

## Short Communication

# 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

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Accepted 7 July, 2013

Warm water extracts of the leaves of *Garcinia kola* and *Vernonia amygdalina* suspended in honey traditionally employed for the treatment of post circumcision wounds, fresh wounds and chronic skin ulcers was prepared and evaluated for its phytochemical properties and antimicrobial activities. The phytochemical analysis of the preparation revealed the presence of polyphenol, reducing sugars, tannins, glycoside, alkaloids, saponins, flavonoids and anthraquinones. Neat (100%) concentration of preparation inhibited the growth of *Escherichia coli*, *Proteus mirabilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Candida albicans*. At 50% concentration, the growth of *P. aeruginosa* while *S. aureus* and *K. pneumoniae* were inhibited while at 25% concentration only *P. aeruginosa* was inhibited. The result suggests that the preparation exhibited significant *in vitro* antimicrobial activity against common wound isolates and may be employed for the routine treatment of wounds and sepsis as an alternative to antibiotics chemotherapy.

**Key words:** Extracts, antimicrobial, honey, *Garcinia kola* and *Vernonia amygdalina*.

## INTRODUCTION

Traditional preparations and medicinal plants with antimicrobial activities have been extensively used in the West African regions (Boyd et al., 1994; Silva et al., 1996). Traditional treatment of circumcision wounds, other wounds and chronic skin ulcers with locally prepared herbs and other natural occurring substances has been known for generations. Extensively employed includes leaves extracts of *Garcinia kola*, *Vernonia amygdalina* (Almagoul et al., 1985) and honey (Efem, 1988). They have been shown to be quite effective even where antibiotics treatments have failed (Efem, 1988; Dunford et al., 2000). With the increasing emergence of multiple antibiotics resistance, wound isolates are posing enormous public

health concerns thus making the need for exploring possible alternatives a necessity. Mboto (2000) in an unpublished study provided evidence of accelerated healing in a combined therapy of *Garcinia kola*, *Vernonia amygdalina* and honey for the treatment of fresh wounds, including wounds resulting from male circumcision and chronic ulcers. In the present study, the phytochemical properties and antimicrobial activities of combined effects of extracts of the leaves of *G. kola*, *V. amygdalina* and honey were evaluated against a select group of commonly encountered microbial pathogens.

## MATERIALS AND METHODS

### Extracts

Approximately 12 g of mature but fresh leaves of *V. amygdalina*

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**Table 1.** Phytochemical components of the extracts in honey.

Phytochemical component	Remarks
Polyphenol	+++
Reducing sugars	++
Tannins	++
Glycoside	++
Alkaloids	+
Saponins	+
Flavonoids	+
Antraquinones	+
Phlobatannins	-
Hydroxymethyl antraquinones	-

+++ High concentration, ++ moderate concentration, + low concentration, - not detected

and *G. kola* were homogenized into 50 ml of sterile but warm water and 30 ml of freshly harvested honey from African wild killer bees was added to the extract to make it up to 80 ml mark. Sealed airtight bottles of extracts were stored at room temperature until required for use.

#### Sterility testing

The sterility of the preparation was evaluated before used by plating it out on blood agar (BA) and nutrient agar (NA) plates and incubated with CO<sub>2</sub> at 37°C for 48 h.

#### Microbial isolates

Stock cultures of some wound infecting organisms (*Escherichia coli*, *Proteus mirabilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Candida albicans*) were obtained from microbiology laboratory of the university of Calabar teaching hospital, Calabar, Nigeria. Standard inoculum density of these isolates for susceptibility testing was prepared using barium sulphate (BaSO<sub>4</sub>) turbidity standard, equivalent to a 0.5 McFarland standard (Trease and Evans, 1989). The correct density of the turbidity standard was verified by measuring its absorbance at 625 nm wavelength using Optima spectrophotometer SP300 (Optima Inc. Japan).

#### Phytochemical screening

Phytochemical analysis of the combined extracts in honey was done following standard methods of Trease and Evans (1989).

#### Susceptibility testing

Tube dilution susceptibility testing method described by Lalitha (2005) was adopted. This was used to assess the minimal concentration of extracts that can kill or inhibit the growth of the tested microbial isolates. 2-fold serial dilution of the combined extracts in honey in nutrient broth (NB) and Sabouraud dextrose broth (SDB) were prepared respectively. 7 concentrations (1.56, 3.13, 6.25, 12.5, 25, 50 and 100% (neat) v/v) were obtained. 4 ml of the extracts nutrient broth concentrations were dispensed into 5 sets of screw-cap tubes in duplicates while a set of tubes for the extracts/SDB concentrations was also prepared in duplicates. 6 control tubes (5 NB and 1 SDB) were prepared. All tubes with their contents were sterilized by autoclaving at 121°C for 15 min.

Upon cooling the tubes were arranged in rows of 7 each to represent the different concentrations. Each row was inoculated with 50 ul adjusted 24 h broth culture of test isolates. Row of tubes with the extracts/SDB was also inoculated with 50 ul of 24 broth culture of *C. albicans*.

Following inoculation, 100 ul of 10<sup>-4</sup> dilutions of different extracts/broth concentrations were spread plated on nutrient agar (NA) (for bacterial isolates) and Sabouraud dextrose agar (SDA) (for *C. albicans*). Plating was done in duplicate, NA plates were incubated at 37°C while SDA plates were incubated at room temperature. Counts from plates were recorded after 24 h incubation.

## RESULTS AND DISCUSSION

Phytochemical analysis of the extracts is as presented in Table 1. Polyphenol was present in high concentration (+++) while reducing sugars, tannins and glycosides were moderately (++) present. Alkaloids, saponins, flavonoids and antraquinones were present in low concentration (+). Phlobatannins and hydroxymethyl antraquinones were not present in the extracts. Some of these bioactive substances have been found to be present in bitter kola (*G. kola*), honey and bitter leaf (*V. amygdalina*) (Almagoul et al., 1985). The phytochemical compounds found in this study are similar to the findings of Adegboye et al., (2008) using *G. kola* and is in line with several other reports (Almagoul et al., 1985; Odiogonyi et al., 2009). The result of antimicrobial activities shows that the extracts exhibited antimicrobial effect on the tested organisms. Neat (100%) concentration of the extracts inhibited the growth of all the isolates. The growth of *P. aeruginosa* was inhibited by 25 and 50% concentration of the extracts while *S. aureus* and *K. pneumoniae* were also inhibited by 50% concentration of the extracts. The inhibiting property of the extracts was established but we cannot categorically state which of the components of the extracts showed the profound antimicrobial activities. However, several studies have shown that saponins, tannins, flavonoids and phenolic compounds possess antimicrobial activities (Subrahmanyam et al., 2001; Osman et al., 2003, while various researchers have shown that honey exerts antimicrobial activities against

various microorganisms (Jeddar et al., 1985; Anand and Shanmugam, 1998; Cooper and Molan, 1999; Allen et al., 1999).

The antimicrobial activities of honey are due to the present of inhibines in honey. These inhibines are flavonoids, hydrogen peroxide, phenolic acid and many other unidentified inhibines (Osman et al., 2003). The contributory effects of the other components of the extracts cannot be ignored in view of the presence of bioactive substances that have antimicrobial effects (Subrahmanyam et al., 2001). The antibacterial activity of extracts of *V. amygdalina* against some gram-negative and gram-positive bacterial has been reported (Okoh et al., 1995; Taiwo et al., 1999). It has been suggested that bitter leaf could be effectively used against drug resistant microorganisms (Iwalokun et al., 2003). Furthermore, *G. kola* has been medicinally used as an antimicrobial. The seeds are used in the treatment of bronchitis and throat infections. The antimicrobial properties of this plant are attributed to the benzophenone and flavonones. Studies have shown it to have very good antimicrobial and antiviral properties (Iwu, 1993). Adegboye et al. (2008) in a recent but similar study has also shown that crude extract of *G. kola* exhibited antimicrobial activities *in vitro* against both gram-positive and gram-negative organisms with zones of inhibition similar to that shown by streptomycin and tetracycline.

Beside the use of *V. amygdalina* (bitter leaf) as a soup condiment and for wound dressing, extracts of *V. amygdalina* (bitter leaf) has also been shown to improve brewing qualities and amino acid profiles of stout drinks (Lasekan et al., 1998), although this later quality was not evaluated in this study.

This study has shown that a combination of *G. kola* and *V. amygdalina* extracts suspended in honey inhibits the growth of *P. aeruginosa*, *K. pneumoniae*, *S. aureus*, *P. mirabilis*, *E. coli* and *C. albicans* at neat concentration. The antimicrobial results of this study confirm the uses of bitter kola (*G. kola*), honey and bitter leaf (*V. amygdalina*) on some of the claimed ethno-medical uses in the treatment of various infectious diseases. We suggest further studies on the individual constituents or extracts of bitter kola (*G. kola*), honey and bitter leaf (*V. amygdalina*) in order to obtain the right combination and or composition that could be used effectively in treatment.

## ACKNOWLEDGMENTS

Our profound appreciation goes to the Chief Medical Laboratory Scientist of UCTH for the isolates used in this work. G. Iwatt is also indebted to the management of the Federal Psychiatric Hospital for granting free access to the use of their spectrophotometer, while CIM thanks Chief Imoke Imoke of Itigidi, for the supply of the fresh plants employed in this study.

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