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RESEARCH PAPER

ANTIBACTERIAL ACTIVITIES OF DIFFERENT SOLVENT EXTRACTS OF CARICA PAPAYA FRUIT PARTS ON SOME GRAM POSITIVE AND GRAM NEGATIVE ORGANISMS

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ABSTRACT

Aside its nutritional values, there are speculations that *Carica papaya*, also known as paw paw, has antibacterial potentials. This study evaluates the antibacterial potentials of different extracts of *C.papaya* parts, in comparison with standard drugs (perflacine and cefuroxine). Dried and grinded papaya leaves (5g), fruit peels (5g); and seeds (5g), respectively mixed with 95ml of extraction solvent (water, ethanol, 1% HCl, acetone and petroleum ether) for 24 hours, were used for this study. The constituent compounds were filtered aseptically and inoculated unto MacConkey and Nutrient agars to verify the sterility of the solutions for utilization. Results showed high antimicrobial activity for the extracts of *C.papaya* in petroleum ether with a Minimun Inhibitory Concentration (MIC) of 2mg/ml as against 4mg/ml and 6mg/ml for perflacine and cefuroxine respectively. Extracts in 1% HCl and ethanol however, showed antimicrobial activity against the gram positive and negative organisms investigated, while extracts in water was only active against *Escherichia coli* and *S. aureus*. No statistical difference ($P<0.05$) was observed in the antimicrobial activities between the extracts of petroleum ether and the standard antimicrobial drugs. These suggest that *C. papaya* may be used as an antibiotic, and extracts in petroleum ether seems more potent.

Key words: ***Carica papaya, Antimicrobial, Extraction solvents, Plant parts.***

INTRODUCTION

The use of local plants as primary health remedies, due to their pharmacological properties, is quite common in Asia, Latin America, USA, China, Japan and Africa (Bibitha et al., 2002). According to Boakye-yiadon and Dwuma-Bada (1997), there are over 10,000 medicinal plants in West Africa, used for disease treatment and prevention. While infectious diseases are reported to be the world's major human threat and accounting for almost 50,000 deaths every day (Ahmad and Beg, 2001), the frequency and diversity of life-threatening infections caused by pathogenic microorganisms has increased steadily; becoming an important cause of morbidity and mortality in immune-compromised patients especially in developing countries (Al-Bari et al., 2006). The situation is further complicated with the rapid development of multidrug resistance to the available antimicrobial drugs (Nirosha and Mangalanayaki, 2013). This has globally challenged research institutions, pharmaceutical companies and the academia, and has led to the search for newer sources of antibiotics that must be more effective, affordable and readily available (Adekunle and Adekunle, 2009; Latha and Kannabiran, 2006).

Plants have been reported to be the cheapest and most effective source of drugs (Mathur et al., 2011; Prince and Prabakaran, 2011; Pretorius and Watt, 2001). In recent times, the use of herbal medicine in third world countries has increased, owing to the fact western orthodox medicines are relatively expensive and readily not available. This

explains the increasing research on various plants, and the upsurge in mass-media advert placement on herbal preparations by countless 'traditional doctors'.

Of interest in this study, is *Carica papaya* (*C. papaya*) commonly known as pawpaw (English), Adiba (Ewe–Ghana) or Bofre (Twi–Ghana). It belongs to the family of *Caricaceae* and several species of *Caricaceae* have been used as medication against a variety of diseases (Mello *et al*., 2008). Available evidence indicate that *C. papaya* can act as an analgesic, amebicide, antibacterial, cardiotonic, cholagogue, digestive, emenagogue, febrifuge, hypotensive, laxative, pectoral, stomachic and vermifuge (Anibijuwon and Udeze, 2009). Many scientific investigations have also been conducted to evaluate the biological activities of their various parts including fruits, shoots, leaves, rinds, seeds, roots or latex (Baskaran et al., 2012; Maisarah et al., 2013). In fact, a number of studies worldwide have investigated the antimicrobial properties of plants and *C. papaya* has been reported to serve as a source for therapeutic alternatives (Adriana et al., 2007).

Studies conducted in different parts of Africa have demonstrated the significant antibacterial activity of various extracts pawpaw tree parts (Doughari et al., 2007; Dawkins et al., 2003; Emeriwa, 1982). Specifically, Osato et al., (1993) reported that the latex of *C. papaya* is bacteriostatic to *Bacillus spp*, *Enterobacter coacae*, *Escherichia coli*, *Saimonella typhi*, *Staphylococcus aureus* and *Proteus vulgaris.* The seed was reported to have antimicrobial activity against *Trichomonas vaginalis* trophozoites and was suggested it could be used in urinogenital disorder like trichomoniasis, however, with care to avoid toxicity (Calzada et al., 2007). In addition to its antimicrobial qualities, scientists have discovered that *C. papaya* can mitigate many side effects associated with synthetic antimicrobial agents (Rajeshwar and Gupta, 2005; Iwu et al., 1999).

Despite these scientific facts on the antimicrobial potentials of *C. papaya*, little information exits on studies that have compared the potencies of extracts from different parts in varied extraction solvents. Hence, this study investigates the antibacterial activity of different parts of *Carica papaya* in different extraction solutions, in comparison with standard drugs (perflacine and cefuroxine).

MATERIALS AND METHODS

Processing of plant samples: Plant materials were collected from in and around Ekpoma, Edo State, Nigeria. The seed, fruit peel and leaves, were collected, washed in tap water, rinsed in sterile distilled water and dried for 5 days at 60^0 C in Lab 1 of the Department of Microbiology, Ambrose Alli University, Ekpoma. The dried plant parts were blended to powder with a clean kitchen blender and stored in airtight glass containers kept in laboratory cupboard, until required for preparation.

Preparation of extracts: 5grams of each *C. papaya* parts was weighed into 100ml reagent bottle and 95ml of extraction solvent was added and left to extract on a mechanical shaker overnight at room temperature. This was done using all the five extraction solvents (water, ethanol, 1% HCl, acetone and petroleum ether) and the three plant parts (seed, leaves and peel).

The extract solution was filtered aseptically into another 100ml reagent bottle using a watt-man No 1 filter paper. All the filtrate were screened for purity by inoculation unto MacConkey agar and nutrient agar plates and incubated at 37^0C for 48 hours. Filtrates yielding growth of any organism was re-filtered and rescreened for purity until a sterile extract solution was obtained, following the methods outlined by Orhue (2004).

Micro organism preparation/growth: The test organisms used are all human pathogenic organisms of clinical origin. They include one strain of Gram positive bacteria (*Staphylococcus aureus*) and two strains of Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). They were obtained from the Department of Microbiology, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma-Nigeria, where they were kept as stock cultures at 4°C. Biochemical analysis was carried out on each of the test organisms for confirmation.

Determination of Minimum Inhibitory Concentration (MIC): Using a 50ml specific gravity bottle, the density of the extract solution was determined. In a similar manner, the density of the plain solvent was also determined. To determine the concentration of the extract, the density of the plain was subtracted from that of the extract solution. This was done for all 5 extract solvents. With the known extract concentrations and the three clinical isolates of *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*, the MIC of the extract solutions, peflacine as well as cefuroxine were determined. The experiments were performed in 3 repetitions for each of the extraction solvents and *C. Papaya* parts, and the average was calculated.

Data analysis: Data were keyed into SPSS (version 16) and the average of each determined MIC was then presented in suitable table for simple descriptive comparism. The MICs of the different *C. Papaya* parts in the different extraction solutions were compared with the values of the standard antibiotic drugs using ANOVA (LSD) at 95% level of confidence.

RESULTS

As shown in table 1, 2, and 3, there was no difference in the minimum inhibitory concentrations (MIC) recorded for the different *C. papaya* parts. However, there were differences in MIC in-terms of the extraction solvent used. Also, differences were observed in the patterns *S. aureus, E. coli* and *P. aeruginosa*, react to the *C. papaya* parts extracted with water, 1% HCl and acetone, while ethanol and petroleum ether react similarly to the organisms herein studied.

Specifically, extraction with water showed the weakest antimicrobial potential while petroleum ether was the most powerful, both with minimum inhibitory concentrations of 30.0 mg/ml and 2.0 mg/ml respectively for *S. aureus* and *E. coli.* However, water extraction did not show any effect on *P. aeruginosa*. The extraction with ethanol presented a minimum inhibitory concentration of 28.0 mg/ml in all the organisms tested upon. Extraction in Acetone presented a minimum inhibitory concentration of 24.0 mg/ml for *S. aureus* and 30.0 mg/ml for *E. coli* and *P. aeruginosa*. Extraction in 1% HCl followed that of petroleum ether in term of antimicrobial potency and presented a MIC of 15.0 mg/ml for *P. aeruginosa* and 9.6 mg/ml for *S. aureus* and *E. coli.*

Compared to the standard antibiotic drugs; perflacine and cefuroxime that showed minimum inhibitory concentrations of 4.0 mg/ml and 6.0 mg/ml respectively to the organism herein study, *C. papaya* parts extraction in petroleum ether was a more powerful antibiotic and presented a minimum inhibitory concentration of 2.0 mg/ml. No statistical difference ($P<0.05$) was observed in the antimicrobial activities between the extracts of petroleum ether and the standard antimicrobial drugs. With other extraction solvent used in this study, perflacine and cefuroxine were more potent compared to the extraction in 1% HCl following with a MIC of 9.6 mg/ml for *S. aureus* and *E. coli* and 15.0 mg/ml for *P. aeruginosa*.

Table 1: MIC of extract from *carica papaya* seed compared with standard antibiotics

Organisms Isolated	Standard anti-biotic drugs		Extraction Solutions from *Carica papaya* seed				
	Perflacine	Cefuroxime	**Water**	**Ethanol**	**1%HCl**	**Acetone**	**Petroleum ether**
S. aureus,	4.0*	6.0*	30.0	28.0	9.6*	24.0	2.0*
E.coli,	4.0*	6.0*	30.0	28.0	9.6*	30.0	2.0*
P. aeruginosa	4.0*	6.0*	0	28.0	15.0	30.0	2.0*

* signifies statistical significant in antimicrobial activities

Table 2: MIC of extract from *carica papaya* leaves compared with standard antibiotics

Organisms Isolated	Standard anti-biotic drugs		Extraction Solutions from *Carica papaya* leaves				
	Perflacine	Cefuroxime	**Water**	**Ethanol**	**1%HCl**	**Acetone**	**Petroleum ether**
S. aureus,	4.0*	6.0*	30.0	28.0	9.6*	24.0	2.0*
E.coli,	4.0*	6.0*	30.0	28.0	9.6*	30.0	2.0*
P. aeruginosa	4.0*	6.0*	0	28.0	15.0	30.0	2.0*

* signifies statistical significant in antimicrobial activities

Table 3: MIC of extract from *carica papaya* peel compared with standard antibiotics

Organisms Isolated	Standard anti-biotic drugs		Extraction Solutions from *Carica papaya* peel				
	Perflacine	Cefuroxime	**Water**	**Ethanol**	**1%HCl**	**Acetone**	**Petroleum ether**
S. aureus,	4.0*	6.0*	30.0	28.0	9.6*	24.0	2.0*
E.coli,	4.0*	6.0*	30.0	28.0	9.6*	30.0	2.0*
P. aeruginosa	4.0*	6.0*	0	28.0	15.0	30.0	2.0*

* signifies statistical significant in antimicrobial activities

DISCUSSION

Being anti-microbial infers that any of these properties: - anti-bacterial (antibiotics), anti-fungal (anti-myotic), anti-cancerous (anti-oncogenic) or anti-viral is inherent (Clark, 1996). The present study showed that the different parts of *C. papaya* possess antimicrobial potential against *S. aureus, E. coli* and *P aeruginosa*. In line with the present finding, several other studies have reported *C. papaya* leaves (Baskaran et al., 2012; Anibijuwon and Udeze, 2009), seeds (Ocloo et al., 2012; Calzada et al., 2007) and peel (Aravind et al., 2013) to have antimicrobial potentials. Several other reports also, have shown that *C.papaya* have significant antibacterial activity in various extracts from different tree parts (Ifesan et al., 2013; Nirosha and Mangalanayaki, 2013; Doughari et al., 2007; Dawkins et al., 2003; Emeriwa, 1982). The observed anti-bacterial results of this study are similar to the reported works on plant extracts and antimicrobial efficacies of the quinolones and cephalsporins (Momoh *et al.*, 2011).

Also, it was observed that the potency of the activity of *C.papaya* against microbes depends on the extraction solvent used. *C. papaya* in organic extracts such as petroleum ether and 1% HCl, were more effective than *C. Papaya* in aqueous extracts like water. This may be due to the fact that there is better solubility of the active components in organic solvents (De Boer et al., 2005). Also, other researchers have reported that organic extracts of the dried seed of C. papaya, produces microbial inhibition (Dawkins et al., 2003; Emeriwa, 1982).

The Therapeutic value of medicinal plants has been reported to lie in the various chemical constituents in it. In fact, active principles singly or in combination inhibit greatly the life processes of microbes, by binding with their protein molecules, acting as chelating agents (selective binding polyvalent metal ions so that the latter loses its biological activities), altering their biochemical systems, preventing utilization of available nutrients to the microorganisms (Garrod *et al.*, 1995). In addition, bioactive substances have been reported to confer resistance to plants against bacteria, fungi and pests and therefore explain the demonstration of antibacterial activity by the plant extracts used in this study (Srinivasan et al., 2001). In these regard, Aravind et al. (2013) reported that the many benefits of papaya, is due to the high content of Vitamins A, B and C, proteolytic enzymes like papain and chymopapain, that have antiviral, antifungal and antibacterial properties.

The results of different studies have previously provided evidence about some medicinal plants and have suggested they might indeed be potential sources of new antibacterial agents (Rahman et al., 2011; Kone et al., 2004). This work has been able to highlight the possible antimicrobial potentials of *C. papaya* parts. Extraction of the active component of the plant and the resulting minimum inhibitory concentrations recorded against well-known Gram positive and Gram negative organisms as well as a *Pseudomonas aeruginosa*, a highly resistant bacterium, gives a head start to pharmaceutical industries willing to undertake and develop potent antibiotics from *C. papaya*. Hence, a strong case is made on the need for government and grant agencies to commit resources to this novel area as its potentials to deliver potent antibiotics against multi-drug resistant (MDR) strains cannot be over emphasized

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AUTHORS' CONTRIBUTIONS

Both authors contributed their technical expertise to this study as well as the presentation of this manuscript.