Full Length Research Paper

Antibacterial activity of bitter leaf (Vernonia amygdalina) soup on Staphylococcus aureus and Escherichia coli

Lovet T. Kigigha¹* and Ebubechukwu Onyema²

Department of Biological Sciences, Niger Delta University Wilberforce Island, P. M. B., 71, Yenagoa, Bayelsa State, Nigeria.

Accepted 25 July, 2015

The study was aimed at assessing the effect of cooking Egusi-soup on the antibacterial activity of bitter leaf (Vernonia amygdalina). Extracts of the leaves of bitter-leaf plant were made using Ethanol, from Bitter-leaf soup and using hot water. The antibacterial activity of the extracts was tested using two pathogenic hospital isolates viz. Staphylococcus aureus and Escherichia coli. The antibiotic, Ampiclox (a broad-spectrum antibiotic in 1 mg ml⁻¹ concentration) was used as control. The test showed that all the three forms of extracts were inhibitory to the two isolates in which the hot water extract indicated the highest inhibitory zone on E. coli and also with S. aureus (P < 0.001). This was followed by the inhibitory effect of bitter leaf soup extract which was significantly higher than the inhibitory effect of Ampiclox on E. coli (representing the enteric bacteria) (P = 0.025). Interestingly, the effect of bitter leaf soup extract was also higher than the effect of Ampiclox on S. aureus (representing superficial etiologic agents) indicating a probable positive effect on the health of probably external and internal mucosa. The observed antibacterial effect of V. amygdalina on the selected bacterial isolates appear to justify the traditional use of bitter leaf as a choice vegetable for African soup recipes and for the cure of a number of human diseases (such as the treatment of gastroenteritis, fever and wound infections etc).

Key words: Vernonia amygdalina, bitter leaf, antibiotic, Enterobacteriaceae, Escherichia coli, Staphylococcus aureus.

INTRODUCTION

Plants extracts are continuously being sort for as effective and cheaper alternative sources of medication all over the world especially in the developing countries. Vernonia amygdalina commonly called bitter leaf (because of its bitter taste) is consumed either as a vegetable for cooking African soups or the aqueous extracts could be drank as tonics for the treatment of various illnesses (Imaga and Bamigbetan, 2003). The bitterness is suspected to be due to factors such as the presence of alkaloids, saponins, tannins and glycosides which have been shown by various authors to be present in bitter leaf (Butler and Bailey, 1973; Ologunde et al., 1992 and Bonsi et al., 1995). In the wild, chimpanzees have been observed to ingest the leaves Vernonia plants when suffering from parasitic infections. According to Huffman et al. (1993), the roots of V. amygdalina have been used for gingivitis and toothache due to its proven antimicrobial activity.

This study was designed to investigate the effect of cooking Egusi soup (a major conduit for using bitter leaf in Nigeria and in Africa generally) on the antibacterial activity of the plant. This is because bitter leaf soup when consumed gives a great soothing, appetizing and unusually satisfying after-effect.

LITERATURE REVIEW

Global use of medicinal plants

According to World Health Organization (WHO, 2007), medicinal plants are plants in which one or more of its parts contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. The local use of natural plants for primary
health remedies is a common practice in Asia, Latin American and Africa (Bibitha et al., 2002). Though many plants are consumed as food without an in-depth knowledge of their exact chemical composition and contribution to health, their utilization through several generations appear to justify their use (Ghani et al., 1989). Use of plant extracts or their active principle may serve as source of new drugs or sources of intermediate compounds for synthesizing analog drugs (Akerele, 1993).

Bitter leaf (Vernonia amygdalina)

Vernonia amygdalina, a member of the Asteraceae family, is a small ever green shrub that grows in tropical Africa. It is a shrub of 1 - 3 m in height with petiole leaf of about 6 mm in diameter and elliptical in shape (Igile et al., 1995). The plant is mostly found in West Africa where the most used part is its leaves (called bitter leaf). The leaves are dark green in colour with a characteristic odour and when chewed has a bitter taste but delicious in meals due to its pleasant nostalgic bitterness when it interacts with protenous ingredients (such as fresh or dry fish) in the soup. Local names by which the plant is called in Nigeria include: Kiriologbo (in Ijaw); Onugbu (in Igbo), Ewuro (in Yoruba) and Shiwaka (in Hausa).

The leaves are used as soup condiment and as vegetable after crushing and washing off using water to remove some of the bitterness (Mayhew and Penny, 1998). Other African soups, in which bitter leaf is used apart from Egusi soup, include: Ogbono and Okra soups. In many parts of west Africa, the leaves could also be used for washing slime off fish and snail before cooking while roots and twigs could be used as chewing-stick.

All parts of the plant are pharmacologically suspected to be useful. Both the roots and leaves are used in the treatment of fever, hiccups, kidney disease and stomach discomfort, among others (Gill et al., 1992; Hoomiona and Saffaf, 1994). The plant is claimed to also exhibit anti-helmitic and anti-malaria properties (Abosi and Raserika, 2003) as well as anti-tumourgenic properties (Izevbige et al., 2004). Locally it is used in treating stomach ache (for immediate relief). The expressed extract is used in treating skin infection such as ringworm, itching, rashes and eczema. It is also claimed to cure diabetes, loss of memory, pneumonia and arthritis. Studies by Oboh and Masodje (2009) indicated that V. amygdalina fresh leaf had moisture content of 83-0%; dry matter of 17-02%; protein 1-30% and ash content 0-50%). Its mineral content (per gram) is Phosphorus 61-55 µg; Selenium 8-2 µg; Iron 4-71 µg and Zinc 1-13 µg.

Previous research works on Vernonia amygdalina

Indication is that various studies have been carried out on V. amygdalina. Fred et al. (2009), researched on the nutritional and antimicrobial properties of V. amygdalina leaves. In the same year, Ibrahim et al. (2009) carried out the assessment of the antibacterial activity of V. amygdalina and Ocimum gratissimum leaves on selected food borne pathogens. The aqueous and ethanol extracts of these plant leaves were tested against Escherichia coli, Staphylococcus aureus, Bacillus cereus, Shigella dysentriae and Salmonella typhimurium in which the plants showed antibacterial activity on the entire test isolates. Imaga and Bamigbetan (2013) carried out an in vivo biochemical assessment of aqueous extracts of V. amygdalina (Bitter leaf). Indication was that there was an improved functionality of the antioxidant system of the test rats due to the probable effect of the phytochemicals and antioxidants in the plant extract. It was inferred that aqueous extract of V. amygdalina could be consumed as food or as an herbal medicine without appreciable toxicity to body organs and tissues.

The present study was designed to investigate the effect of cooking Egusi soup (a major conduit for using bitter leaf in Nigeria and Africa generally) on the antibacterial activity of the plant. This is because bitter leaf soup when consumed gives a great soothing, appetizing and unusually satisfying after-effect; thus it would be of medicinal interest to ascertain the extent of depreciation or otherwise of the known antibacterial activity of the plant when consumed in Egusi soup.

MATERIALS AND METHODS

Preparation of plant extracts

Samples of fresh plant of V. amygdalina leaves were bought at Amassoma market in Bayelsa state of Nigeria. The plant was used to prepare three different extracts: i) the leaf sample was rinsed three times with distilled and deionized water. 100 g of the leaf (in three batches) were pounded using sterile mortar and pestle. The first batch was soaked in 100 ml of ethanol which was stoppered with cotton wool covered with aluminum foil and left for 24 h. in the refrigerator. This was then filtered using Whatman No.1 filter paper and the extract stored in the refrigerator for use. ii) The second batch of 100 g of pounded bitter leaf was filtered using 100 ml of boiled water and stoppered with cotton wool covered with aluminum foil and left for 24 h in the refrigerator. The extract was then filtered using what man No.1 filter paper and stored in the refrigerator for use. iii) The third batch of 100 g pounded bitter leaf was used for preparing Egusi soup. Bitter leaf soup preparation was started with an initial 800 ml of water. Ingredients used alongside with V. amygdalina include; fresh pepper, salt, soup thickener, dry fish, meat, crayfish, palm oil, onion, magi. After cooking, the final volume of the soup was 100 ml.
This was strained with sterilized and then filtered using Whatman No.1 filter paper and the extract stored in a refrigerator for use.

**Media and apparatus**

All media were prepared according to manufacturer’s specifications while all glassware were washed with detergent and rinsed with deionized and distil water three times and were then autoclaved for 15 min.

**Test bacterial isolates**

Clinical isolates of *S. aureus* and *E. coli* were obtained respectively from Gloryland Clinic, Yenagoa, Bayelsa state and the Department of Pharmaceutical Microbiology Laboratory, College of Health Sciences, Niger Delta University, Bayelsa state. They were characterized with respect to their morphological, biochemical and antibiotic susceptibility.

**Preparation of antibacterial discs from leaf extracts**

The disk-diffusion technique was used to test the antibacterial activity of the different extracts using 10 mm discs (Kigigha and Atuzie, 2010). Antibiotic discs were prepared from Whatman No. 1 filter paper using cock borer. The solution containing the extracts (0.1 ml each) were impregnated on the disc in triplicates using sterile pipette and air-dried.

**Antibacterial assessment of extracts**

Using flamed-sterilized and cooled pair of forceps the discs which had been impregnated with the bitter-leaf extracts including the one percent Ampiclox control were (in triplicates) placed on separate Nutrient agar plates which had been confluent inoculated with the test *E. coli* and *S. aureus* isolates. The discs were well spaced in the agar plates to avoid overlapping of the zones of inhibition. The plates were inoculated at 37°C for 72 h to observe the zones of inhibition.

**RESULTS**

The result as shown in Figure 1, indicated that all the three extracts were inhibitory to the two bacterial test isolates viz. *E. coli* and *S. aureus* (which represent the enteric forms and the superficial etiologic agents respectively). From Figure 1, the hot water extract indicated the highest inhibitory zone on *E. coli* and also
on *S. aureus* compared to the Ampiclox control (P < 0.001). This was followed by the inhibitory effect of bitter leaf soup extract on *E. coli*, which was significantly higher than the inhibitory effect of Ampiclox on *S. aureus* (P < 0.001) and also higher than the inhibitory effect of Ampiclox on *E. coli* (P = 0.025).

In Table 1, the test isolates were characterized accordingly. The antibiotic resistance pattern in Table 2, indicated that *E. coli* was resistant to six out of the ten antibiotics tested viz. Tetracycline, Cefuroxime, Ampicillin, Ampicillin, Gentamycin and Norfloxacin. While the *Staphylococcus* isolate was resistant to three out of the ten antibiotics tested viz. Clindamycin, Cephalexin and Ominoxazole.

### Statistical analysis

The differences in the mean values among the treatment groups were greater than would be expected by chance; there was statistically significant difference (P < 0.001) using *SigmaStat* statistical package. All pair wise multiple comparison procedures at level of Comparison P < 0.050, (or Tukey Test) indicated as follows:

- Hot W Ec vs. AMPI Sa (P <0.001); Hot W Ec vs. AMPI Ec (P<0.001);
- Hot W Ec vs. BLS Sa (P <0.001); Hot W Ec vs. Et Ex Ec (P = 0.004); Hot W Ec vs. Hot W Sa (P = 0.036);
- BLS Ec vs. AMPI Sa (P = <0.001); BLS Ec vs. AMPI Ec (P = 0.025);
- Hot W Sa vs. AMPI Sa (P = <0.001); Hot W Sa vs. AMPI Ec (P = 0.036);
- Et Ex Sa vs. AMPI Sa (P = <0.001); Et Ex Ec vs. AMPI Sa (P = <0.001).

### Discussion

The antibiotic resistance pattern indicated that the test
isolates were resistant to a number of antibiotics; invariably, they were viewed to have originated from some pathogenic forms. The inhibition on both \textit{E. coli} and \textit{S. aureus}, indicated that extraction of bitter-leaf with hot water, was significantly higher than that obtained from bitter-leaf soup extraction. This appears to indicate a probable positive effect on the health status of enteric etiologic agents and on the superficial internal and external mucosa. Of the known 17 species of the genus \textit{Vernonia} all have been shown to exhibit therapeutic characteristics viz. as blood purifier, uterus toner and also to prevent atherosclerosis (Erasto et al., 2007; Nwanjo, 2005). Many herbalists and naturopathic practitioners recommend aqueous extracts for their patients as treatment for anaemia, nausea, diabetes, loss of appetite, dysentery and other gastrointestinal track ailments. \textit{V. amygdalina} extracts have also been reported to help suppress, delay, or kill cancerous cells (Kupchan et al., 1969).

The present study showed that the cooking of Egusi soup (a major conduit for using bitter leaf in Nigeria and Africa generally) decreased the antibacterial activity of the plant probably due to the presence of different proteinous ingredients such as dried fish, crayfish etc. But then it was yet to be ascertained if the presence of the same proteinous ingredients may not be responsible for detoxifying bitter leaf extract and thus giving the soothing and pleasant feeling of satisfaction that accompanies bitter-leaf Egusi-soup consumption. The study also appears to justify the traditional use of bitter-leaf as vegetable and as a medicinal plant.

**REFERENCES**


Bonsi MLK, Osuji PO, Tuah AK, Umunna MN (1995). \textit{Vernonia amygdalina} as supplement of teff straw (Eragrostis tef) fed to Ethiopian Menz sheep. \textit{Agroforestry Syst.}, 31: 229-244.


Further observations on the use of the medicinal plant, \textit{Vernonia amygdalina} (Del) by a wild chimpanzee it’s possible effect on parasite load, and its phytochemistry. \textit{Afr. Study Monogr.}, 14(4): p227-240.


Further observations on the use of the medicinal plant, \textit{Vernonia amygdalina} (Del) by a wild chimpanzee it’s possible effect on parasite load, and its phytochemistry. \textit{Afr. Study Monogr.}, 14(4): p227-240.

