kola* and honey on the test bacteria.

Test organism	5.0 mg/ml	10.0 mg/ml	15.0 mg/ml	20.0 mg/ml
E.coli	9.00 ±1.00	15.33 ± 0.58	22.33 ±0.58	25.67 ±0.58
K. pneumoniae	4.33 ± 0.58	9.00 ± 1.00	13.33 ±1.53	17.33 ± 2.89
S. aureus	5.33 ± 0.58	13.70 ± 0.58	19.00± 1.00	22.67 ± 0.58
B. subtilis	0.00	2.67 ± 0.58	7.33 ± 0.58	13.00 ± 1.00
Salmonella sp.	4.67 ± 0.58	11.00 ± 1.00	18.67 ±0.58	21.00 ± 1.00
P. aeruginosa	12.00±1.00	17.30 ± 0.58	23.67±0.58	27.67 ± 0.58

*Mean **±** standard deviation.

agar plates and absence of growth on incubation for 24 h was considered as the MBC.

Measurement of bactericidal and bacteriostatic property

This was carried out to assess the concentrations of the samples that can kill or inhibit the growth of the tested organisms. Sterile inoculating loop was rubbed on the clear zones produced during the tests for antibacterial activities and streaked in fresh chocolate agar plates and then incubated as earlier described. Absence of growth was interpreted as bactericidal action while growth represented a bacteriostatic action (Madunagu et al., 2001).

Statistical analysis

Statistical analysis as carried out to determine whether there was significant difference between the synergistic action of the bitter kola and honey mixture and the separate inhibitory actions of bitter kola and honey.

RESULTS AND DISCUSSION

The present study shows the evaluation of the antibacterial properties and synergistic effect of *G. kola* and honey on some bacteria. The antibacterial activity in the study was expressed as a measure of the diameter of the inhibition of growth in millimetres. Tables 1 to 3 show the antibacterial activity (as shown by zones of inhibition of the different concentrations) of honey, aqueous bitter kola extract, and mixture of bitter kola and honey, respectively. Tables 4 and 5 show the MIC and MBC of the various samples on the test organisms. The results of this work show that the bitter kola, honey and the mixture of bitter kola and honey had antibacterial property by preventing the growth of the test organisms.

The investigation done on *G. kola* extract and honey revealed that samples possessed antimicrobial property against the tested bacterial isolates. The antibacterial action

Table 4. Minimum inhibitory concentration.

Test organism	Aqueous *G. kola* extract (mg/ml)	Honey (%)	Honey + *G. kola* extract mixture (mg/ml)
E. coli	2.5	25.0	2.5
K. pneumoniae	7.5	25.0	2.5
S. aureus	7.5	100.0	2.5
B. subtilis	12.5	0.0	7.5
Salmonella sp.	7.5	25.0	2.5
P. aeruginosa	2.5	25.0	2.5

Table 5. Minimum bactericidal concentration.

Test organism	Aqueous *G. kola* extract (mg/ml)	Honey (%)	Honey + *G. kola* extract mixture (mg/ml)
E. coli	5.0	50.0	5.0
K. pneumoniae	10.0	50.0	5.0
S. aureus	10.0	100.0	5.0
B. subtilis	15.0	0.0	10.0
Salmonella sp.	10.0	50.0	5.0
P. aeruginosa	5.0	50.0	5.0

occurred at varying concentrations (Tables 1 to 3), indicating that the plant extract had broad antibacterial spectrum (Bankole, 1992). Presence of an inhibine factor in honey which is hydrogen peroxide and flavonoids have been observed to be responsible for its antimicrobial property. The data obtained showed that the inhibitory effects of the samples on the various investigated microorganisms was dose-dependent. This observation is in agreement with the findings of Agbaje et al. (2006) and Akinnibosun et al. (2009) who found that the efficacy of honey and most plant extract was concentration-dependent. Honey at 25, 50 and 75% concentration inhibited all the test orgainisms except *S. aureus* and *B. subtilis* (Table 1). Honey at 100% concentration inhibited growth of all the test organisms except *B. subtilis*. This is similar to the findings of Agbaje et al. (2006), as well as DeMera and Angert (2004), who noted that non-susceptibility of some test organisms to honey could be due to the emergence of resistant strain. Besides, several factors such as botanical and entomological origin may influence the antibacterial activity of honey. DeMera and Angert (2004) reported that honey from different phytogeographic regions varied in their ability to inhibit the growth of bacteria, suggesting that botanical origin plays an important role in influencing the antimicrobial activity. Various researchers have shown that honey exerts antimicrobial activities against various microorganisms (Allen et al., 1991; Anand and Shanmugam, 1998; Cooper and Molan, 1999).

Honey has been successfully used in the treatment of surgical wounds, burn wounds and decubitus ulcers. It has also been shown as a good medium to store skin grafts and honey has anti-leishmanial effect also (Vishnu et al., 2012). Honey may inhibit bacterial growth due to a number of different mechanisms. High sugar concentration, low pH, hydrogen peroxide generation, proteineceous compounds, or other unidentified compounds present in the honey may all provide antimicrobial activity (Mundo et al., 2004). Shrinkage and disruption of the bacteria may be due to its osmotic effect, low pH and also due to the presence of antibacterial substance such as inhibine (Vishnu et al., 2012). Besides its antimicrobial properties, honey can clear infection in a number of ways *in vivo,* like boosting the immune system, anti-inflammatory, and antioxidant activities and via stimulation of cell growth (Al-Jabri, 2005). Due to its antimicrobial properties, honey may serve as a natural food preservative (Mundo et al., 2004). Previous research has demonstrated preservative power of honey by reducing enzymatic browning of fruits (Chen et al., 2000), and preventing lipid oxidation in meat (Mckibben and Engeseth, 2002).

Aqueous bitter kola extract was most active against *P. aeruginosa* with zones of inhibition ranging from 16.70 ± 0.21 to 18.00 ± 1.00 mm and least active against *B. subtilis* with zone of inhibition of 0.00 to 6.00 ± 1.00 mm. This result is similar to the work of Adegboye et al. (2008), who showed that the crude extract of *G. kola* exhibited antimicrobial activities *in vitro* against both Gram-positive and Gram-negative organisms. *G. kola has* been medicinally used as an antimicrobial. The seeds are used in the treatment of bronchitis and throat infections (Mboto et al., 2009). The antimicrobial properties of this plant are attributed to the benzophenone

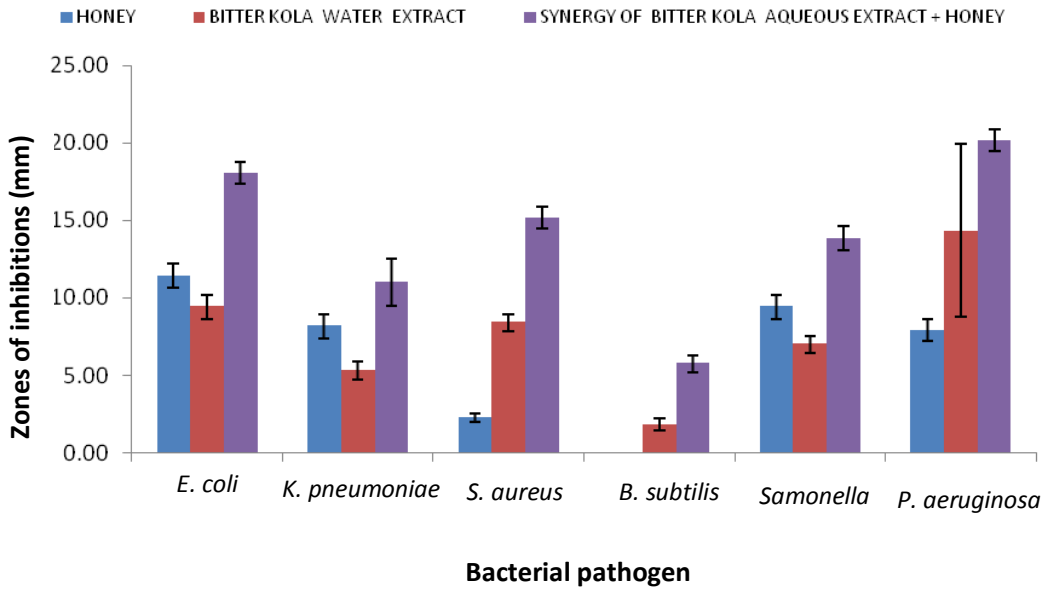

Figure 1. Average zones of inhibition in mm of the test bacteria with different treatments (honey, bitter kola and the synergy).

Table 6. Susceptibility testing (zone of inhibition in mm).

Test organism	Gentamicin (positive control)	Water (negative control)
E. coli	23.0	0.00
K. pneumoniae	16.0	0.00
S. aureus	0.0	0.00
B. subtilis	13.0	0.00
Salmonella sp.	22.0	0.00
P. aeruginosa	20.0	0.00

and flavonones. Studies have shown it to have very good antibacterial and antiviral properties (Adesuyi et al., 2012; Terashima et al., 2002). Traditionally, the plant is used as a natural antimicrobial. Other medicinal properties of the plant include usage in the treatment of skin infections in Liberia and Congo Democratic Republic. The powdered bark of the plant is applied to malignant tumors, cancers etc., the plants latex is taken internally for gonorrhoea and externally to seal new wounds and prevent sepsis (Adesuyi et al., 2011). In Nigeria, a cold water extract of the roots and bark with salt are administered to cases of bronchial asthma or cough and vomiting. *G. kola* seed is believed to contain a wide spectrum of organic compounds such as flavonoids which confer on it some antibacterial and antifungal actions against Gram negative and Gram positive microorganisms (Adesuyi et al., 2011).

The mixture of honey and bitter kola extract produced greater zone of inhibition for the test bacteria than bitter kola and honey used separately as shown in Figure 1. The synergistic effect of the *G. kola* and honey mixture on the test organisms showed in descending order as *P. aeruginosa* > *E. coli* > *S. aureus* > *Salmonella* > *K. pnemoniae* > *B. subtilis.* The mixture showed better antibacterial activity against the test organisms when compared with the standard antibiotic as shown in Table 6. Statistical analysis showed that *E. coli, S. aureus* and *P. aeruginosa* at $P < 0.05$ showed significant difference in their susceptibility to the synergistic mixture and separate use of *G. kola* extract and honey.

Susceptibility testing with standard antibiotic, gentamicin, showed zone of inhibition which ranged from 0.0 to 23 mm. From this observation, *G. kola* extract, honey and their synergy compared favourably with the standard antibiotic. The MIC and MBC of the samples against the test organisms were also determined. The MIC varied between 2.5 and 12.5, 0.0 and 100.0, as well as 2.5 and 7.5 mg/ml for *G. kola* extract, honey and *G. kola* extract and honey mixture, respectively. The MBC varied between 5.0 and 15.0, 0.0 and 100.0 as well as 5.0 and 10.0 mg/ml for *G. kola* extract, honey and *G. kola* extract and honey mixture, respectively. The results of

MIC and MBC showed that the synergistic use of honey and *G. kola* extract is more potent against the test organisms even at low concentrations, as shown in Tables 4 and 5. The broad spectrum of activity displayed by the samples in this study appears to justify and explain the scientific basis for their uses in traditional medicine.

Indiscriminate use of antibiotics has led to the emergence of drug-resistant strains which have a significant impact on patient's morbidity and mortality (Bhatia and Narain, 2010). It is hoped that this study would lead to the preparation of antibacterial drugs of natural origin for the treatment of infections caused by the test organisms.

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REFERENCES

Adegboye MF, Akinpelu DA, Okoh A (2008). The bioactive and phytochemical properties of *Garcinia kola* (Heckel) seed extract on some pathogens. Afr. J. Biotechnol. 7(21):3934 - 3938.

Adesuyi AO, Elumm IK, Adaramola FB, Nwokocha AGM (2012). Nutritional and Phytochemical Screening of *Garcinia kola.* Adv. J. Food Sci. Technol. 4(1):9-14.

Agbaje EO, Ogunsanya T, Aiwerioba OIR (2006). Conventional use of honey as Antibacterial agent. Ann. Afr. Med. 5(2):78-81.

Ajayi IA, Jonathan SG, Adewuyi A, Oderinde RA (2008). Antimicrobial screening of essential oil of some herbal plants from Western Nigeria. World Appl. Sci. J. 3(1):79-81.

Ajebesone PE, Aina JO (2004). Potential African substances for hops in tropical beer brewing. J. Food Technol. Afr. 9(1):13-16.

Akinnibosun FI, Akinnibosun HA, Ibeh IN, Osaghae F (2008a). Antibacterial activity of *Phyllanthus amarus* Schum and Thorn. on five vegetative organisms. Plant Archives 8(2):563-568.

Akinnibosun HA, Akinnibosun FI, Adieme BC (2009). Biotherapeutic potential of aqueous and ethanolic extracts of *Solenostemon monostachyus* (P. Beauv.) Brig. Leaves on some vegetative Gram-negative bacteria. Biol. Environ. Sci. J. Trop. 6(3):33 - 37.

Akinnibosun HA, Akinnibosun FI, German BE (2008b). Antibacterial activity of aqueous and ethanolic leaf extracts of *Peperomia pellucid* (L) H. B & K.(Piperaceae) on three Gram-negative bacterial isolates. Sci. World J. 3(4): 33-36.

Al-Jabri AA (2005). Honey, milk and antibiotics. Afr. J. Biotechnol. 4:1580-1587

Allen KL, Molan PC, Reid GM (1991). A survey of the antibacterial activity of some New Zealand honeys. J. Pharm. Pharmacol. 43:817-822.

Anand S, Shanmugam J (1998). Antistaphylococcal properties of natural honey. Biomed. 18:15-18.

Bankole AA (1992). The antibacterial activity of the crude seed extracts of three plants used in Nigerian ethnomedicine. University of Benin, Benin City, p. 45.

Bhatia R, Narain JP (2010). The growing challenge of antimicrobial resistance in the South-East Asia region- are we losing the battle? Ind. J. Med. Res. 132:482-486.

Cheesebrough M (2002). Biochemical tests to identify bacteria. *In* Laboratory practice in tropical countries. Cambridge Edition pp. 63-70.

Chen L, Mehta A, Berenbaum M, Zangerl A, Engeseth N (2000). Honeys from different floral sources as inhibitors of enzymatic browning in fruit and vegetable homogenates. J. Agric. Food Chem. 48:4997-5000.

Cooper RA, Halas E, Molan PC (2002b). The efficacy of honey in inhibiting strains of *Pseudomonas aeruginosa* from infected burns. J. Burn Care Rehabil. 23:366 - 370.

Cooper RA, Molan PC (1999). The use of Honey as an antiseptic in managing *Pseudomonas* infection. J. Wound Care 8:161-164.

Cooper RA, Molan PC, Harding KG (1999). Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds. J. R. Soc. Med. 92:283-285.

Cooper RA, Molan PC, Harding KG (2002a). The sensitivity to honey of Gram positive cocci of clinical significance isolated from wounds. J. Appl. Microbiol. 93:857-863.

Cowan MM (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12:564-582.

Dunford CR, Cooper P, Molan P (2000). Using honey as a dressing for infected skin lesions. Nurs. Times 96(14 Suppl):7-9.

El-Mahmood AM, Doughari JH, Chanji FJ (2008). *In* vitro antibacterial activities of crude extracts of *Nauclea latifolia* and *Daniella oliveri.* Sci. Res. Essays 3(3):102-105.

Fabiola BH, Tania U, Benedito PDF, DiogenesAparicio GC, Jose AM, Celso VN (2003). Effect of essential oil of *Ocimum gratissimum* on the Trypanosomatid. *Herpetomonas samuelpessoai.* Protozoologica 42:269-276.

Gill LS (1992). Ethnomedicinal Uses of plants in Nigeria. University of Benin Press, Benin City, Edo State, Nigeria p. 276.

Idu M, Omogbai EKI, Aghimien GEI, Amaechina F, Timothy O, Omonigho SE (2007). Preliminary phytochemistry, antimicrobial properties and acute toxicity of *Stachytarpheta jamaicensis* (L.) Vahl. Leaves. Trends in Med. Res. 2:193-198.

Iwu MW, Duncan DR, Okunji CO (1999). New antimicrobials of plant origin. *In*: Janick, J. (Ed.) Perspective on New Crops and New uses. ASHS Press. Alexandria, VA. pp. 107-108.

Jonathan G, Ohimain E, Kigigha L (2007). Antagonistic effects of extracts of some Nigerian higher fungi against selected microorganisms. Am-Eur. J. Agric. Environ. Sci. 2(4):364 - 368.

Jonathan SG, Fasid, IO (2005). Antimicrobial activities of some selected Nigerian mushrooms. Afr. J. Biomed. Sci. 8(2):83 - 87.

Jonathan SG, Fasidi IO (2003). Antimicrobial activities of *Lycoperdon pusilum* (Bat.Ex.) and *Lycoperdon giganteum* (Pers.) Nigerian edible macrofungi. Afr. J. Biomed. Res. 6:88-90

Levy SB, Marshall B (2004). Antibacterial resistance worldwide: causes, challenges and responses. Nat. Med. 10: S122 - S129.

Madunagu BE, Ebana RUB, Udo SM, Ndifon LT (2001). Antimicrobial effects of *Ixora divaricata* and *Citrus aurantifolia* on some pathogens and drug resistant *Neisseria gonorrhoeae.* Nig. J. Bot. 14:63-69.

Mahesh B, Satish S (2008). Antimicrobial activity of some important medicinal plants against plant and human pathogens. World J. Agri. Sci. 4:839-843.

Mboto CI, Eja ME, Adegoke AA, Iwatt GD, Asikong BE, Takon I, Udo SM, Akeh M (2009). Phytochemical properties and antimicrobial activities of combined effect of extracts of the leaves of *Garcinia kola, Vernonia amygdalina* and honey on some medically important microorganisms. Afr. J. Microbiol. Res. 3(9):557-559.

McKibben J, Engeseth NJ (2002). Honey as a protective agent against lipid oxidation in ground turkey. J. Agric. Food Chem. 50: 592-595.

Mundo MA, Padilla-Zakour OI Worobo RW (2004). Growth inhibition of foodborne pathogens and food spoilage organisms by select raw honeys. Int. J. Food Microbiol. 97:1-8

Mythilypriya R, Shanthi P, Sachdanandam P (2007). Oral acute and subacute toxicity studies with Kalpaamruthaa, a modified indigenous preparation on rats. J. Health Sci. 53(4):351-358.

Odugbemi T, Akinsulire O (2006). Medicinal plants by species names. *In*: T. Odugbemi (Ed.). Outlines and pictures of medicinal plants from Nigeria. University of Lagos Press, Lagos State, Nigeria.

Okemo P, Nwanta W, Chabra S, Fabry W (2001). The kill kinetics of *Azadirachta indica* A. Juss. (Meliaceae) extracts on *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans.* France J. Sci. Technol. 2:113-118.

Okunji CO, Tantalia AW, Hicks RP, Iwu MM, Skanchy DJ (2002). Capillary electrophoresis determination of biflavonones from *Garcinia*

kola in three traditional African medicinal formulations. Plant Med. 68:440-444.
Prescott LM, Harley JP, Klein OA (2005). Microbiology. 6th Edition. McGraw Hills, New York.
Rabe T, Vanstoden J (2000). Isolation of an antimicrobial sesquiterpenoid *from Warbugie salutaris.* J. Pharmacol. 93: 171-174.
Sahm DF, Washington JA (1990). Antibacterial susceptibility test dilution methods: *In*: Manuals of Clinical Microbiology. Lennette E. H. 5th Edition American Society for Microbiology, Washington D. C. pp. 1105-1116.
Stoke EJ (1975). Clinical Bacteriology. 4th Edition. Edward Arnold Publishers.
Terashima K, Takaya Y, Niwa M (2002). Powerful antioxidative agents based on garcinoic acid from *Garcinia kola.* Bioorganic Med. Chem. 10(5):1619-1625.
Uniyal SK, Singh KN, Jamwal P, Lal B (2006). Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalayan. J. Ethnobiol. Ethnomed. 2:1-14.
Vishnu PS, Mamatha B, Shivananda PG Indira B (2012). Honey as an antimicrobial agent against *Pseudomonas aeruginosa* isolated from infected wounds. J. Global Infect. Dis. 4(2):102-105.
Walsh C (2003). Antibiotics: Actions, Origins, Resistance, American Society for Microbiology (ASM) press, Washington, DC.
Zumla A, Lulat A. (1989). Honey- a remedy rediscovered. J. R. Soc. Med. 82:384-385.