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# Phytochemical and Antimicrobial Screening of Root Extracts of *Carica papaya*

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## ABSTRACT

Resistance to antimicrobial agents has been on increase in recent time due to a number of factors. There is the need to explore new agents to address the rising failure of some of the existing antimicrobials to therapy. This work is designed to confirm the folklore claim of the antimicrobial activity of the root extract of the plant *Carica papaya*. The antimicrobial activity of ethanol extract of male *Carica papaya* (EMCP), female specie (EFCP) collected at 8.00a.m were tested against some selected bacteria and fungal isolates namely *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Staphlococcus aureus* and *Candida albicans* respectively. The antimicrobial activity were evaluated using cup plate diffusion method. The root extracts of the plant were also screened phytochemically for the presence of secondary metabolites. The two extracts, EFCP and EMCP exhibited certain degree of antimicrobial activity from concentration of 62.5mg/ml to 1000mg/ml .The result further indicated some form of similarity in antimicrobial activity for both EMCP and EFCP at most concentrations tested. The Extracts of both EMCP and EFCP contains Saponin, Flavonoid, and Tannins in pronounced amount. They also contain Akaloids, anthraquinones, anthocyanide in moderate amount. The antimicrobial activity of EMCP and EFCP was however, lower than that of ciprofloxacin and clotrimazole that were used as reference standards

Keywords: *Carica papaya*, Antimicrobial, Antifungal, Ciprofloxacin, Secondary metabolites, Clotrimazole

## INTRODUCTION:

The rapid development of multi-resistant bacterial strains have attracted the interest of scientists to develop newer broad spectrum antimicrobial agents (Weisser *et al*,1966). Infectious diseases are major public health issues in the resource-poor countries. They contribute to higher rate of morbidity and mortality related indices, due to fragile primary care settings and people's low socio-economic status to access the modern healthcare facilities. Despite recent scientific advancement and globalization, WHO estimates that in the developing countries, nearly 80% of the population remains to rely upon the traditional system of medicine (TSM) as a primary health care modality in the resource-constrained health settings (Karunamoorthi *et al*,2013). Globally, since time immemorial, each and every society has had its unique way of indigenous health practice system in order to treat various ailments (Karunamoorthi *et al*,2012). The development of resistance and high cost of new generation antibiotics necessitates investigation into newer substances especially of natural origin with claimed antimicrobial activity. A number of herbs with significant antimicrobial activity have been reported in different traditional literatures (Balandrin *et al*,1985; Satish *et al*, 1999; Jones,1996). *Carica papaya* Linn (Caricacae) with common names Ibepe (Yoruba), Gwandau (Hausa), Paw (Australia), Mamao (Braxil), exists in America, parts of Asia, India, Africa as tree-like herb with an extensive rooting system, 2 – 10m tall, usually unbranched, although sometimes branched due to injury, containing white latex in all parts (Fatope *et al*,1993). Carica species particularly the fruits, seeds and roots are known to contain papain among many biologically active compounds, papain a proteolytic enzyme with wide pH range has been used variously medicinally in combating dyspepsia and other digestive disorders, dewormer in traditional veterinary medicine, antibacterial, antifungal

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among others (Emeruwa,1982; Gurorrdani *et al*, 1996). Also the latex from the fruit has been used in the manufacture of rubber (Morton, 1997), tanning purposes in textile industry (Duke, 1984) .Till date most uses of *Carica papya* has been in traditional medicine particularly in veterinary practice. Various investigations are on to establish and standardize the use in human medicine. Owoyele *et al*, 2010 reported antipyretic and analgesic activity of *Carica papaya* leaves and went further in their work to report from literature various other medicinal uses such as the use of the fallen dry leaves of *Carica papaya* along with some other leaves like *Azadirachta. indica*, being used for the treatment of malaria fever. Likewise, the fallen dry leaves with *Rawolfia.vomitoria* and *Azadirachta. leiocarpus* being used locally in the western part of Nigeria to reduce body temperature (Gill, 1992). Other medicinal uses of the leaves reported include treatment of syphilis, amoebic dysentery, asthma, inflammation and also as a purgative (Akah *et al*, 1997; Oloyede, 2005). The ripe and unripe fruits are used as laxative and as a diuretic. The ripe fruit is eaten fresh or cooked. The roots and leaves are used as abortifacient agents (Gill, 1992). Although a number of investigations have been carried out on some parts of the plant namely, leaves, seeds, stem from the foregoing but rarely the roots. The present aim therefore is to explore scientifically the antimicrobial potential of the root extract of *Carica papaya* plant and substantiate the folklore claims.

## MATERIALS AND METHODS

### Extraction of *Carica papaya* roots:

The roots of *Carica papaya* were collected at 8:00 a.m. from a cultivated plot behind Faculty of Pharmacy, Olabisi Onabanjo, University, Shagamu, Nigeria. The matured roots were collected separately from both male and female plants. The samples were washed immediately after collection, cut into pieces, surface sterilized with methylated spirit and dried in oven at 45$^{o}$C for twelve hours. The dried samples were grinded with pestle and mortar and later blended in a blender (Model 857 chrome white, Osterizer, U.S.A). The solvent chosen to extract the roots was 96% ethanol at root weight solvent ratio 1:10. The roots were allowed to macerate in the solvent for two days at room temperature. The solvent was then filtered through a whatman filter paper (No 1) to remove the coarse root material into pre-weighed sterile containers. The solvent was evaporated off using rotary evaporator and then transferred to heating mantle to remove any residual solvent. The weight of the residue was calculated and the extracts were kept at room temperature.

### Phytochemical tests:

Plant extract was screened for the presence of secondary metabolites using the method previously described by (Sofowora, 2008; Trease and Evans, 2009)

### Susceptibility Test:

Activity of the extracts was tested using cup plate diffusion method, 0.1ml of I in 100 dilution biochemically identified 24 hours broth culture of clinical isolates of *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *Staphlococcus aureus* were separately inoculated into each 20ml cooled molten nutrient agar (at 45$^{o}$C) in universal bottles. The contents of the universal bottles were mixed gently, then poured into sterile petri dishes and allowed to set. The surfaces of the plates were dried at 37$^{o}$C for 30 minutes in an incubator. Thereafter, 5 holes equidistant from each other and the edge of the plate were bored using sterile cork borers and then different concentrations (1.953mg/ml – 1000mg/ml) of each test extracts and reference, ciprofloxacin purchased from Oxoid Ltd (Basingstoke Hampshire, U.K.)

(0.098mg/ml – 50.00mg/ml) solution was instilled into the cups in duplicate using sterile 0.1 ml micropipettes. The plates were left at room temperature for one hour to allow for diffusion and then incubated at 37$^{o}$C for 24 hours. Similarly, 20ml sterile molten sabouraud dextrose agar cooled to 45$^{o}$C was poured into sterile petri dishes, 0.1ml of 1in 100 dilution of culture of *Candida albicans was* spread over the plates. Thereafter, 5 holes were dug with sterile cork borer and then extracts concentrations (1.953mg/ml-1000mg/ml) and reference clotrimazole (0.098mg/ml-50.00mg/ml) were introduced. The plates were incubated at room temperature. The experiments were repeated once. Diameter of inhibition zones were measured in millimeters with vernier caliper.

## RESULTS

The yield of the extracts is 10.2% w/w (EMCP) and 9.7%w/w (EFCP). The presence of secondary plant metabolites as revealed in *Carica papaya*, following phytochemical tests, is as shown in Table 1. Both extracts (EMCP and EFCP) have similar constituents e.g. Flavonoids, Tannins, Saponin, Alkaloids and

Anthraquinones. The in *vitro* antibacterial activity of EMCP, EFCP and Reference compound is as presented in Table 2 while that of antifungal activity is presented in Table 3. It appears EMCP in Table 2 has higher spectrum than EFCP ($p<0.05$) against test organisms. In Table 3, There is similarity in the inhibition zones against *Candida albican* for both EMCP and EFCP ($p> 0.05$). The results of minimum inhibitory concentration (MIC) for bacterial and fungus were shown in Table 4. The result showed that EFCP (MIC 62.5mg/ml) is more sensitive against *Proteus mirabilis* than EMCP (125.0mg/ml). Also from Table 4, sensitivities of the extracts against gram positive organism (*Staph aureus*) is higher than that of gram negative organisms screened. Generally the other of sensitivities of the test agents as presented in Table 4 is ciprofloxacin>EFCP>EMCP ($p<0.05$) while clotrimazole, antifungal reference sample has higher sensitivity ($p<0.05$) (0.098mg/ml) than either EMCP or EFCP (15.625mg/ml) as antifungal agents.

**DISCUSSION**

The antimicrobial activities of various plants have been reported by many workers (Cowan, 1999; Sherriff, 2001). In the present study a variety of gram positive, gram negative bacteria and fungus strains were selected for the screening of antimicrobial effect of both male and female species of *Carica papaya* root. Ciprofloxacin and clotrimazole were used as reference standards for both bacterial and fungus organisms respectively. The yield of the extracts (10.20%w/w and 9.70%w/w) for EMCP and EFCP appear low for it to be able to support continuous herbal formulation. However, chemical constituents may need to be isolated for further investigation; the low yield may not be unconnected with the geographical location and ecological factors of where this plant was collected from. The yield of EMCP is slightly higher than that of EFCP; this is probably so because male specie of *Carica papaya* does not bear fruits like the female specie does. There is high probability that the constituents are distributed throughout the various parts of the female plant. The secondary metabolites found in the extract are as stated in Table 1.

Table 1: Qualitative Estimation of Secondary Metabolites

| Bioactive constituent | Chemical Test | Remarks |
|---|---|---|
| **Saponin** | Frothing test | + |
| | Emulsifying | + |
| **Tannins** | $Fecl_3$ | + |
| **Alkaloid** | Wagner's | + |
| | Meyer's | + |
| **Cardiac glycoside** | Keller kiliani | – |
| | Kedde | – |
| **Anthraquinone** | Combined ( Modified Borntrager's Test) | – |
| | Free ( Borntrager's Test) | + |
| **Flavonoid** | Shinoda's test | + |
| **Terpenoids** | Lieberman-burchard | - |
| | | |

**KEY:**
-, Absence of component; + Presence of component.

Table 2: Assessment of the Antibacterial properties of the root extracts and Reference showing inhibition zone in (mm) at various concentrations.

| Test Agents | Conc. In mg/ml | Test Organisms | | | | |
|---|---|---|---|---|---|---|
| | | *Staph. aureus* | *E.coli* | *Ps aeruginosa* | *Proteus mirabilis* | *Klebsiella spp* |
| EMCP | 1.95 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 3.91 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 7.81 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 15.63 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 31.25 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 62.50 | 3.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 125.00 | 6.0 | 3.0 | 0.0 | 0.0 | 3.0 |
| | 250.00 | 6.0 | 3.0 | 2.0 | 2.0 | 3.0 |
| | 500.00 | 7.0 | 4.4 | 2.6 | 3.6 | 4.0 |
| | 1000.00 | 8.0 | 6.0 | 3.3 | 5.1 | 6.3 |
| EFCP | 1.95 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 3.91 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 7.81 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 15.63 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 31.25 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 62.50 | 3.0 | 2.5 | 0.0 | 3.0 | 2.0 |
| | 125.00 | 3.0 | 2.5 | 0.0 | 3.0 | 2.0 |
| | 250.00 | 4.0 | 2.8 | 2.8 | 3.5 | 2.5 |
| | 500.00 | 7.0 | 3.4 | 3.3 | 4.4 | 4.2 |
| | 1000.00 | 8.0 | 4.0 | 3.3 | 4.4 | 4.2 |
| Ciprofloxacin | 0.10 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 0.20 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 0.39 | 0.0 | 3.0 | 0.0 | 0.0 | 0.0 |
| | 0.78 | 6.0 | 4.0 | 3.0 | 8.0 | 3 |
| | 1.56 | 9.0 | 6.0 | 6.0 | 10.0 | 6.0 |
| | 3.13 | 12. | 8.0 | 12.0 | 17.0 | 9.0 |
| | 6.25 | 15.0 | 10.0 | 16.0 | 20.0 | 12.0 |
| | 12.50 | 15.0 | 12.0 | 19.0 | 22.0 | 16.0 |
| | 25.00 | 23.0 | 15 | 21.0 | 22.0 | 18.0 |
| | 50.00 | 23.0 | 19.0 | 22.0 | 25.0 | 20.0 |

Key: EMCP – Ethanolic root extract of mature male *Carica papaya plant*, EFCP – Ethanolic root extract of mature female *Carica papaya plant*.

One-way ANOVA statistical tool was used to analyze sensitivity of the bacteria at various concentrations to EMCP, EFCP differently at significant level of 0.05. The calculated "F statistic" was less than critical value of "F" leading to acceptance of Null hypothesis i.e. there is no statistically significant difference in the sensitivity of test organisms (bacterial) to the test agents

Table 3: Assessment of the antifungal properties of the root extracts and reference compound showing zones of inhibition in (mm) at various concentrations.

| Test Agents | Conc. In mg/ml | Test Organism *Candida albicans* |
|---|---|---|
| | 9.531 | 0.0 |
| | 3.906 | 0.0 |
| | 7.813 | 0.0 |
| | 15.625 | 0.0 |
| EMCP | 31.250 | 3.0 |
| | 62.500 | 5.0 |
| | 125.000 | 5.3 |
| | 250.000 | 6.0 |
| | 500.000 | 8.0 |
| | 1000.000 | 9.0 |
| | 9.531 | 0.0 |
| | 3.906 | 0.0 |
| | 7.813 | 0.0 |
| | 15.625 | 0.0 |
| EFCP | 31.250 | 3.0 |
| | 62.500 | 5.0 |
| | 125.000 | 6.0 |
| | 250.000 | 6.7 |
| | 500.000 | 6.7 |
| | 1000.000 | 8.4 |
| | 0.098 | 0.0 |
| | 0.195 | 3.0 |
| | 0.039 | 4.0 |
| | 0.781 | 8.0 |
| Clotrimazole | 1.563 | 14.0 |
| | 3.125 | 18.0 |
| | 6.250 | 18.0 |
| | 12.500 | 20.0 |
| | 25.000 | 20.0 |
| | 50.000 | 26.0 |

Key: EMCP – Ethanol root extract of mature male *Carica papaya plant,* EFCP – Ethanol root extract of mature female *Carica papaya plant.*

Student t-test (paired, independent, two-tailed) at significant level of 0.05 was used to compare the sensitivity of EMCP and EFCP against *Candida albicans.* It was found out that the calculated t- statistic is less than critical "t" value leading to acceptance of null hypothesis that there is no difference in the sensitivity statistically.

Table 4: Minimum inhibitory concentration of Extracts and Reference in (mg/ml)

| Test Organisms | | | Test Agents | |
|---|---|---|---|---|
| | EMCP | EFCP | Ciprofloxacin | Clotrimazole |
| *Staphylococcus aureus* | 31.250 | 31.250 | 0.195 | - |
| *Eschericia Coli* | 62.500 | 62.500 | 0.098 | - |
| *Pseudomonas aeruginosa* | 125.000 | 125.000 | 0.391 | - |
| *Proteus mirabilis* | 125.000 | 62.500 | 0.195 | - |
| *Klbsiella spp* | 62.500 | 62.500 | 0.195 | - |
| *Candida abicans* | 15.625 | 15.625 | - | 0.098 |

Key: EMCP – ethanol root extract of mature male *Carica papaya* plant, EFCP – Ethanol root extract of mature female *Carica papaya* plant.

One-way ANOVA at significant level of 0.05 was used to compare statistically the MICs of EMCP,EFCP and Ciprofloxacin in terms of sensitivity. Calculated F Statistic was greater than critical value " F" leading to rejection of null hypothesisthat is there is real difference in the sensitivity of the test agents in the order of Ciprofloxacin > EFCP=EMCP.

The outcome agrees with the earlier work (Bhathacharga *et al*, 1982; Madrigal *et al*, 1980). Phytoconstituents present in plants namely flavonoid, alkaloids, tannins and triterpenoids are producing exciting opportunities for the expansion of modern chemotherapies against wide range of microorganisms (Lutterodt et *al*, 1999; Marjorie, 1999). Tannins especially according to Shimoda, 2006 has been found to form irreversible complex with proline rich protein resulting in the inhibition of cell wall synthesis thereby leading to the death of the organisms. Furthermore, EMCP and EFCP tend to have similar antimicrobial activities against the tested organisms ($p>0.05$). The constituents present in the plant extracts are not too much varied which may account for similarity in antimicrobial activities (Wenbebe, 1998). Also from Tables 2 and 4 there was observed pronounced activity of both extracts against gram positive *Staphylococcus aureus* and *Bacilli substilis* better than those of gram negative organisms tested. This can be explained through the mode of action of antibacterial agents. Antimicrobial agents that act by inhibiting cell wall synthesis (bacteriostatic) tend to have more activity against gram positive organisms. Whereas those agents that inhibit protein synthesis (bactericidal) have more activity against gram negative organisms (Dugid *et al*, 1998). Earlier, some parts of *Carica papaya* plant e.g. seed and fruit have been shown to have antimicrobial activities. The reference standard (Ciprofloxaxin) from Tables 2 and 4 exhibited broader spectrum of activity<0.05) against all organisms tested than the extracts. The antifungal outcome in Table 3 is quite interesting; the reference compound had marked activity($p<0.05$) against *Candida albicans* as compared with test extracts .This may be due to the fact that extracts being not isolated bio- molecule contained some other non-medicinal constituents interfering with its biological activities. It is a known fact that purified isolated compounds exert more potent effect than the crude extracts. However, herbal formulation of this extract may still be useful in certain mild ailment conditions like diarrhea, oral thrush among others. Further work will however be necessary to isolate, purify, characterize, and formulate into stable dosage forms the active bio- molecule contained in the plant.

## CONCLUSION

From the foregoing, it has been shown that both extracts of male and female plant, *Carica papaya* have promising activity against some commonly implicated organisms in infectious diseases although lesser activity as compared with reference standards. The extracts may still be explored in some specific cases of infections particularly when it is a suspected gram positive organism.

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