**ANTIBACTERIAL ACTIVITIES OF *Carica papaya* SEED AGAINST ENTERIC ORGANISMS**

**BY**

**NWOSU, JONADAB CHIKA**

**A RESEARCH PROJECT SUBMITTED TO THE DEPARTMENT OF MICROBIOLOGY,**

**FACULTY OF NATURAL AND APPLIED SCIENCES**

**GODFRY OKOYE UNIVERSITY UGWUOMU NIKE ENUGU STATE**

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**GODFRY OKOYE UNIVERSITY UGWUOMU NIKE ENUGU STATE**

**IN PARTIAL FULFILMENT OF THE REQUIRMENT FOR THE AWARD OF BACHELOR OF SCIENCES (B.Sc.) DEGREE IN MICROBIOLOGY**

**SUPERVISOR**

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**JULY, 2018**

**APPROVAL PAGE**

This project has been presented and approved by Godfrey Okoye University, Enugu in partial fulfilment of the requirement for the award of Bachelor of Science, degree in microbiology from the department of microbiology, faculty of natural and applied sciences.

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**DEDICATION**

This work is dedicated to God Almighty whose unfathomable love and inexhaustible grace made it possible for this work to be accomplished.

**ACKNOWLEDGEMENT**

I want to give thanks to God Almighty who has been guiding and protecting me throughout my project period. My deepest appreciation goes to my late mum, Mrs. Ngozi Nwosu and to my Dad Mr. Chukwubuike Nwosu, who started my academic foundation and for taking care of me. In a special way, my appreciation goes to my Sponsor, Dr. Anyozie David Kekeocha for making it possible for my tertiary education to be a reality and for providing for all my necessary needs. My spiritual director, Rev. Sis. Mary Odinakachukwu Nwosu for always being there spiritually, physical and financially.

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**ABSTRACT**

Lots of research has been conducted on *Carica papaya* and its antibacterial efficacy on bacterial pathogens and this led to this study on antibacterial activities of *Carica papaya* seed extract on enteric microorganisms. The seed extract was prepared in two ways; Aqueous and methanolic with varying concentration such as 25, 50, 75, 100 mg/ml respectively. The agar well diffusion method was used to assay the effect on standardized test organisms such as *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Salmonella typhi.* Also positive and negative control were used to truly have an accurate idea of how potent the seed extracts were when used in close compsarism with conventional drugs. The zone of inhibition was calculated at different concentrations of the seed extracts for both (aqueous and methanolic). There was inhibition of the test organisms at varying concentrations except *Salmonella typhi* which was inhibited at 100mg/ml for both forms of extract. Though it was revealed that both forms of the seed extract (aqueous and methanolic) were effective against the bacterial pathogens, however the methanolic had more potency compared to the aqueous extract.

**CHAPTER ONE**

**1.0 INTRODUCTION**

*Carica papaya* belongs to the family of *Caricaceae,* and several species of *Caricaceae* have been used as remedy against a variety of diseases (Alabi *et al.,* 2012). *Carica papaya* is a neutraceutical plant having a wide range of pharmacological activities. The whole plant has its own medicinal value. Papaya is a powerhouse of nutrients and is available throughout the year. It is a rich source of threes powerful antioxidant vitamin C, vitamin A and vitamin E; the minerals, magnesium and potassium; the B vitamin pantothenic acid and folate and fiber (Aravind *et al.,* 2013). The black seeds of the papaya are edible and have a sharp, spicy taste. They are sometimes ground and used as a substitute for black pepper. Dried papaya seeds actually look quite similar to peppercorns and can be used in just the same way. Sprinkle a little quantity over a meal, especially protein rich meals, is a simple way to add extra enzymes to your diet and improve your digestive appetite. The papaya seeds are very pungent and peppery, making them almost unpalatable. However the seeds seem to have more potent medicinal values than the flesh. Papaya seeds have antibacterial properties and are effective against *Escherichia coli*, *Salmonella* and *Staphylococcus* infections. Papaya seeds may protect the kidneys from toxin induced kidney failure. Papaya can eliminate intestinal parasites. It is used as heamoroid Cure for (piles) and typhoid and anti-helminthic and anti-amoebic properties (Aravind *et al.,* 2013).

Enteric bacteria naturally live in the intestine of animals and humans; however some of these bacteria cause diarrhoea. Diarrhoea disease is one of the leading cause of illness in young children in Nigeria (Parashar *et al*; 2013). Diarrhoea disease are the cause of almost three million deaths annually mainly among children younger than five years of age, (Seung-Hak *et al.,* 2006). Available report in Nigeria indicates that more than 315,000 deaths of pre-school age children are recorded annually as a result of diarrhoea disease (Babaniyi *et al.,* 1991; Alabi *et al*; 1998).

The seed of papaya has antimicrobial activity against *Trichomonas vaginali strophozoites*. It could also be used in urinogenital disorder like trichomoniasis with care to avoid toxicity. The seeds, irrespective of its fruit maturity stages have bacteriostatic activity on gram positive and negative organisms which could be useful in treating chronic skin ulcer. However, little information exits on the antimicrobial property of *Carica papaya* dried and fresh leaves (Alabi *et al.,* 2012). Recently, antifertility (Lohiya *et al.,* 1999) antihelminthic (Satrija *et al.,* 1995) and anti-inflammatory activity (Osho *et al.,* 2007) have been reported. *Carica papaya* seeds possess moisture, proteins, fatty acids, and phospholipids, such as phosphotidylcholine and cardiolipin. Other compounds present in seeds are c farpaine, benzyl isothiocynate, benzyl glucosinolate, beta-sitosterol,caricin, enzyme myrosin. The well-studied proteinases from papaya are papain, chymopapain, caricain, and glycylendopeptidase. Papain occurs in all parts of the tree except the root (*Anonymous, 1992*).

Fruit and seed extracts have antibacterial activity against *Bacillus cereus*, *Escherischia coli*, and *Pseudomonas aeuroginosa* (Tang *et al.,* 1972; Emeruwa *et al.,* 1982). The juice is used for curing warts, cancer, and tumors. Leaves have beenpoulticed into nervous pains, elephantoid growths (Asolkar *et al.,* 1992). The antihyperglycemic effect of unripe mature fruits and seeds of *Carica papaya* have also been reported (Olagunja, *et al* 1995; Adeneye and Olagunja, 2009).

Papaya leaves are made into tea as a treatment for malaria. Antimalarial and antiplasmodial activity has been noted in some preparations of the plant, the leaves of the papaya plants contain chemical compounds of karpain, Substance which kills microorganisms that often interfere with the digestive function (Udoh *et al.,* 2005). Papaya leaf extracts have phenolic compounds, such as protocatechuic acid, p-coumaric acid, 5, 7- dimethoxycoumarin, caffeic acid, kaempferol, quercetin, chlorogenic acid (Romasi *et al.,* 2011).Antimicrobials of plant origin effective in the treatment of infectious diseases and simultaneously mitigating many of the side effects often associated with synthetic antimicrobial agents have been discovered. Medical uses of plants range from the administration of the roots, barks, stems, leaves and seeds to the use of extracts and decoction from the plants (Iwu *et al.,* 1999).

* 1. **AIM**

To determine the antibacterial effect of *Carica papaya* seed on enteric microorganism.

* 1. **OBJECTIVES**

To extract the active ingredients from *Carica papaya* seeds using two different solvents (water and methanol)

To determine the potency and efficacy of aqueous and methanolic seed extract of *Carica papaya* seed.

**CHAPTER TWO**

**LITERATURE REVIEW**

**2.0 Classification and Nomenclature**

*Carica papaya* is a member of family *Caricaceae*. This family consists of 4 genera. Three of tropical American origin namely: *Carica, jarilla*, *jacaratia* and one from equatorial American namely: *Cylicomorpha. Carica* is the largest genus, which composes of 22 species. *Carica papaya* is the most economically valuable and widely cultivated in tropical and subtropical areas for its edible fruit and latex production. Papain, thiol protease abundant in the milky latex of fruits, is use for food, textile and perfume industries. Common names of papaya include papaya, papaw or pawpaw (Australia), *manbo* (Brazil), *papayer* (French), *iechosa* (spanish), *mugua* (chainese) *amd malakol* (Thailand) (Anjum *et al.,* 2013)

**2.1 Origin and Distribution**

The papaya is a native of Central and South America. It was recorded that seeds were distributed to the Caribbean and Southeast Asia during Spanish exploration in the 16th Century, from there, it spread rapidly to India, The Pacific and American. It is now present in every tropical and subtropical country between latitudes 32oN and 32oS (Anjum *et al.,* 2013)

**2.2 Common Uses**

Papaya is a multi-purpose fruit, not only as dessert but also as a source of chemical compounds use like papain, chymopapain and carpaine for medicinal cure (cancer, heart disease, and anti-aging) (Parle *et al.,* 2011). The International Tropical Fruits Network (TFNet) and Food and Agricultural Organization Statistic (FAOSTAT) reported that the world production of papayas has reached 5,951,000 metric tons (RM 100 million) in 2004 (Tropical Fruit Net, 2004).

**2.3 Papaya Morphology**

**2.3.1 Stem**

Papaya is a fast-growing tree like herbaceous plant. Papaya normally has a monoaxial stem without branching .The plant is 5-7 m in height. The stem is semi woody and hollow. The bark is smooth, grayish in color. The stem contains copious white milky latex (Anjum *et al.,* 2013).

**2.3.2 Leaves**

The cluster of leaves at the apex and along the upper part of the stem makes up the foliage of the tree. New leaves emerge directly from the upper part of the stem in spiral on nearly horizontal petioles 30-100 cm feet long, hollow, succulent, green or more or less dark purple. Leaves are palmate lobed with prominent venation. Petioles are cylindrical, hollow and 60-90 cm in length. The life span of a leaf is 4 to 6 months. The leaves contain copious white milky latex (Parle *et al.,* 2011).

**2.3.3 Flowers**

Papaya flowers are borne on modified cymose inflorescences, which appear in the axils of the leaves. The type of inflorescences depends upon the sex of the tree. The plant normally flowers within 9-12 months (Nakasone & Paul, 1998). Papaya is a dioecious species with three sex types; male, female and hermaphrodite (Okunola *et al.,* 2012).

**2.3.4 Fruits**

A papaya fruit is berry, melon-like, oval to nearly round. It has pyriform or elongated club shape. Fruits from female trees are spherical and those from hermaphrodite trees can show diverse shapes depending upon modifying factors affecting flower morphology during ontogeny. The fruits range in size from 7-30 cm. The fruit is normally composed of five carpels. When the fruit is green and hard, it is white latex rich. The skin is waxy, thin and smooth. As it ripens the skin color changes from green or greenish to yellow or orange. A number of small, dark brown seeds, each with a mucilaginous sarcotesta are attached to the walls inside the fruits (Nakasone *et al.,* 1998).

**2.3.5 Seeds**

The black seeds of the papaya are edible and have a sharp, spicy taste. They are sometimes ground and used as a substitute for black pepper.

**(a) Nephro** - protective activity- In wistar rat’s nephro- protective activity was observed in dose. Concentration of urine and creatinine were evaluated.

**(b) More potent** - The papaya seeds are very pungent and peppery, making them almost unpalatable. However the seeds seem to have more potent medicinal values than the flesh. Papaya seeds have antibacterial properties and are effective against E. coli, Salmonella and Staphylococcus infections, Papaya seeds may protect the kidneys from toxin - induced kidney failure. Seeds can eliminate intestinal parasites, and help detoxify the liver. Used as a skin irritant to lower fever. Cure for piles and typhoid and anti-helminthic and anti- amoebic properties. Dried papaya seeds actually look quite similar to peppercorns and can be used in just the same way. Grinding a couple over a meal, especially protein rich meals, is a simple way to add extra enzymes to your diet and improve your digestive health (Arvind *et al.,* 2013).

**2.3.5 Peel**

Papaya peel is often used in cosmetics. The papaya peel can also be used in many home remedies.

a) Sunscreen and soothing slave- The presence of vitamin A helps to restore and rebuild damaged skin. Applied papaya peel used as skin lightening agent. When peel mixed with honey and applied it can act as soothe and moisturizers the skin.

b) Fight dandruff- The papaya vinegar with lemon juice can be applied to the scalp for 20 minutes prior to shampooing to fight dandruff.

c) Muscle Relaxant- Adding papaya oil and vinegar to bath water, along with essential oils like lavender, orange and rosemary can be nourishing, refreshing and relaxing, and can work as a pain reliever and muscle relaxant (Aravind *et al.,* 2013).

Roots Juice from papaya roots is used in some countries of Asia to ease urinary troubles. Papaya leaf when dried and cured like a cigar is smoked by asthmatic persons. An infusion of fresh papaya leaves is used by person to expel or destroy intestinal worms. Fresh young papaya are also used to remedy colic, a certain stomach disorder or cramp. A decoction formed by boiling the outer part of the roots of the papaya tree in the cure of dyspepsia (Aravind *et al.,* 2013).

**2.3.6 Latex**

The milky sap of an unripe papaya contains Papain and chymopapain. chymopapain was approved for intradiscal injection in patients with documented herniated lumbar intervertebral discs and who had not responded to "conservative therapy". Papain is also used to treat commercial beer, to degum natural silk, as a meat tenderizer and in the production of chewing gums. Cosmetically it is used in Shampoos and in a number of face-lifting operations. In humans papain slows down the heart and thus reduces blood pressure. It is also used in Anthelmintic, relives dyspepsia, cures diarrhea, pain of burns and topical use, bleeding hemorrhoids, stomachic, whooping cough (Aarvind *et al.,* 2013).

**2.4 Other Uses**

**a) Colon cancer-** The fiber of papaya is able to bind cancer- causing toxins in the colon and keep them away from the healthy colon cells. These nutrients provide synergistic protection for colon cells from free radical damage to their DNA.

**b) Anti-Inflammatory effects-** Protein enzymes including papain and chymopapain and antioxidant nutrients found in papaya; including vitamin C, vitamins E, and beta- carotene, reduce the severity of the conditions such as asthma, osteoarthritis, and rheumatoid arthritis.

**c) Rheumatoid arthritis-** Vitamin C - rich foods, such as papaya, provides humans with protection against inflammatory polyarthritis, a form of rheumatoid arthritis involving two or more joints. d) Promote lung health- If you are smoker, or if you are frequently exposed to second hand smoke. Eating vitamin A rich foods, such as papaya, help your lung healthy and save your life.

**d) Promote Lung Health**

If you are smoker, or if you are frequently exposed to second hand smoke. Eating vitamin A rich foods, such as papaya, help your lung healthy and save your life.

**e) Anti-sickling activity-** Current research proves that papaya is having an anti-sickling activity. **f) Prevent prostate cancer-** Men consuming lycopene - rich fruits and vegetables such as papaya, tomatoes, apricots, pink grape fruit, watermelon, and guava were 82 % less likely to have prostate cancer compared to those consuming the least lycopene - rich foods.

**g) Anticoagulant effect-** Injection of papain extract in a dog increases prothrombin and coagulation threefold. It is also claimed that the enzyme eliminates necrotic tissues in chronic wounds, burns and ulcers. Papain is also of commercial importance in the brewery industry, in the food industry and in the textile industry (Arvind *et al.,* 2013).

Papaya also contains several unique protein-digesting proteolytic enzymes including papain and chymopapain.

**(a)Papain**

This enzyme is similar to pepsin, a digestive enzyme in our body.

**(b) Chymopapain**

A drug made from chymopapain used to be very popular in treating slipped disk. Both papain and chymopapain can help lower inflammation and improve healing from burns.

**(c) Carpaine**

The alkaloid, Carpaine, slows the heart rate in humans and thus reduces blood pressure. Its action is similar to the drug prescribed for heart patients, digitalis. The alkaloid is reported to be able to kill worms and amoebas.

**(d) Lycopene**

Papaya has an abundance of cancer fighting lycopene. It is a key intermediate in the biosynthesis of many important carotenoids, such as beta-carotene and xanthophylls.

**(e) Fibrin**

Another useful compound not readily found in the plant kingdom is Fibrin. It reduces the risk of blood clots and improves the quality of blood cells, optimizing the ability of blood to flow through the circulatory system. Fibrin is also important in preventing stoke.

**2.6 Cosmetic Benefits of Papaya**

Rubbing the white pulp of raw papaya improves pimples as well as wrinkles. Papaya works as a good bleaching agent. It is an important ingredient in bath soaps, astringents, detergent bars and hand washes.Home Recipe for Papaya Skin Lightner Experts suggest that papaya can help in removing dead worn-out skin cells and replace it with healthy new cells, thereby lightening the color of our skin. For this, one can prepare a paste of raw papaya and apply it on the skin once for few days.

**2.7 Allergies and Side Effects**

Papaya is frequently used as a hair conditioner, but should be used in small amounts. Papaya releases a latex fluid when not quite ripe, which can cause irritation and provoke allergic reaction in some people. The latex concentration of unripe papayas is speculated to cause uterine contractions, which may lead to a miscarriage. Papaya seed extracts in large doses have a contraceptive effect on rats and monkeys, but in small doses have no effect on the unborn animals. Excessive consumption of papaya can cause carotenemia, the yellowing of soles and palms, which is otherwise harmless. However, a very large dose would need to be consumed; papaya contains about 6% of the level of beta carotene found in carrots (the most common cause of carotenemia)

**2.7.1 Toxicity**

Externally the papaya latex is an irritant to the skin and internally it causes severe gastritis. Some people are allergic to various parts of the fruit and even the enzyme papain has its negative properties.

**2.7.2 Skin Discoloration**

Eating too much of a yellow, green or orange- colored food that contains beta carotene can cause a benign form of skin discoloration called carotenemia. The palms of the hands and soles of the feet are the most visible areas of the body affected by carotenemia. Cutting back on your papaya consumption will resolve the discoloration of the skin.

**2.7.3 Free Radical Scavenging Activity**

Papaya has many phenolic groups which may scavenge free radicals. Aqueous extract of papaya leaves shows anti-oxidant activity

**2.7.4 Respiratory Distress**

Papain is also a potential allergen, according to Purdue University, people who eat too much papaya and ingest high levels of papain may develop symptoms consistent with hay fever or asthma, including wheezing, breathing difficulties and nasal congestion.

**2.7.5 Gastrointestinal Symptoms**

Ironically, the same papain that calms your stomach can cause an upset stomach when taken in large amounts. The high fiber content of papaya can also contribute to unrest of the digestive system. The latex of the fruit's skin can also cause irritation of the stomach.

**2.8 Nutritional Value of *Carica papaya***

Papaya is common’s man fruit, which is reasonably priced and has a high nutritive value. It is low in calories and rich in natural vitamins, and minerals. The comparative low calories content (32 Kcal / 100 g of ripe fruit) make this a favorite fruit of obese people who are into weight reducing regime. Papaya has low carotene compared to other fruit such as apples, guava, sitaphal and plantains, which helps to prevent damage by free radicals. Unripe green papaya is used as vegetable, it does not contain carotene but also all other nutrients are present. The fruit is a rich source for different types of enzymes. Papain, vegetable pepsin present in good amount in unripe fruit is an excellent aid to digestion, which helps to digest the protein in food at acid, alkaline and neutral medium. The celiac disease patients, who cannot digest the wheat protein gliandin, can tolerate it, if it is treated with crude papain, papaya has the property of tenderizing meat. This knowledge is being put to use by cooking meat with raw papaya to make it tender and digestible (Marotta *et al.,* 2006). The fermented papaya fruit is a promising nutraceutical as an antioxidant. It improves the antioxidant defense in elderly patients even without any overt antioxidant deficiency state at the dose of 9 g/day orally. The papaya lipase, a hydrolase enzyme tightly bonded sto the water insoluble fraction of crude papain, is considered as a “naturally immobilized” biocatalyst (Marotta *et al.,* 2006). Papaya markedly increases iron (Fe) absorption from rice meal, which was measured in parous Indian women, using the erythrocyte utilization of radioactive Fe method. The black seeds edible and have a sharp, spicy taste. They are sometimes ground up and used as a substitute for black pepper. In some parts of Asia the young leaves of papaya are steamed and eaten like spinach (Dominguez *et al.,* 2006) .Nutritive value of 100 gm of *Carica papaya* fruit are described.

**Table 1: Nutritive value of 100gm of Carica papya fruit**

**Constituents** **Ripe papaya**  **Green papaya**

Protein 0.6g 0.7g

Minerals 0.5g 0.7g

Fibre 0.8g 0.9g

Fat 0.1gb 0.9g

Carbohydrates 7.2g 0.2g

Energy 3.2g 5.7g

Carotene 888 0

Total 2,740um 27kcal

**Related Studies:**

A journal written by (Jyotsna *et al.,* 2014), on the antibacterial activity of seed and leaf extract of *Carica papaya*.

The study dealt with the antibacterial activity of aqueous, chloroform extract of leaves and aqueous, methanolic extract of seeds of *Carica papaya* var. pusa dwarf through agar well diffusion assay against *Pseudomonas aeruginosa*, *E.coli* and *Salmonella typhi*. They found that the aqueous as well as the methanolic extract of seeds were effective to inhibit the bacterial pathogens while in case of chloroform extract of *Carica papaya* leaves did not show any inhibition against the bacteria and the aqueous leaf extract was potent to inhibit them.

Also Anibjuwon *et al.,* (2009) from the department of microbiology, University of Ilorin wrote on the antimicrobial activity of *Carica papaya* (pawpaw leaf) leaf on some pathogenic organism of clinical origin from south-western Nigeria.

They found some bioactive compound in leaf and root extracts of *Carica papaya* wen extracted, using water and organic solvents, and investigated for antibacterial activity against some human pathogenic bacteria using the agar diffusion method. The aqueous extracts of the root extracts did not show significant activity, but the organic extracts had significant activity with the methanol extracts demonstrating the highest activity against the test bacteria. The root extracts demonstrated higher activities against all the gram-positive bacteria than the gram-negative bacteria tested, with the highest activity (14 mm zone of inhibition) demonstrated against *Pseudomonas aeruginosa* while the aqueous leaf extract showed pronounced inhibition demonstrating higher activities against the test bacteria than the organic solvents. The extracts demonstrated higher activities against all the gram-positive bacteria than the gram-negative bacteria tested, with the highest activity (4.2 mm zone of inhibition) demonstrated against *Pseudomonas aeruginosa*. Increase in temperature enhanced the activity of the extracts, while alkaline pH decreased the activity. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the root extracts ranged between 50-200 mg/ml (Anibjuwon *et al.,* 2009) Preliminary phytochemical analyses showed that the extracts contain alkaloids, tannins, saponins, glycosides and phenols. *Carica papaya* may be used for the treatment of gastroenteritis, uretritis, otitis media and wound infections.

Aravind *et al.,* (2013) from the department of pharmacognosy, Nimra College of pharmacy, India wrote in the journal of traditional and medicinal on the uses of *Carica papaya*.

Papaya, botanical name *Carica papaya*, as a lozenge tropical fruit, often seen in orange-red, yellow-green and yellow-orange hues, with a rich orange pulp. The fruit is not just delicious and healthy, but whole plant parts, fruit, roots, bark, peel, seeds and pulp are also known to have medicinal properties. The many benefits of papaya owed due to high content of Vitamins A, B and C, proteolytic enzymes like papain and chymopapain which have antiviral, antifungal and antibacterial properties. *Carica papaya* can be used for treatment of a numerous diseases like warts, corns, sinuses, eczema, cutaneous tubercles, glandular tumors, blood pressure, dyspepsia, constipation, amenorrhoea, general debility, expel worms and stimulate reproductive organs and many, as a result *Carica papaya* can be regarded as a Neutraceutical(). The present article reviews the pharmacological uses of *Carica papaya* and side/toxic effects. *Carica papaya* contains an enzyme known as papain which is present in the bark, leaves and fruit. The milky juice is extracted, dried and used as a chewing gum for digestive problems, toothpaste and meat tenderizers. It also contains many biological active compounds including chymopapain and papain which is the ingredient that aids digestive system, and again used in treatment of arthritis.

**CHAPTER THREE**

**MATERIALS AND METHOD**

**3.0 Collection of Plant Materials (Of *Carica papaya* seed)**

The mature seeds of *Carica papya* fruit were bought from the market and it was air dried. And the dried seed was grinded into fine powder with a mortar and a piston.

**3.1 Extraction of Plant Materials**

The seed extract was prepared as described in WHO, 1983 with slight modification through hot extraction method. Air dried ripened papaya seeds were powdered using mortar and pestle. For aqueous extract, 5g of dried and powdered seeds were mixed in 100 ml distilled water while for solvent extract preparation, methanol was used. The contents were kept in water bath for 1h at 40˚C. After cooling both extracts were filtered successively through ordinary cheese cloth and Whatmann filter paper. The extracts were then air dried using a water bath at 40˚C and dissolved in dimethyl sufoxide (DMSO) to form different concentrations of viz. 25, 50, 75 and 100% aqueous and methanolic extracts respectively. The hot water seed extract was prepared according to the as described by (Oyagade *et al.,* 1999). The hot water extraction was done at 80°C in a water bath for 1/2hours. The extracts were then decanted and filtered through a Whatman filter paper. The filtered extract was then sterilized using a membrane filter and evaporated to dryness at 45°C.

**3.2 Antibacterial Activity**

Bacterial samples were isolated from Godfrey Okoye University clinic, *Pseudomonas aeruginosa*, *Salmonella typhii* and *Escherichia coli* were used in the study.

**3.3 Standardization of test organisms**

Marcfarland 0.5 turbidity standard was prepared by adding 0.5ml of 1% w/v barium chloride dehydrate (BaCl2.2H2O) solution and 99.5ml of 1% sulphuric acid (H2SO4). This was mixed well and then aliquoted into test tubes identical to the ones used in the preparing innoculum suspensions of the test organisms.

A sterile wire loop used to pick a loopful of inoculums from a pure culture of the test organism. This was then transferred and suspended in a tube of sterile normal saline (NaCl 8.5g, distilled water 1 litre). The tube was compared with the turbidity standard and the density of the organism was adjusted to that of the standard by adding more bacteria or more sterile saline (Vandepitte *et al.,* 2003).

**3.4 Well diffusion assay**

An inoculum suspension was swabbed uniformly to solidified Nutrient agar for bacteria, and the inoculum was allowed to dry for 5 min., holes of 5 mm in diameter were made in the seeded agar using cork borer. Aliquot of 20 µl from each plant crude extract was added into each well on the seeded medium and allowed to stand on the bench for 1 hour for proper diffusion and thereafter incubated at 37°C for 24 hour. Respective solvents were used as the negative control and gentamicin (10µg/ml) as the positive control. The resulting inhibition zones were measured In mm (Obeidat *et al.,* 2012).

**CHAPTER FOUR**

**RESULT**

Table 2: Antibacterial activity of the Methanolic extract of *Carica papaya* seeds on enteric organisms.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Methanolic  Extract | Conc. in mg/ml  (DMSO) | Zones of inhibition in (mm) | | | |
|  |  | *Pseudomonas* Spp. | *E. coli* | *S. typhi* |
| 1 | 25 | 3.1mm | 3.5mm | 0.0mm |
| 2 | 50 | 4.2mm | 4.3mm | 0.0mm |
| 3 | 75 | 6.8mm | 6.5mm | 0.0mm |
| 4 | 100 | 7.9mm | 7.5mm | 2.5mm |

Table 3: Antibacterial activity of the aqueous extract of *Carica papaya* seeds on enteric organisms.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Aqueous  Extract | Conc. in mg/ml  (DMSO) | Zones of inhibition in (mm) | | | |
|  |  | *Pseudomonas* Spp. | *E. coli* | *S. typhi* |
| 1 | 25 | 2.6mm | 3.0mm | 0.0mm |
| 2 | 50 | 3.8mm | 4.0mm | 0.0mm |
| 3 | 75 | 6.0mm | 5.0mm | 0.0mm |
| 4 | 100 | 7.0mm | 7.0mm | 2.0mm |

Zones of inhibition (mm)

Fig 1: Graphical representation of the antibacterial activity of the aqueous extract of carica papya seeds on enteric organisms.

Zones of inhibition (mm)

Fig 2: Graphical representation of the antibacterial activity of the methanolic extract of *Carica papaya* seeds on enteric organisms

**CHAPTER FIVE**

**DISCUSSION**

Aqueous extract of *Carica papaya* seeds showed increasing zones of inhibition of *Pseudomonas aeruginosa* and *Escherichia coli* with increasing seed extract concentrations as 25, 50, 75, and 100 mg/ml while the extract concentration of 25, 50 and 75mg/ml were unable to show zones of inhibition against *Salmonella typhi*, but the pathogen was inhibited at 100mg concentration of the extract with a zone of inhibition of 2.0mm.

The methanolic extract extract of *Carica papaya* seeds showed increasing zones of inhibition of*, P.aeruginosa and E.coli* with increasing seed extract concentrations of 25, 50, 75 and 100mg/ml while the extract concentration of 25, 50 and 75 were unable to show zone of inhibition against *S.typhii*, but the pathogen was inhibited at 100mg concentration of the extract with a zone of inhibition of 2.5mm.

The fact that the extracts were active against both gram +ve and gram –ve bacteria tested may indicate a broad spectrum of activity. This may be as a result of the phyto constituents inbedded in the seed extracts. This observation is very significant because of the possibility of developing therapeutic substance that will be active against multi-drug resistant organisms and this shows that it is also in line with findings of Zakira *et al.,* 2006 and also Romasi *et al.,* 2011.

**CONCLUSION**

The aqueous and methanolic seed extracts of *Carica papaya* exhibits antibacterial activity against the common enteric bacteria used for the study which are; *P.aeruginosa,* *E.coli* and *S.typhii* respectively. From these findings it was noticed that the methanolic seed extracts showed more efficacy against the aqueous seed extract because of the synergy in which was formed between the methanol and the seed extract which showed more antibacterial effect. The presence of various bioactive compounds justifies the use of the whole plant for various ailments by traditional practioners. It could be concluded that *carica papya* seed is of phytopharmaceutical importance. Studies with these compounds may yield nature friendly strong antimicrobial agents of agricultural and anti-cancer drugs.

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