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Research Article

# CHEMICAL CONSTITUENTS OF THE LEAF ESSENTIAL OIL OF *CARICAPAPAYA* FROM SOUTH EAST NIGERIA AND ITS ANTIMICROBIAL ACTIVITY

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**ABSTRACT**

The leaves of *Carica papaya* are used in herbal medical practices in South East Nigeria to treat malaria and typhoid fever which engendered a probe of its chemical constituents. With isopropanol as a choice of solvent, the chemical constituents of the extract of the leaves of *C. papaya* were characterized using Gas Chromatography-Mass Spectrometry (GC/MS) technique and six compounds were identified which include hexahydro-1-aH-naphtho[1,8a-b]oxiren-2(3H)-one (2.17%), 3,7-dimethyloct-7-en-1-ol (8.08%), 3-methyl-4-(phenylthio)-2-enyl-2,5-dihydrothiophene-1,1-dioxide (11.78%), cyclopentaneundecanoic acid methyl ester (12.02%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (37.78%) and 9-octadecenamide (28.18%). These compounds possess pharmacological profiles. The extract showed potent antimicrobial activity against *Staphylococcus aureus*,*streptococcus faecalis*, *Escherichia coli* and *Proteus mirabilis*. The sensitivity of each test microorganism to the extract was determined using the Disc Diffusion Technique. Highest sensitivity was shown with *S. aureus*(14.33 mm at 100 % concentration) followed by *E. coli* (12.98 mm at 100 % concentration) and *P. mirabilis* (12.37 mm at 100 % concentration) while the least was shown with *S. faecalis*(11.39 mm at 100 % concentration). This research lends credence to the use of *C. papaya* in herbal medicine in Nigeria.

**Keywords:** *Carica papaya*, Chemical constituents, GC/MS analysis, Antimicrobial activity**.**

## INTRODUCTION

Plants are sources of chemicals used as drugs in herbal medicine. Some of these plant extracts are incorporated in cosmetic preparations like soaps, lotions, pomades and tooth pastes because of their curative properties. The quest to fully elucidate and authenticate these plant chemicals and their efficacy is born by the fact that theyare used in synergy (in herbal medicine) as infusions, decoctions, concoctions, compresses, tinctures, ointments, poultices, powders, syrups, salves and teas.

*C. papaya* commonly known as pawpaw belongs to the family *Caricaceae*. The papaya is a large, tree-like plant, with a single stem growing from 5 to 10 m (16 to 33 ft) tall, with spirally arranged leaves confined to the top of the trunk. The lower trunk is conspicuously scarred where leaves and fruits were borne. The leaves are large, 50–70 cm, deeply palmately lobed, with seven lobes. Unusually for such large plants, the trees aredioecious. The tree is usually unbranched, unless lopped. The flowers appear on the axils of the leaves, maturing into large fruit, 15–45 cm long and 10–30 cm in diameter. The fruit ripens when it feels softand its skin has attained amber to orange hue[1].

*C.papaya* leaves are made into tea as a treatment for malaria in some parts of the world[2]. Antimalarial and antiplasmodial activities have been noted in some preparations of the plant, but the mechanism

is not understood and no treatment method based on these results has been scientifically proven[2]. The leaves of *C. papaya* may be used as vegetable and in the treatment of jaundice, urinary complaintsand gonorrhoea, fever, asthma and dressing wounds[3]. *C. papaya* is marketed in tablet form to remedy digestive problems. *C. papaya* is also applied topically for the treatment of cuts, rashes, stings and burns.*C. papaya* ointment is commonly made from fermented papaya flesh, and is applied as a gel-like paste[4]. Women in India, Bangladesh, Pakistan, Sri Lanka, and other countries have long used green papaya as an herbal medicine for contraception and abortion. Enslaved women in the West Indies were noted for consuming papaya to prevent pregnancies and thus preventing their children from being born into slavery[4]. The latex is used locally as antiseptic. Infusion of the rootis said to remove urine concretions[5]. The young leaves, and to lesser degree, other parts, containcarpain, an active bitter alkaloid, which has a depressing action on the heart. The plant is a strong amoebicide. Latex from the plant is used as dyspepsia cure and can also be applied externally to burns and scalds[5].

Papaya roots have been reported to contain antibacterial properties against *S. aureus. S. pyogenase, B. cereus, S. pneumonia, E. coli, P. mirabilis*and*S. typhi*[6]. Papaya seed is used as carminative, emmenagogue, vermifuge, abortifacient, counter irritant, paste in the treatment of ringworm and psoriasis, anti-fertility agents in males[3]. Theroots are said to cure piles and yaws. In Asia, the latex is smeared on the mouth of the uterus as ecbolic. The root infusion is used for syphilis in Africa. The leaf is smoked for asthma relief in various remote areas. Japanese believe that eating papaya prevents rheumatism[7]. Dietary papaya does reduce urine acidity in humans. The flowers have been used for jaundice. Experimentally, papaya is hypoglycemic. The inner bark is used for sore teeth. The latex is used in psoriasis, ringworm and it's prescribed for the removal of cancerous growths in Cuba[7,8]. It has been reported that the extract of unripe pawpaw possesses anti-sickling and reversal of sickling properties[9]. The tea prepared with the green papaya leaf, promotes digestion and aids in the treatment of ailments such as chronic indigestion, overweight and obesity, arteriosclerosis, high blood pressure and weakening of the heart[10,11].

Alkaloids carpain, pseudocarpain and dehydrocarpain I and II, choline, carposide, Vitamin C and E have been isolated from the leaves of *C. papaya*[3]. It is noteworthy that many other organic compounds have been identified in the leaves of *C. papaya;* hence this research lends a contributory role to the number of chemical constituents in the leaf with respect to South East Nigeria as a geographical location as well as with isopropanol as a choice of solvent.

## MATERIALS AND METHODS

### Plant Materials

*C. papaya* leaves were collected from Oyimo, Ubakala, Umuahia South, Abia State, Nigeria, where it was planted as an economical plant. Identification and authentication were done at the Taxonomy Section of Forestry Department, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, where a herbarium specimen is on file. The leaves were then dried under a shade and thereafter milled into a uniform and fine powder by a mechanically driven attrition mill.

### Extraction of Plant Materials

The powdered leaves (500 g) were successfully extracted with 2 L of isopropanol (8 h / 3 times /30°C). The extract was concentrated under reduced pressure and the supernatant extract was decanted (8.02 g) after complete removal of the solvent. The extract was centrifuged at 10,000 rpm for 20 min and the clear supernatant extract was subjected to systematic GC/MS analysis[12].

### Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

GC analysis was carried out in SHIMADZU JAPAN gas chromatography 5890-11 with a fused GC column (OV-101) coated with polymethyl silicon (0.25 mm × 50 m) and the conditions were as follows: temperature programming from 60-280°C held at 60°C for 1 min, and at 160 °Cfor 2 min (rate 10 °C/min), at 220°C for 2 min (rate 10°C/min) and finally at 280°C for another 2 min (rate 10°C/min). The injection temperature was 220°C. GC/MS analysis was conducted using GCMS-QP 2010 Plus Shimazu Japan with column oven temperature of 60°C. The carrier gas was Helium with a pressure of 100.2 Kpa and linear velocity of 46.3 cm/s. Total flow was 20.7 mL/min, column flow was 1.61 mL/min, injection mode was split, flow control mode was linear velocity, purge flow was 3.0 mL/min and split ratio was 10.0. Also, ion source temperature was 200°C, interface temperature was 250°C, equilibrium time was 3.0 min, solvent cut time was 2.5 min., detector gain was 0.00 KV, detector gain mode was relative and the threshold was 1000. For the mass spec., start time was 3.0 min., end time was

27.0 min, event time was 0.5 s, scan speed was 1250, and start m/z was 50 while end m/z was 600.The mass spectrum was also equipped with a computer fed mass spectra data bank. Hermle Z 233 M-Z centrifuge, Germany, was used. All solvents used were of analytical grade and were procured from Merck, Germany.

**Components Identification**

The components of the extract were identified by matching the peaks with computer Wiley MS libraries and confirmed by comparing mass spectra of the peaks and those from literature[13].

**Antimicrobial Activity**

The *in vitro* antimicrobial activity of the leaf extract of *C. papaya* was carried out for 24 h culture of four selected microorganisms. The organisms used were *Staphylococcus aureus, streptococcusfaecalis, Escherichia coli* and *Proteus mirabilis*. All the test organisms were clinical isolates of human pathogens obtained from stock cultures at the Federal Medical Centre, Umuahia, Abia State, Nigeria. With the aid of a single hole punch office paper perforator, circular discs of 5 mm diameter were cut from Whatman No 1 filter paper. The paper discs were boiled in distilled water for an hour to remove any residual preservatives. The boiled paper discs were allowed to drain dry and they were wrapped in aluminium foil and sterilized in an autoclave at 121°C for 15 min. They were however, used within 48 h of production. The sensitivity of each test microorganism to the extract was determined using the Disc Diffusion Technique[14,15]. A loopful of each test sample organism was aseptically transferred into the surface of a sterile solid medium, appropriate for the test organism. Using a flamed glass hockey, the inoculums was spread evenly over the surface of the medium, and then with the aid of a flamed pair of forceps, the extract bearing paper discs was carefully placed on the surface of the inoculated medium at some distance from one another. The inoculated plates were incubated for 24 h in an incubator at 37°C. They were examined daily for growth and for the presence of inhibition zones around the paper discs. The level of sensitivity was determined by the diameter of the inhibition zone as measured with a transparent millimetre rule. The minimum inhibitory concentration (MIC) was determined by comparing the different concentrations of the extract having different zones and selecting the lowest concentration. Gentamycin was used as a standard antimicrobial agent.

**RESULTS AND DISCUSSION**

The isopropanolic extract of *C. papaya* leavesshowed six peaks from the chromatogram of the extract (Fig. 1). These peaks indicated the presence of six compounds in the extract (1-6, in Fig. 2). The nomenclature, molecular formula, molecular weight, retention time and percentage peak area of these compounds are shown in Table 1. The chemical constituents comprise cyclic ketonic ether (2.17 %), alkene alcohol (45.86 %), aromatic thiophene dioxide (11.78 %), fatty acid ester (12.02 %) and alkaloid (28.18 %). The major constituents in the leaf extract of *C. papaya* were 3,7,11,15-tetramethyl-2-hexadecen-1-ol (37.78 %) and 9-octadecenamide (28.18 %).

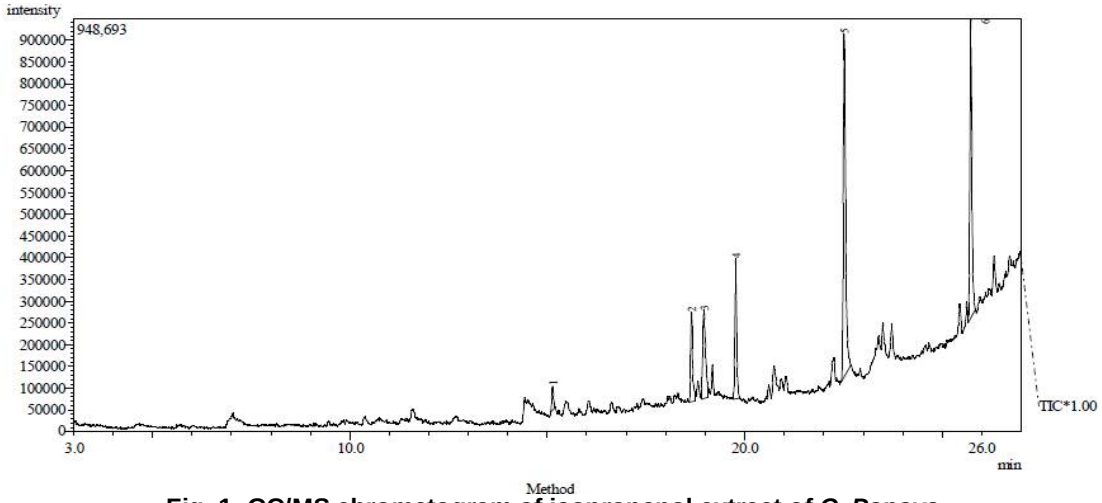


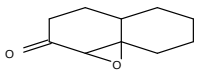

**Fig. 1: GC/MS chromatogram of isopropanol extract of *C. Papaya***

Compound **5** also known as phytol was the highest chemical constituent analysed. It had a peak area of 37.78 %. It is an acyclic diterpene alcohol that can be used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K1[16,17]. Phytol is used in the fragrance industry and in cosmetics, shampoos, toilet soaps, household cleaners and detergents[18,19]. Phytol uses may also include increasing energy and fighting infection and are natural alternatives to use for hypertension and cancer[17-19]. Phytol has been reported to have anti-mycobacterial activity against mycobacterium tuberculosis[17,19,20]. The high quantity of phytol in the leaf of *C. papaya* suggests that the plant might be used in the treatment of tuberculosis.

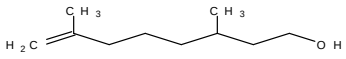
Alkaloid constituted 28.18 % of the extract from *C. papaya* leaves and was the second highest constituent. Alkaloids are a group of naturally occurring chemical compounds which mostly contain basic nitrogen atoms. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants and animals and are part of the group of natural products called secondary metabolites [21]. Many alkaloids are toxic to other organisms and are used as antimicrobial agents. They often have pharmacological effects and are used as anaesthetics, stimulants, analgesics and anti-malarial [22,23,24]. Although alkaloids act on a diversity ofmetabolic systems in human and other animals, they almost uniformly invoke a bitter taste[21]. The detection of 28.18% alkaloid in the leaves of *C. papaya* suggests the use of the plant in treating malaria and as anaesthetics, analgesics or as a stimulant.

Organosulphur compounds (OSCs) prevent or slow down the carcinogenic process induced by a variety of chemical carcinogens. OSCs offer protection against cancer. These include inhibition of the carcinogens, dermatitis and other minor wounds[25]. Organosulphur compounds have been reported to have numerous beneficial health effects including protection from oxidative damage[24,26,27]. 3-methyl-4-(phenylthio)-2-enyl-2,5-dihydrothiophene-1,1-dioxide was detected in the *C. papaya* leaves. This compound may lend anticarcinogenic property to the extract since OSCs offer protection against cancer.

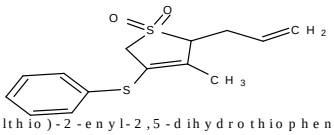
Compounds **1**, **2** and **5** are terpenoids. This means that the extract consists 48.03 % of terpenoids. Terpenoids constitute part of the essential oils from plants. Compound **1** belongs toketonicterpenoids which are not very common in the majority of essential oils; they are relatively stablemolecules and are not particularly important as fragrance or flavour substances[28]. In somecases, ketones are neurotoxic and abortifacients such as camphor and thujone (note that the composition of compound **1** is just 2.17 % which is low)[28,29] but have some therapeutic effects. They may be mucolytic, cellregenerating, sedative, antiviral, analgesic and digestive. Due to their stability,ketones are not easily metabolized by the liver. Other examples of ketones found inessential oils include carvone, menthone, pulegone, fenchone, camphor, thujone andverbenone[28]. Compound **2** and **5** are alcoholic terpenoids. In addition to their pleasant fragrance, alcohols are the most therapeutically beneficial ofessential oil components with no reported contraindications. They are antimicrobial, antiseptic, tonifying, balancing and spasmolytic. Other examples of essential oil alcoholsare linalol, menthol, borneol, santalol, nerol, citronellol and geraniol[28].



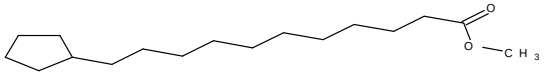

[1] hexahydro-1aH-naphtho[1,8a-b]oxiren-2(3H)-one



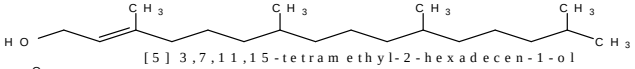

[2] 3,7-dimethyloct-7-en-1-ol



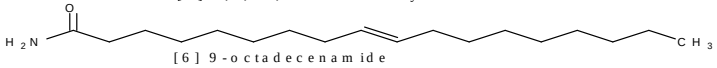

[3]3-methyl-4-(phenylthio)-2-enyl-2,5-dihydrothiophene-1,1-dioxide




[4] cyclopentaneundecanoic acid, methyl ester




[5] 3,7,11,15-tetramethyl-2-hexadecen-1-ol




[6] 9-octadecenamide

Fig. 2: Structures of the chemical constituents of isopropanol extract of *C. papaya* leaves

**Table 1: Chemical constituents identified from the GC/MS analysis of the leaf extract of *C. papaya***

| Chromatogram peak | Compound name | Molecular formula | Molecular weight | Retention time(min) | Peak area(%) | Nature of compound |
|---|---|---|---|---|---|---|
| 1 | Hexahydro-1-aH-naphtho[1,8a-b]oxiren-2(3H)-one | $C_{15}H_{24}O_2$ | 166 | 15.135 | 2.17 | cyclic ketonic ether |
| 2 | 3,7-dimethyloct-7-en-1-ol | $C_{10}H_{20}O$ | 156 | 18.655 | 8.08 | Alkene alcohol |
| 3 | 3-methyl-4-(phenylthio)-2-enyl-2,5-dihydrothiophene-1,1-dioxide | $C_{14}H_{16}O_2S_2$ | 280 | 18.969 | 11.78 | Aromatic thiophene dioxide |
| 4 | Cyclopentaneundecanoic acid, methyl ester | $C_{17}H_{32}O_2$ | 268 | 19.773 | 12.02 | Fatty acid ester |
| 5 | 3,7,11,15-tetramethyl-2-hexadecen-1-ol | $C_{20}H_{40}O$ | 296 | 22.526 | 37.78 | Alkene alcohol |
| 6 | 9-octadecenamide | $C_{18}H_{35}NO$ | 281 | 25.732 | 28.18 | Amide (alkaloid) |

**Table 2: Antimicrobial activity of the leaf extract of *C. papaya***

| Test Microorganism | Concentration (%) | | | | |
|---|---|---|---|---|---|
| | 25 | 50 | 75 | 100 | MIC (%) |
| *Staphylococcus aureus* | 8.21 | 11.23 | 13.22 | 14.33 | 25 |
| *streptococcus faecalis* | - | 7.43 | 9.47 | 11.39 | 50 |
| *Escherichia coli* | 7.77 | 10.23 | 12.31 | 12.98 | 25 |
| *Proteus mirabilis* | 6.67 | 8.37 | 9.01 | 12.37 | 25 |

Values are in mm and include the diameter of the paper disc (5 mm).
Data are means of triplicate determinations.
MIC = Minimum Inhibitory Concentration
-= Zone of No Inhibition

Gentamycin which was used as a standard antimicrobial agent showed the highest inhibitory effect against all the microorganisms with inhibition zone of 37 mm for *S. aureus,* 43 mm for *S. faecalis*, 39 mm for *E. coli* and 35 mm for *P. mirabilis*,all at 100 % concentration. The presence of a considerable amount of alkaloid in the extract could be one of the reasons behind its antimicrobial effect.Alkaloids have established broad-spectrum antibacterialactivity[22]. Also, as mentioned above, essential oils possess antimicrobial activity. Because of the variability of amounts and profiles of the components of essential oils, it islikely that their antimicrobial activity is not due to a single mechanism, but to several sites ofaction at the cellular level. Then, different modes of action are involved in the antimicrobialactivity of essential oils[28]. One of the possibilities for action is the generation of irreversible damage to the membraneof bacterial cells, that induce material losses (cytoplasmic), leakage of ions, loss of energysubstrate (glucose, ATP), leading directly to the lysis of bacteria (cytolysis) and therefore toits death. Another possibility of action is inhibition of production of amylase and proteasewhich stop the toxin production, electron flow and result in coagulation of the cell content [28,30,31,32,33].

## CONCLUSION

*C. papaya* is widely used as herbal medicine in South East Nigeria. This work describes the GC/MS analysis results of the isopropanolic extract of the leaves of *C. papaya,* and six chemical constituents have been characterized. This research reveals that the major constituent ofthe extract are amide, alkene alcohol, cyclic ketonic ether, aromatic thiophene dioxide and fatty acid ester, revealing that 48.03 % of these compounds are terpenoids. The compounds observed could alsobe as a result of the use of isopropanol as a choice of solvent which has a particular polarity and eluent power. The synergistic effect of the essential oils and alkaloid in the extract of the leavesof *C. papaya*could be the reason behind its use as herbal remedies in Eastern Nigeria.

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