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Phytochemical screening, antimicrobial and antioxidant properties of assorted *Carica papaya* leaves in Ghana

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Abstract
The bioactive compounds of the leaves of *Carica papaya*; solo and solomix were extracted using ethanol and n-hexane, and investigated for the presence of secondary metabolites. Both ethanol and n-hexane extracts revealed the presence of alkaloids. Flavonoids, glycosides and saponins were present in only the ethanol extract whereas tannins were present in the n-hexane extract. The bioactivities of the leaf extracts were attributed to their phytochemical constituents. Antimicrobial activity of the extracts were determined against some human pathogenic bacteria and fungi such as *Staphylococcus aureus, Escherichia coli, Streptococcus pneumoniae, Bacillus subtilis* and *Candida albicans* using the agar well diffusion and broth dilution methods with the polar extract being more effective. The ethanol extract demonstrated a significant broad-spectrum antimicrobial activity against both gram-positive and gram-negative bacteria, with the highest activity having a zone of inhibition of 10 mm. Antioxidant activity was determined using the DPPH assay method and the absorbance measured using UV- visible spectrophotometer with ascorbic acid as control. The antioxidant activities of solo and solomix showed IC_{50} of $1.465x10^{-2}$ and $1.364x\ 10^{-2}$ respectively. This study demonstrates the efficacy of ethanolic leaf extracts of *C. Papaya* as an alternative antibiotic for the development of newer antibacterial agents.

Keywords: *Carica papaya*, solo, solomix, antimicrobial, antioxidant

1. Introduction
Biological activity is the basis for traditional medicine, which uses the pharmacological efficacy of natural compounds present in herbal preparations for treating human diseases [1]. Plants constitute a good source of cheap and affordable drugs and medicinal plants possess therapeutic efficacy like their orthodox drugs counterpart, yet they show little or less side effects [2]. Plants and their parts such as roots, stems, barks, leaves, flowers, fruits, seeds and exudates form an important major constituent of drugs used in traditional herbal medicinal systems. The therapeutic efficiency of the drugs used in these systems greatly depend on the use of proper and genuine raw materials [3]. The screening of medicinal plant extracts and plant products for antimicrobial and antioxidant properties show that many of such plants are primary sources of antibiotics [4]. Indigenous groups have used curing plants as their personal phytomedical remedies [5]. Pawpaw (*Carica papaya*) belongs to the family *caricaceae* with over twenty species but only one member of the genus *Carica* is cultivated as a fruit tree, while the other three genera (*Cyclicomorpha, Jarilla* and *Jacaratia*) are grown primarily as ornamentals [6]. *C. Papaya* leaves have been used in the treatment of various ailments including urinary tract infections [2]. The *C. Papaya* plant produces a natural compound (Annonaceous acetogenins) in its leaf, bark and twig tissues that possess both highly anti-tumour and pesticidal properties [7]. Antimalarial and anti-plasmodial activities have also been demonstrated by the leaf extract of the plant. The leaves of the *C. Papaya* plants contain karpain, a substance that kills microorganisms that often interfere with the digestive function [8]. Antioxidants are a special group of nutrients produced by the cell, which removes supplements that scavenge free radicals [9]. The free radicals impair the proper functioning of the glutathione peroxidase and regulates the action of immune system, leading to various disease conditions. Nutrient antioxidants such as vitamins C and E within the flavonoids are naturally occurring phenolic compounds in the body [10, 11]. An antimicrobial is a substance that kills or prevents the growth of microbes.

Antibiotics are from natural sources whiles antimicrobials are from synthetic sources [12]. Bromelain (a digestive enzyme extracted from the pineapple plant) has been used for centuries as a folk remedy for digestive problems, and to promote wound healing [13]. Over the years, the use of medicinal plants have been a source of economic value to many parts of the world. The use of antibiotics in medicine is limited, because bacteria have developed resistance against certain antibiotics. Intrinsic resistance and acquired resistance are the two types of resistances developed by bacteria over the years. Methods by which bacteria develop resistance are exclusion of the drug from the target by either decreased cell wall permeability or destruction of the drug, decrease of agent modifying enzymes, increase concentration of metabolites antagonising the drug action and formation of adaptive-drug inactivating enzymes [14].

2. Materials and Methods

2.1 Chemicals

All chemicals and reagents used for this work were purchased from Sigma Chemicals (Huge Ltd, Accra, Ghana). All the solvents used for extraction were suitable for industrial food use and were used as received without any further purification or treatment.

2.2 Sample Collection and preparation

The *C. Papaya* leaves used for this research were collected and identified by a horticulturist at the Department of Horticulture, KNUST.

2.3 Sample Preparation and Extraction of Phytoconstituents

The leaves were washed with distilled water, air-dried for two weeks and ground to fine powder with a mill. The leaves of solo and solomix varieties of *C. Papaya* were separately extracted with ethanol and n-n-hexane using the soxhlet extractor as described by Oyagade and co-workers [15]. Typically, 55 g of ground leaves of solo and solomix varieties were extracted separately in 200 ml of 95% ethanol and n-n-hexane under heat for 8 hours to ensure complete extraction. The solvents were removed by rotary evaporation and the concentrates, stored in the refrigerator prior to analysis [16].

2.4 Phytochemical Screening

Phytochemical constituents such as flavonoids, alkaloids, glycosides, steroids, saponins and anthraquinones were determined qualitatively using standard procedures as described by Edeoga *et al*.,(2005) [17] with slight modification.

2.5 Test for alkaloids

Each leaf sample (0.5 g) was dissolved in 5 ml dilute HCl in a steam bath and filtered. 1 ml of the filtrate was treated with few drops of Mayer's reagent, giving rise to a cream or pale yellow precipitate, indicating the presence of alkaloids.

2.6 Test for tannins

About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blueback colouration, indicating the presence of tannins.

2.7 Test for phlobatannins

Deposition of a red precipitate when an aqueous extract of each plant sample was mixed with 1% aqueous hydrochloric acid was taken as evidence for the presence of phlobatannins.

2.8 Test for saponins

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with three drops of olive oil and shaken vigorously, then observed for the formation of emulsion, indicating the presence of saponins.

2.9 Test for flavonoids

5 ml of dilute ammonia solution was added to a portion of the aqueous filtrate of each plant extract, followed by addition of concentrated sulfuric acid. The yellow colouration disappeared on standing. Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed, indicating the presence of flavonoids.

2.10 Test for steroids

2 ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml sulfuric acid. The colour changed from violet to blue or green in the samples indicating the presence of steroids.

2.11 Standardization of test organisms

All inoculums were standardized using the McFarland Nephelometer method [18]. To prepare this, eleven large test tubes, 10 ml of 1% each of barium chloride and sulfuric acid was added in the various tubes. The reaction gave rise to turbid solutions but the degree of turbidity differed in each test tube. These were then kept on the workbench for use. Afterwards, liquid broth of the test organisms were made in other test tubes and their turbidities were used to match the turbidity of the standard solutions, such that any one that had turbidity similar to the standard solution was considered as having the corresponding number of bacterial suspension per millilitre. The standard used in this work corresponds to 15×108 bacteria suspension per millilitre. To prepare this, 0.5 ml of already prepared nutrient broth was pipetted into a sterile test tube aseptically and the pure culture of the particular test organism was dissolved into it until the bacterial suspension was corresponding to the standard.

2.12 Antimicrobial Analysis

The ethanol and n-hexane leaf extracts were tested against some common organisms such as; *Streptococcus aureus, Streptococcus pneumoniae, Candida albicans, Escherichia coli and Bacillus Subtilis* to determine their zone of inhibition and minimum inhibitory concentration. Agar well method of the agar diffusion technique was used to determine the antibacterial activity of the plant extracts.

2.13 Agar Well Diffusion

Nutrient agar was put in each sterile petri dish and allowed to set and then labelled. A sterile 8 mm cork borer was then used to punch holes (i.e. 5 wells) in the inoculated agar and the agar was then removed. Four wells that were formed were filled with different concentrations of the extract which were labelled accordingly as follows; 200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml, while the 5th well contained the solvent used for the extraction to serve as control. These were then left on the bench for 1 hour for adequate diffusion of the extracts and incubated at 37 °C for 48 hours. After incubation, the diameter of the zones of inhibition around each well was measured to the nearest millimetre along two axes 90° to each other and the mean of the two readings were then calculated.

Minimum Inhibitory Concentration (MIC)

The MIC of the extracts were determined by using the broth dilution technique [19]. The MIC helps to measure more exactly the concentration of an antibiotic necessary to inhibit growth of standardized inoculum under defined conditions [20]. Serial dilutions of the extract in liquid medium were prepared. These were then challenged with small inoculums of an overnight broth culture of the test organisms. The culture was then incubated at 37 °C for 24 hours. The smallest concentration that inhibits the growth was taken as the MIC.

2.14 Zone of inhibition

The zone of inhibition was determined using the nutrient agar method. Ten (10) petri-dishes with each petri-dish corresponding to one test organism for each extract were well labelled and used. 20 ml nutrient agar was put in each petri-dish for the organism. The nutrient agar was allowed to solidify and wells created in them using the cork borer (6 mm) with each well filled with its respective concentration of the plant extract and left for about 1 hour for complete diffusion of the extract within the nutrient agar. The petri-dishes containing the nutrient agar were then incubated between 37 °C and 42 °C for a period of 18 hours after which the zone of inhibition was determined.

2.15 Antimicrobial activity index

Antimicrobial index (AI) for ethanol and n-hexane extracts of *C. Papaya* leaves were calculated as the mean value of the antimicrobial activity obtained against the sum of all individual microorganisms. Weight-age was assigned to activity of extracts against each microbe. For zone of inhibition up to 10 mm, a weight-age of one (1) was given and that ranging from 11 to 20 mm, weightage of two (2) was assigned. For zone of inhibition greater than 20 mm, weightage of three (3) was assigned and for no antimicrobial activity, weight-age of zero was assigned. The sum total of weight-ages obtained by each extract divided by the total number of pathogens tested gave the AI of the extract.

2.16 Antioxidant Analysis Using DPPH

A stock solution of the two extracts, ethanol, and n-hexane were prepared and eight different concentrations of each prepared through serial dilution of the stock solution, using methanol. The UV-Visible spectrophotometer was used to read the absorbance between 515 nm and 517 nm. The absorbance of the 0.06 mM DPPH solution to be used was measured at 515 nm. 1 ml of each of the concentrations from each extract was measured into separate test tubes and 2 ml of the DPPH added and incubated in the dark for 30 minutes, after which the absorbance was read at 515 nm. The scavenging ability on DPPH radicals by each extract concentration was thereafter calculated.

3. Results and Discussion

3.1 Phytochemical Screening

Results obtained for the phytochemical screening of the two varieties of pawpaw leaves are shown in table 1. The phytoconstituents of the two varieties were similar as they contained the same secondary metabolites in both the n-hexane and ethanol extracts. This shows that the have similar secondary metabolites [21]. Both varieties, solo and solomix contained alkaloids, flavonoids, saponins, tannins, glycosides, and steroids. From the results obtained the presence or absence of secondary metabolites in different extracts are dependent on the polarity of the solvent used for extraction. In addition, the ethanol extract contained more phytoconstituents than the non-polar solvent, implying that most of the phytoconstituents were polar and therefore easily extracted with polar solvents [22]. Similar report on the presence of the leaf extracts of *C. Papaya* has been put to bare by Onaku and co-workers [23], however, a report on the methanol extract of the roots from Nigeria showed otherwise [24]. The pharmacological properties of *C. Papaya* lies in the various chemical constituents it contains. For instance, plants rich in tannins have antibacterial potential due to their character that allows them to react with proteins to form stable water-soluble compounds, which kills bacteria by directly damaging their cell walls [7]. Phytotherapeutically, tannins containing plants are used to treat nonspecific diarrhoea, inflammations of mouth, throat, and slightly injured skins [25]. Therefore, the n-hexane extract of *C. Papaya* leaves can be used for the treatment of such ailment. Infections may also be controlled by the presence of saponins. Earlier reports showed that saponins have antibiotic properties and so help the body to fight infections and microbial invasion [22]. The presence of saponins supports the fact that *C. Papaya* leaf has cytotoxic effects such as permeabilization of the intestine as saponins are cytotoxic [26, 27]. It also gives the leaves the bitter taste. Another important action of saponins is their expectorant action through the stimulation of a reflex of the upper digestive tract [7]. Flavonoids have been recognized to possess anti-allergic, anti-inflammatory, antiviral, anti-proliferative, and anti-carcinogenic activities [7]. The anti-allergic function of flavonoids is particularly advantageous since it may help in the treatment of immune system disorders that are responsible for 5–10% of recurrent miscarriages [28, 29]. Since flavonoids prevent platelet stickiness (platelet aggregation), they are probably wonderful remedies for the treatment of all types of miscarriages [21, 30]. Through this preventive function, flavonoids 'thin the blood' and thereby inhibit the clotting pathway. Alkaloids are the most efficient therapeutically significant plant substances. Pure isolated alkaloids and the synthetic derivatives are used as basic medicinal agents because of their analgesic properties. They show antispasmodic and bacterial properties marked physiological effects when administered to animals. The potency of the leaf extract as an anti-malarial agent is because of the presence of alkaloids in the leaves. The alkaloids consist of quinine, which is anti-malarial [7]. Li *et al*. [31] reported that several drugs have been obtained from alkaloid-containing plants because of its pharmacological importance although higher doses can be toxic. A review by Krishna *et al*. [32] outlines the medical values of various parts of the *C. Papaya* tree.

Table 1: Results of phytochemical screening with ethanol and n-hexane extracts of Solo and Solomix varieties

Secondary metabolites	Results of ethanol extracts		Results of n-hexane extracts	
	solo	solomix	solo	solomix
Alkaloids	+	+	+	+
Flavonoids	+	+	-	-
Phenolics	-	-	-	-
Glycosides	+	+	-	-
Tannins	-	-	+	+
Saponins	+	+	-	-

+ indicates presence of metabolites; - indicates absence of metabolites

3.2 Antimicrobial Assay - MIC

The results obtained from the antimicrobial study (tables 2 and 3) indicate that the ethanol extracts of both solo and solomix showed activity against the various test organisms; *Staphylococcus aureus, Streptococcus pneumoniae,*

Escherichia coli, Bacillus subtilis and Candida albicans. The MIC helps to measure more exactly the concentration of an antibiotic necessary to inhibit growth of standardized inoculums under defined conditions [20]. The results obtained show the ethanolic leaf extract of *C. Papaya* is sensitive to both the gram-negative bacteria and the gram-positive bacteria, and fungus with varying sizes of zones of inhibition [33]. Several factors predispose bacteria to antibacterial agents, such as, previous encounters with the agents or the nature of medium used that may affect the diffusion ability of the agent. The demonstration of activity against the test bacteria provides scientific bases for the local usage of these plants in the treatment of certain ailments [7]. The fact that the extracts were active against both gram-negative and gram-positive bacteria tested may be an indication of the broad-spectrum activity of the extract. This observation is significant because of the possibility of developing therapeutic substances that will be active against multidrug resistant organisms. Therefore, this result shows the importance of the leaf extracts in antibiotics to control resistant bacteria that are becoming a threat to human health. The antibacterial activity is because of the phytoconstituent present in the ethanol extract of the leaves of C. Papaya. These include the presence of alkaloids, tannins and flavonoids, which have been shown to possess antibacterial properties [34]. This study opens the possibility of finding new clinically effective antibacterial compounds and formulated reparations for enhancing potency and stability are needed for several bacteria associated disease.

Table 2: Antimicrobial activity of diluted extracts of solo and solomix varieties

Test organisms	Concentration (mg/ml)							
	90	**64**	**32**	**16**	**8**	**4**	**2**	**1**
Bacillus subtilis	-	-	-	-	+	+	+	+
Staphylococcus aureus	-	-	-	-	+	+	+	+
Streptococcus pneumoniae	-	-	-	-	+	+	+	+
Escherichia coli	-	-	-	-	+	+	+	+
Candida albicans	-	-	-	-	+	+	+	+

+ indicates microbial growth; - indicates no microbial growth

Table 3: Agar diffusion assay of ethanol extracts of solo and solomix varieties

Extract	Zone of inhibition for Agar diffusion assay (mm)					
	Test organisms					
	Staphylococcus aureus	***Streptococcus pneumoniae***	***Escherichia coli***	***Bacillus subtilis***	***Candida albicans***	**Antimicrobial Activity Index**
Ethanol (solo)	8.0	9.0	8.5	10.0	8.5	1.0
Ethanol (solomix)	8.5	8.9	8.4	10.2	8.7	1.0
N-hexane (solo)	0	0	0	0	0	0
N-hexane (solomix)	0	0	0	0	0	0
Ciprofloxacin	25.0	27.0	30.0	26.0	20.0	2.8

3.3 Antioxidant Activity

Antioxidants are known to be substances that reduce oxidation of other molecules. In the process, one antioxidant molecule replaces one free radical. Since these antioxidants are essential for protection of the human body against reactive oxygen species, they need to be constantly replaced. Many antioxidant methods have been proposed to evaluate the antioxidant activity. In this study, the DPPH assay method was used with ascorbic acid as control. According to Koleva *et al*. (2002) [35], DPPH assay method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extract. The maximum and minimum scavenging abilities (%) of the *Carica papaya* were 65.79% at 0.001 mg/ml and 34.53% at 0.122 mg/ml respectively whiles the ascorbic acid (control) had maximum and minimum scavenging abilities of 93.91% and 73.52% respectively (Fig. 1). In addition, both varieties of the *Carica papaya* demonstrated inhibition of the desired activity IC_{50} (mg/mL) and had significant IC_{50} (mg/mL) values calculated.

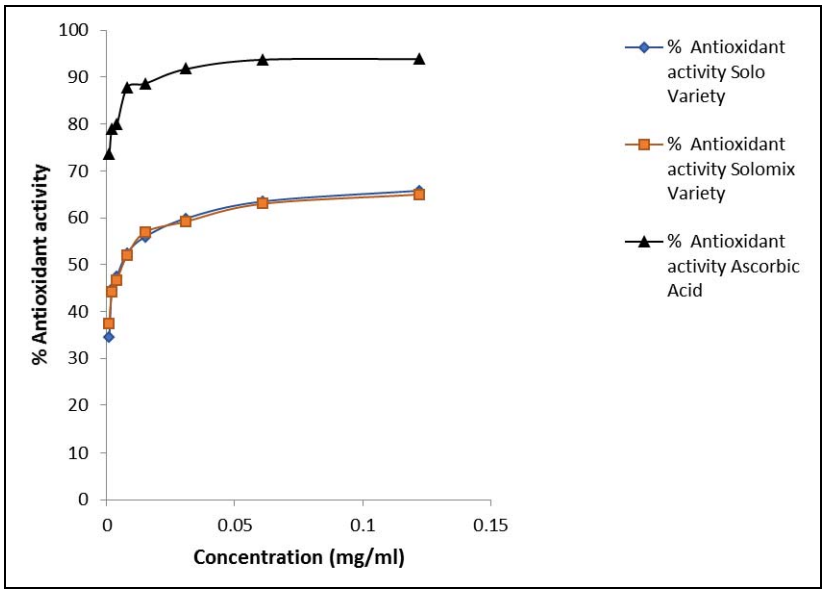

Fig 1: DPPH radical scavenging activity of extracts of solo and solomix varieties and control (Ascorbic acid).

Table 6: DPPH radical scavenging activities of extracts of solo and solomix varieties and ascorbic acid expressed as IC_{50}.

Extract	IC_{50} (mg/mL)
Solo	1.465×10^{-2}
Solomix	1.364×10^{-2}
Ascorbic acid (control)	7.012×10^{-3}

4. Conclusion

This study has shown the phytochemicals, antimicrobial and antioxidant activities of *C. Papaya* leaf extracts. Phytochemicals such as alkaloids, saponins, flavonoids, and glycosides were present in the ethanol extract. These phytoconstituents were responsible for the antimicrobial activity of the plant. This was evidenced in the antimicrobial activity against tested organisms used for the study. The zone of inhibition for the various extracts suggests the degree of efficacy of the extracts on target organisms. Both varieties of the *C. Papaya* leaves demonstrated antioxidant activated. This research has confirmed the antimicrobial and antioxidant properties of the leaves extracts of *C. papaya*.

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