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Antibacterial Screening of Crude Extract of Oven-Dried Pawpaw and Pineapple

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Abstract- Epidemiological surveillance of antimicrobial resistance is indispensable for empirically treating infections, implementing resistance, control measures and preventing the spread of antimicrobial resistant micro-organisms. Crude extracts from oven-dried pawpaw (Carica *papaya*) and pineapple (*Ananas comosus)* were tested against *Staphylococcus aureus*, *Pseudomonas aureginosa, Escherichia coli* and *Streptococcus pneumonia*. Even though, these test bacteria showed multidrug resistance against controlled conventional antibiotics, it was observed that the extract from *C. papaya* showed the highest potency at a concentration of 1.00g/ml on all the test bacteria, the efficacy of *A. comosus* was also pronounced against all the bacteria at the same concentration. The sensitivity of *P. aeruginosa* and *Staph aureus* were not significantly different ($p \leq 0.05$) at all concentrations of *A. comosus* extract, except at the concentration of 0.4g/ml and the sensitivity of all the test organisms were not significantly different at a concentration of 0.8g/ml. The sensitivity of all the test organisms at different concentrations of *C. papaya* extract varied significantly ($p \leq 0.05$), though *E. coli* and *Strept pneumonia* were not significantly different at a concentration of 0.2g/ml. The efficacy of fruits in therapy against diseases or infections from which these bacteria were isolated cannot be underestimated.

Index Terms- Antibacterial screening, *Ananas comosus, Carica papaya*, crude extract.

I. Introduction

The search for newer sources of antibiotics is a global challenge preoccupying research institutions, pharmaceutical companies and academia. This is because many infectious agents are becoming resistant to synthetic drugs. Infectious diseases are the world's major threat to human health and account for almost 50,000 deaths everyday (Cowan, 1999). The situation has further been complicated with the rapid development of multidrug resistant micro-organisms.

In developing countries, orthodox medicine is often the only accessible and affordable treatment available (Kare, 2007). Medicinal plants consisting compounds of therapeutic properties and have been in use as remedies for human diseases since early civilization. In recent time, the demand for the medicinal plants is increasing in both developing and developed countries due to growing recognition of natural products as being non-toxic, devoid of side effects, easily available and of affordable prices. In Nigeria, there are several medicinal plants with potent antibacterial, antidiabetic, antihyperlipidemic and antidysuria activities that lack scientific scrutiny, examples of such plants are pineapple (*Ananas comosus*) and pawpaw (*Carica papaya*) (Anibijuwon and Udeze, 2009).

Ananas comosus is commonly known as pineapple, it is an herbaceous and perennial plant. This plant is known for its folklore medicinal utility besides its use for nutritional food. Sixty percent (60%) of fresh pineapple is edible (FAO, 2005). It is composed of several nutrients such as digestible carbohydrates, raw fibres, protein, vitamins A and C and water which are essential for human health. It also contains essential nutrient such as calcium, copper, iron, magnesium, manganese, phosphorus and potassium which are also useful to the body (Praveen *et al*., 2014). *A. comosus* fruits contain bromelain, a proteolytic enzyme which is used as a meat tenderizing agents and for its medicinal purposes such as interfering with the growth of malignant cells, inhibiting platelet aggregation, fibrinolytic and anti- inflammatory action. It also acts in the same way as pepsin present in the stomach and facilitates digestion in the digestive tract (Mondaol *et al*., 2011). According to Khare (2007), a poultice of pineapple leaf is applied to the skin to alleviate rheumatism and other infections like eczema. The juice of the fruits is taken orally as herbal remedy of arthritis, heamaturia and liver ailment (Rejendra *et al*., 2012). *A. comosus* possesses a wide array of pharmacological properties such as antibacterial activities, antihyperlipidemic activities, antidysuria activities and antitumor activities (Praveen *et al*., 2014).

Carica papaya commonly known as pawpaw has been used as remedy against a variety of diseases (Anibijuwon and Udeze, 2009). The plant is widely found in Nigeria, it is a lozenge tropical fruit, often seen in orange –red, yellow-green and yellow-orange hues, with a rich orange pulp (Ghani, 2003). The fruit is not just delicious, but the whole plant parts fruits, roots, bark, peels, seeds and pulp are also known to have medicinal properties (Akujobi and Ofodiam, 2010). The numerous benefits of *C. papaya* is due to its high content of vitamins A, B and C, calcium, beta-carotene, thiamine, lycopene, proteolytic enzymes like papain and chymopapain which have antiviral, antifungal and antibacterial properties (Bansode and Chavan, 2013). It can be used for the treatment of numerous diseases like wart, corns, sinuses, eczema, cutaneous tubercles, glandular tumors, blood pressure, dyspepsia, constipation, amoebic dysentery, convulsion and amenorrhea as a result of this; *C. papaya* can be regarded as a nutraceutical (Doughari *et al*., 2007). The fruits, leaves and flowers of pawpaw are edible, its seeds have anthelmintic activity and the roots can be used as medicine for renal and urinary bladder problem, the extracts have phenolic compounds such as protocatechuic acid, P-coumaric acid and caffeic acid (Ahmad and Beg, 2001).

The aim of this research is to evaluate and compare the antibacterial activity of the crude extract of oven dried pawpaw and pineapple on some selected bacteria and to provide a scientific basis for the use of the fruits to prevent and treat infections from which the tested bacteria are isolated.

II. MAERIALS AND METHOD

A. Collection and treatment of samples

Fruit samples of pineapple and pawpaw were collected from Aba Igbira opposite Fajuyi housing estate, along Ilawe road in Ado Ekiti. They were washed with tap water, cut into small sizes and oven dried at 60^0c for 10 days. They were homogenized into fine powder and stored separately in sterile containers.

B. Extraction of Bioactive Compound

100g of each dried powder of (pineapple and pawpaw) was added separately to 500ml of local gin. The samples were allowed to stand for six hours (6hrs) at room temperature ($28^oC\pm1^oC$) with intermittent shaking. Each sample was filtered using Whatman no 4 filter paper and evaporated to dryness by using rotary evaporator.

C. Reactivation of Microorganisms

The bacteria used were *Streptococcus pneumomoniae, Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli.* They were removed from stock, streaked onto nutrient agar (oxoid) plates and incubated for 24hr at 37^oC to reactivate them.

D. Susceptibility Testing of Organisms on the Crude Extracts of C. papaya and A. comosus

Different concentrations (0.2g, 0.4g, 0.6g, 0.8g, and 1.0g) of each extract was weighed and dissolved separately in 2ml of distilled water. These extracts were incorporated into sterilized paper disks made from No1whatman filter paper. The medium used (nutrient agar) was prepared according to the manufacturer specifications, the broth culture of each organism was inoculated into the prepare agar plates using sterilized cotton swabs .The disks with the different concentrations were placed on the inoculated plates, incubated at 37^oC for 18-24 hr and observed for growth and the diameter of the zones of inhibition was measured in millimeter using a meter-rule.

E. Susceptibility Testing of Organisms on Convetional Antibiotics

Conventional antibiotics such as septrin, erythromycin, amoxacillin, ampiclox, streptomycin and gentamycin were used as control against the test organisms at a concentration of 20µg.

III. RESULTS

The results of the screening are shown in the tables below. In table 1, the highest zone of inhibition (12.00mm) was observed in all the test bacteria at a concentration of 1.0g /ml. Table 2 shows the susceptibility of different concentration of extract from pawpaw, the highest zone of inhibition (13.50mm) was evident against *E. coli* at a concentration of 1.00g/ml; other susceptibilities were shown against all the test bacteria at concentration ranging from 0.8g/ml to 1.0g/ml. In table 3, the test organisms showed multidrug resistance to all the conventional antibiotics at a concentration of 20µg.

Table 1: Susceptibility Pattern of Test Organisms to Extract of *A. comosus*

Test organisms	Concentration(g/ml) Diameter of zones of inhibition (mm)				
	0.20	0.40	0.60	0,80	1.00
Streptococcus pneumoniae	5.00 ± 0.14^a	8.00 ± 0.14^b	10.00 ± 0.14^b	11.00 ± 0.14^a	$13.00\pm0,14^b$
Pseudomonas aeruginosa	6.00 ± 0.14^b	7.00 ± 0.14^a	9.00 ± 0.71^a	$11,00\pm0.14^a$	12.00 ± 0.71^a
Staphylococcus aureus	6.00 ± 0.70^b	8.00 ± 0.71^b	9.00 ± 0.14^a	11.00 ± 0.14^a	12.00 ± 0.14^a

Values are means of duplicates ± standard deviation, mean within the same column carrying the same superscript are not significantly different at $p \leq 0.05$.

Table 2: Susceptibility Pattern of Test Organisms to Extract of *C.papaya*

Test organisms	Concentration (g/ml) Diameter of zones of inhibition(mm)				
	0.20	0.40	0.60	0.80	1.00
Streptococcus pneumoniae	6.00 ± 0.14^a	8.00 ± 0.14^b	$7.5\pm0,07^a$	11.00 ± 0.14^a	14.00 ± 0.14^c
Pseudomonas aeruginosa	7.50 ± 0.07^b	9.00 ± 0.07^c	10.00 ± 0.14^c	12.50 ± 0.07^c	13.00 ± 0.14^a
Escherichia coli	6.00 ± 0.14^a	7.00 ± 0.14^a	9.00 ± 0.07^b	12.00 ± 0.14^b	13.50 ± 0.01^b

Values are means of duplicates ±standard deviation, mean within the same column carrying the same superscript are not significantly different at $p \leq 0.05$.

Table 3: Susceptibility Pattern of Test Organisms to Conventional Antibiotics

Test bacteria	Antibiotics					
	SXT	E	AM	APX	S	CN
Staphylococcus aureus	R	R	R	R	R	S
Eschericia coli	R	R	I	I	R	R
Pseudomonas aeruginosa	R	R	R	S	R	I
Streptococcus pneumoneae	R	R	R	R	S	S

SXT: septrin, E: ethrythromycin, AM: amoxacillin, APX: ampiclox, S: streptomycin CN: gentamycin.
R: resistant, I: intermediate, S: sensitive.
≤10mm: resistant, 11mm -14mm: intermediate, ≥15mm: sensitive

IV. DISCUSSION

The results obtained from table 1 and 2 showed that pineapple and pawpaw extracts are effective on the test bacteria due to the exhibition of wide zones of inhibition. As evident in table 1, the sensitivity of *Pseudomonas aeruginosa* and *Staphylococcus aureus* are not significantly different at all concentrations of *A. comosus* extract, except at the concentration of 0.4g/ml and the sensitivity of all the test organisms are not significantly different at a concentration of 0.8g/ml. In table 2, the sensitivity of all the test organisms at different concentrations of *C. papaya* extracts vary significantly, though *E. coli* and *Streptococcus pneumonia* are not significantly different at concentration of 0.2g/ml. In all, the zones of inhibition are greater than the standard dimension of 5.00mm at concentrations of 0.20 to 1.00g/ml. The susceptibility of the test bacteria to the fruit extracts is high, the potency of the extracts are due to the presence of active bio-agents like E-citral, D-limonene, a-felandren in pineapple and chymopapin, pectin and benzilsothiocyanate in pawpaw. The efficacy and potency of the extracts on the test bacteria corroborate the earlier findings of Akujobi and Ofodemi, (2010). They authenticated the antibacterial activities of pineapple and pawpaw and discussed that the efficacy was due to the numerous active components possessed by the fruits. Table 3 shows multidrug resistance of all the test bacteria to the convectional antibiotics, such multidrug resistant has important implications for the empirical therapy of infection caused by *E. coli*, *Staph. aureus, Pseudomonas aeruginosa and Streptococcus pneumoniae.*

The folklore uses of plants have been observed to be very important because of the high percentage of bioactive components possessed by them. Fruits have been proven not to be an exemption, thus Pineapple and pawpaw apart from their nutritive values and the supply of vitamins, their extracts have been used for specific purposes especially in food and in the treatment of infectious diseases like yaws, syphilis, eczema and gonorrhea (Timesis, 2000). Phytochemical agents present in them can synergistically be effective in therapy. However, because of their heterogeneous composition and antimicrobial activities of their active components, it seems unlikely that there is only one mechanism of action or that only one of their constituents is responsible for this action. Pineapple phytochemicals of interest in therapy have been identified as E-citral which has strong antimicrobial properties, D-limonene that has potent antioxidant, anti-cancer and anti-inflammatory properties and offers assistance for a variety of metabolic and health problem. Others of interest are neral, germacrene D, g-terpinen, a-felandren and a-copaene (Doughari *et al*., 2007). Papin, chymopapin, pectin and benzilsothiocyanate are phytochemicals of interest in therapy that are present in pawpaw. They have antiviral, antifungal and antibacterial properties, these active components act singly and in combination (Timesis, 2000). At single activity, they show a high therapeutic effect on pathogenic and spoilage food micro-organisms. The observation made by Akujobi and Ofodemi, (2010) correlates with the findings of this screening.

From the above, it can be inferred that the extracts possess significant growth inhibitory activity on test organisms like *Staph aureus* and *strept pneumonia* that are gram positive and *E. coli and P. aeruginosa* that are gram negative bacteria. The efficacy of pineapple and pawpaw extracts against these bacteria may provide a scientific ground for the application of the fruits in the prevention and treatment of bacterial infections caused by the test bacteria. The usage of fruits, especially the ones tested in this research work, for therapeutic purposes require continuous examination, because the antimicrobial resistance pattern are continually evolving and *E. coli*, *Staph. aureus*, *Pseudomonas aeruginosa* and *Strep pneumonia* undergo progressive antimicrobial resistance continuously. Undated data and antimicrobial susceptibility profile will continue to be essential to ensure the provision of safe and effective empiric therapy. Fruit-therapy has since been a research for thought and it has been observed that it has a reasonable therapeutic implication in combating diseases from which the above test bacteria have been isolated.

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