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Evaluating the antimicrobial potency of crude extracts of *Psidium guajava* bark, leaves of *Vernonia amygdalina*, *Carica papaya* and whole plant of *Phyllanthus niruri* against specific pathogenic bacteria

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The potency of hot water, cool water and ethanoilc extracts of bark and leaves of *Psidium guajava*, leaves of *Vernonia amydalina*, *Carica papaya* and whole plant of *Phyllanthus niruri* were assessed against isolates *of Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus*, and *Candida albicans* obtained from the Mampong Research Institute of Herbal medicine in the Eastern region of Ghana. Antibacterial tests were carried out using the agar wells diffusion method. The susceptibility test results showed that extracts of *P. niruri* are very effective on all gram negative bacteria and extracts of *Psidium guajava* were effective on both gram negative and positive bacteria. Although *V. amygdalina* extracts showed inhibition to some bacteria in both hot water and ethanolic extract, hot water extract of *C. papaya* showed inhibition to *S. aureus* and *S. typhi* but room water extracts of both *V. amygdalina* and *C. papaya* showed quite a good results. Hence the potency of these plants base on their inhibition zones can be effectively used on some infectious disease caused by the test bacteria.

Key words: Antimicrobial, Crude extracts, *Psidium guajava*, *Phyllanthus niruri, Vernonia amydalina*, *Carica papaya,* bacteria.

INTRODUCTION

The plant kingdom has become a medicinal goldmine due the quest for suitable and affordable alternatives in the face of increasing antibiotic resistance by various strains of bacteria. This has led researchers into exploring the use of plant extracts in the treatment of bacterial infections. Antibiotics provide the main basis for the therapy of microbial infection. Since the discovery of these antibiotics and their uses as chemotherapeutic

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agents, there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases (Rosina et al., 2009). In the light of the emrgence of rapid global spread of resistant clinical isolates, the need to find new antimicrobial agent is of paramount importance. However, the past record of rapid, wide spread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy (Coates et al., 2002). The use of plants, plant extracts or plant-derived chemicals to treat diseases is a therapeutic modality that has been explored for centuries. Over 40,000 species of tropical flowering plants are said to possess medicinal properties (Idu et al., 2008) and are currently in use for various medical conditions. The majority of the people of African descent patronize herbal or traditional medicine for their health needs. It is estimated that 70-80% of patients in Africa are treated by traditional healers and herbal practitioners (Diallo et al., 1996; Nyika, 2007). Furthermore, about 40% of all medicines on the market today have been derived directly or indirectly from natural sources; 25% being from plants, 13% from microorganisms and 2% from animals (De Smet, 1997; Blumenthal, 1999).

Phyllanthus niruri is an annual plant best known by the common names Stonebreaker (Eng.). It is a small erect herb that grows 30-40 cm in height. It is a relative of the spurges**,** belonging to the leaf flower genus of Family *Phyllanthaceae*. All parts of the plant exhibit medicinal properties. It is used medically as a diuretic and an astringent (Umarani, 1985). One *in vitro* study and four *in vivo* studies (with rats and mice) document that extracts of *P. niruri* effectively protect against liver damage from various chemical liver toxins (Syamasundar et al., 1985). *P. niruri* was an effective single drug in the treatment of jaundice in children, (Bhumyamalaki, 1983) and that children treated with a *P. niruri* extract for acute hepatitis had liver function return to normal within five days (Thabrew,1996).

Vernonia amygdalina, variously known as bitter leaf (English), suwaka (Dagaare) oriwo (Edo), ewuro (Yoruba), shikawa (Hausa), and olubu (Igbo), is a tropical shrub, 1-3 m in height with petiole leaf of about 6 mm in diameter, and elliptic in shape (Igile et al., 1995). All parts of the plant are pharmacologically useful. Both the roots and leaves are used in phyto-medicine to treat fever, hiccups, kidney disease and stomach discomfort, among others (Gill, 1992; Hamowia and Saffaf, 1994). The plant has been proved in human medicine to possess potent antimalarial and antihelminthic properties (Abosi and Raseroka, 2003) as well as antitumorigenic properties with an amazing antiparasitic efficacy in zoo pharmacognosy as it is easily recognized and used for self-medication by parasitized chimpanzees (Huffman, 2003). Both aqueous and alcoholic extracts of the stem, bark, roots and leaves have been extensively used as a purgative, antimalarial and in the treatment of eczema (Kupcham, 1971). Pharmacological studies have also shown that the leaf extract has both hypoglycaemic and hypolipidaemic properties in experimental animals and so could be used in managing diabetes mellitus (Akah and Okafor, 1992; Nwanjo, 2005). *V. amygdalina* Del. contains significant quantities of lipids (Ejoh et al., 2007; Eleyinmi et al., 2008), proteins with high essential amino acid score, carbohydrates and fibre (Igile et al., 1994; Udensi et al., 2002) that compare favorably with values reported for *Telfairia occidentalis* and *Talinum* triangulare (Ijeh et al., 1996). The plant has also been shown to contain appreciable quantities of ascorbic acid and caroteinoids (Udensi et al., 2002; Ejoh et al., 2007). Calcium, iron, potassium, phosphorous, manganese, copper and cobalt have also been found in significant quantities in V. *amygdalina* (Bonsi et al., 1995; Ejoh et al., 2007; Eleyinmi et al., 2008).

Psidium guajava is a low evergreen tree or shrub 6 to 25 feet high, with wide-spreading branches and square, downy twigs. *P. guajava* is used worldwide for different purposes. Parts of the plant mostly used include; leaves, bark, roots, and flowers. The plant provides astringency, wounds healing, ulcers and skin damage repair properties. In India, decoction of the leaves and bark of guava is used to cure diarrhea, dysentery, vomiting and sore throats, and to regulate menstrual cycles. It was shown that *P. guajava* leaf extracts might be beneficial in treating acne especially those that have anti-inflammatory activities. In Ghana and in Nigeria the leaves are chewed to relieve toothache. A decoction of the root-bark is recommended as a mouthwash for swollen gums and decoction of the leaves makes an efficacious gargle for swollen gum and ulceration of the mouth and also for bleeding gums. Some of the ethno-medicinal uses includes the crushing of the leaves and the application of the liquids coming out from them on wounds, cuts, ulcers, boils, skin and soft tissue infectious site, rheumatic places (Bala, 2006) and the chewing of the leaves to relieve toothache, oral ulcers, inflamed gums, throat, chest pains, treatment of leucorrhea, diarrhea, dysentery, convulsions and epilepsy, as well as the use of the decoctions and infusions as a douche for vaginal discharges and to tighten and tone of vaginal walls after childbirth (Burkil, 1994). In some cultures, a decoction of the leaves is drunk to regulate menstrual periods and expel the placenta after child birth (Lozoya et al., 2002). It has anti-amoebic and antimalarial effects (Morton, 1987;Tona et al., 1998). The leaves and bark of *P. guajava* tree have a long history of medicinal uses that are still employed today (Nwinyi et al., 2008). The phytochemical components of the guava plant have been established in previous studies. Guava is rich in tannins, phenols, triterpenes, lectins, quercetins, leucocyanidins, sequiterpenes hydro-carbons, caryophyllenes, sterols, gallic acid, guavins A, C and D, carotenoids, vitamins, fibres and fatty acids (Akinpelu and Onakoya, 2006; Kamath et al., 2008). Several in-vitro studies have shown

significant antimicrobial activities against Staphylococcus, Shigella, Salmonella, Bacillus, *E. coli*, Clostridium, Pseudomonas and Candida spp. (Akinpelu and Onakoya, 2006; Mbuh et al., 2008). The leaves are used to treat diarrhea and stomach ache in Columbia, Mexico, USA, Ghana, Nigeria etc. They are also used in USA as antibiotic in the form of poultice or decoction for wounds, ulcers and tooth ache (Heinrich, 1998; Leonti et al., 2001).

Carica papaya is an erect, fast-growing, usually unbranched tree or shrub, 7-8 m tall, with copious latex, trunk about 20 cm in diameter, soft, leaves clustered near top of plant, alternate, long-petiolate, blade suborbicular, to 80 cm long, palmately 7-11-lobed; lobes glabrous, toothed, flat; Papaya is cultivated for its ripe fruits, favored by tropical people, as breakfast fruit, and as an ingredient in jellies, preserves, or cooked in various ways; juice makes a popular beverage; young leaves, shoots, and fruits cooked as a vegetable (Duke, 1984b). The high level of natural self-defence compounds in the tree makes it highly resistant to insect and disease infestation (Peter, 1991). The seed is used for intestinal worms when chewed. The root is chewed and the juice swallowed for cough, bronchitis, and other respiratory diseases. The unripe fruit is used as a remedy for ulcer and impotence, (Elizabeth, 1994). Fresh, green pawpaw leaf is an antiseptic, whilst the brown, dried pawpaw leaf is the best as a tonic and blood purifier (Atta, 1999). Chewing the seeds of ripe pawpaw fruit also helps to clear nasal congestion, (Elizabeth, 1994). The green unripe pawpaw has therapeutic value due to its antiseptic quality. It cleans the intestines from bacteria, more so that (only a healthy intestine is able to absorb vitamin and minerals, especially vitamin B12). The tea, prepared with the green papaya leaf, promotes digestion and aids the in treatment of ailments such as chronic indigestion, overweight and obesity, arteriosclerosis, high blood pressure and weakening of the heart (Mantok, 2005). Roots said to cure piles and yaws.

The root infusion is used for syphilis in Africa. In Asia, the latex is smeared on the mouth of the uterus as ecbolic. Javanese believe that eating *papaya* prevents rheumatism. Dietary *papaya* does reduce urine acidity in humans. Flowers have been used for jaundice. Fruit and seed extracts have pronounced bactericidal activity against *Staphylococcus aureus, Bacillus cereus, Escherischia coli, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumoniae and Shigella flexneri*. Among the gram-positive and gram-negative bacteria tested, the gram-negative bacteria were more susceptible to the extracts. The fact that the extracts were active against both gram-negative and gram-positive tested indicates a broad spectrum of activity (Emeruwa, 1982). The main objective of this research is to assess the anti-bacteria potency of extract from *P. Guadiana*, *V. amygdalina, C. papaya,* and *P. ninuri* on bacterial activities.

MATERIALS AND METHODS

Sampling and Sample preparation (extracts)

Fresh leaves of the plant materials (*P. niruri, P. guajava*, *V. amydalina,* and *C. papaya*) were collected from the three Northern Regions of Ghana. The leaves of the plants were air dried (in a shade) at room temperature for 2 weeks and ground to coarse powder (about 0.5 mm mesh size). 5 g(2 teaspoons) of the powder was placed in 50 ml each of cold water(27° ± 2°C), hot water (by boiling-100°C) and 70% ethanol in conical flasks and kept for 30 minutes after it was thoroughly mixed by shaking it. For the ethanolic extract, the ethanol was evaporated by exposing the extract to atmospheric air because the ethanol has effect on most bacterial. The extracts were filtered and stored at 4°C prior to use (Alabi et al., 2012). Clinical strains of *E. coli, S. aureus*, *P. aeruginosa, C. albicans* and *S. typhi* were obtained from the Mampong Research Institute of Herbal Medicine, maintained on nutrient agarand stored at 4°Cbefore use.

Standardization of Inoculums

Exactly one loop of cultured test organisms in nutrient broth were inoculated into 5 ml of peptone water for 2 h for standardization of the culture for use. This standard method is in agreement with those of Evans (2002) and Sofowora (2008).

Antibacterial Testing

The agar wells diffusion method was used. 0.5 ml of 2 h old culture in peptone of each clinical isolate was aseptically transferred to the solidified Mueller Hinton agar and spread evenly on the agar surface using a sterile glass spreader. Four 6 mm wells were bored unto the agar and filled with the extract while distilled water served as the control. The Petri dishes were incubated at 37°C for 24 h and the inhibition zones were measured.

RESULTS

Table 1 shows the results of hot aqueous crude extract of *P. niruri, P. guajava, C. papaya* and *V. amydalina* on clinical isolates of *C. albicans*, *E. coli, S. aureus, P. aeruginosa* and *S. typhi. C. albicans* were resistant to *C. papaya* extract but highly susceptible to extract of *P. niruri, P. guajava* and *V. amydalina. E. coli* was resistant to *C. papaya* extract but susceptible to extract of *P.niruri, P. guajava* and *V. amydalina. S. aureus* were resistant to *P. niruri*, and *V. amydalina* extract but susceptible to extract of C*. papaya* and *P. guajava. P. aeruginosa* was resistant to *C. papaya* extract but susceptible to extract of *P. niruri, P. guajava* and *V. amydalina* and *Salmonella typhi* was susceptible to extract of all the four plantsmost especially *Phyllanthus niruri* and *V. amydalina*.

Table 2 shows the susceptibility pattern of cold aqueous crude extract (27 ± 2°C) of *P. niruri, P. guajava C. papaya* and *V. amygdalina* on clinical isolates of *C. albicans, E. coli, S. aureus*, *P. aeruginosa* and *S. typhi*. *C. albicans* was resistant to *C. papaya* and *V. amydalina* extract but highly showed inhibition to extract of *P. niruri* and *P. guajava. E. coli* was susceptible to all the various

Table 1. Inhibition zones of hot aqueous extract against clinical bacterial isolates.

Clinical bacterial isolates	Diameter of inhibition zone in millimeters(mm)			
	P. niruri	***P. guajava***	***C. papaya***	***V. amydalina***
Candida albicans	21±0.14	20±0.84	-	21±0.21
Escherichia coli	19±0.14	13±0.21	-	18±0.00
Staphylococcus aureus	-	13±0.14	10±0.28	-
Pseudomonas aeruginosa	11±0.28	11±0.56	-	8±0.84
Salmonella typhi	23±0.14	20±0.14	9±0.28	19±0.14

Legend: (-) means no inhibition.

Table 2. Inhibition of cool aqueous extract against clinical bacterial isolates.

Clinical bacterial isolates	Diameter of inhibition zone in millimeters (mm)			
	P. niruri	***P. guajava***	***C. papaya***	***V. amydalina***
Candida albicans	6±0.28	11±0.28	-	-
Escherichia coli	14±0.28	11±0.42	12±0.28	12±0.24
Staphylococcus aureus	-	21±0.21	9±0.56	23±0.14
Pseudomonas aeruginosa	14±0.56	25±0.56	-	13±0.14
Salmonella typhi	20±0.28	13±0.14	20±0.14	21±0.28

Legend: (-) means no inhibition.

Table 3. Inhibition zones of ethanolic extract against clinical bacterial isolates.

Clinical bacterial isolates	Diameter of inhibition zone in millimeters(mm)			
	P. niruri	***P. guajava***	***C. papaya***	***V. amydalina***
Candida albicans	21±0.42	17±0.14	-	14±0.28
Escherichia coli	26±0.42	16±0.28	-	19±0.28
Staphylococcus aureus	-	22±0.28	-	-
Pseudomonas aeruginosa	-	12±0.14	-	-
Salmonella typhi	22±0.28	20±0.14	-	-

Legend: (-) means no inhibition.

extracts, *S. aureus*s showed inhibition to *C. papaya* and *V. amydalina* but resistant to *P. guajava*; *P. aeruginosa* was resistant to *C. papaya* extract but susceptible to extract of *P. niruri, P. guajava* and *V. amygdalina* but most especially *P. guajava. S. typhi* was highly susceptible to extract of all the four selected plants. Table 3 shows the outcome of the ethanolic herbal extracts against the bacterial isolates. The extract of *P. niruri, P. guajava* and *V. amygdalina* inhibited the growth of *C. albicans* except *C. papaya* that it was resistant to. *E. coli* showed resistance to *C. papaya* susceptibility to *P. niruri, P. guajava* and *V. amydalina*; *P. aeruginosa* exhibited susceptibility only to *P. guajava* extract. *P. guajava* extract was also the only effective product on *S. aureus*. *S. typhi* was inhibited by *P. niruri, P. guajava* and *V.amydalina* but resistant to *Carica papaya*.

DISCUSSION

Hot water extract of *P. guajava* has proven to be the most effective in bacterial inhibition. This is followed by its ethanolic extract and cold water extracts. Hot and cold water extracts of *P. niruri* and *V. amygdalina* showed good inhibition of the test bacterial but their ethanolic extract did not do so well. *C. papaya* cold water extract inhibited bacterial better than its hot water extract but its ethanolic extract did not yield any positive results; this could be due the denaturing of chemical elements in the plant by the ethanol. Hot water may have denaturing effect on *C. papaya.* This finding is in consonance with research work reported (Bhasha et al., 2014; Uma and Beena, 2014).

This work revealed great potential of plant for

therapeutic purposes in spite of the fact that they have not been completely investigated. It is interesting to know that the extracts were effective against at least one or more of the bacterial isolates. *E. coli* has been known to be multi-drug resistant and *P. aeruginosa* which is very difficult to control by therapeutic means were all by one extract or more inhibited. In this study, the extracts showed considerable antibacterial activity against the clinical isolate: the gram positive isolate *S. aureus* as the most susceptible to extracts of *P. guajava.* This activity could be attributed to the tannins present (Lutete et al., 1994). The effectiveness of Guava as an antimicrobial was confirmed by Abdelrahim et al. (2002). *P. niruri,* and *V. amydalina* extracts were the most effective ones on *S. typhi.* These finding are in consistence with those of Okechukwu et al. (2012) and Nirosha and Mangalanayaki (2013).

Candida was effectively inhibited by extracts of *P. guajava, P. niruri*, and *V. amydalina* except cool extract of *P. niruri*, and *V. amygdalina. P. guajava* was effective for *P. aeruginosa*. This is in agreement with Aruljothi*et al*., (2014).

CONCLUSION

Hot, cool and ethanolic extracts of *P. guajava* inhibit all the test organisms. Both hot and ethanolic extract of *P. niruri* and *V. amygdalina* had no effect on *S. aureus*. All extract of *C. papaya* had no effect on all the test organisms except cool extract only which showed slight inhibition on candida and Pseudomonas. Depending on the type of infectious diseases, an effective extracts of plant materials should be prepared to inhibit the disease concerned. It can also be concluded that the combination of these extracts can yield an effective herbal products/ remedy that can cure a good number of bacterial infection.

RECOMMENDATION

Various plants should be prepared under hygienic condition.

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Conflict of interest

The authors hereby declare that there was no conflict of Interest in the preparation of this manuscript.

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