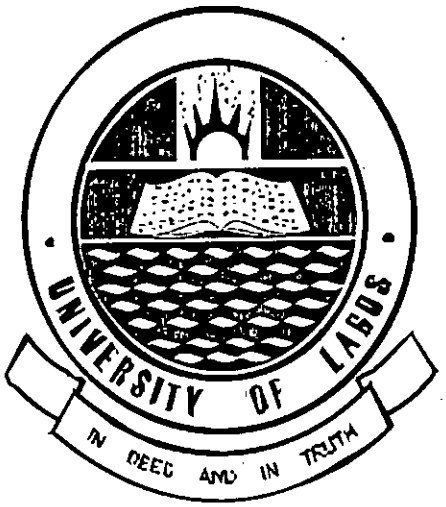

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AIMS AND SCOPE

It is the official vision of the University of Lagos "*to be a top class institution for the pursuit of excellence in knowledge through learning and research, as well as in character and service to humanity*".

The University in furtherance of, and informed by this vision proclaims the official objective "*to produce graduates who will be well-qualified to operate and develop the public service, engage in industrial management and pursue development and research*". One of the strategies instituted by the University towards the achievement of this objective is manifested by the publication of a world-class scholarly journal, the *UNILAG Journal of Medicine, Science and Technology*, ***UJMST***.

The *UNILAG Journal of Medicine, Science and Technology* is a peer-reviewed publication with focus on basic and applied research in the physical and biological sciences, medical and pharmaceutical sciences, as well as disciplines of engineering, technology and environmental sciences. The Journal, which is a biennial publication, has an Editorial Advisory Board constituted by eminent scientists and researchers spread across the globe, and accepts original contributions from authors in the global scientific community.

Authors are encouraged to submit manuscripts that typically include descriptions of the problem formulation, the establishment of an appropriate model, method(s) of problem solution, results and applications of interest to ideal practice. Where applicable experimental data are required to be complete and should include sufficient description of experimental set-up, methods and relevant experimental conditions, as well enable replication. Original research papers are hereby invited not only from Higher and Research Institutions in Nigeria but all over the world.

PREFACE

The 1962 Act of parliament which established the University of Lagos mandated us to, inter alia:
"Encourage, promote and conduct research in all fields of learning and human endeavour".

World class universities are known and judged by the quality of their teaching, research facilities and research outputs. A true academic, therefore, justifies his/her position in academia through teaching and quality research.

At the University of Lagos, each faculty has its journal that focuses on publishing research findings within the compass of the various disciplines in the faculty.

Unilag Journal of Medicine, Science and Technology (UJMST), a science-based journal, has been established to provide another platform for the publication of quality research findings that will contribute to national development in the fields of engineering, environmental science, medicine, science and technology. UJMST is dedicated to increasing the depth of the subject across disciplines within the already defined scope with the aim of expanding knowledge of the subject in the field that it captures. It is a biennial publication and all the articles are peer-reviewed.

The journal welcomes the submission of original manuscripts of significant contributions to knowledge. They should meet the general criteria for academic excellence and must be consistent with the Author guidelines.

We are grateful to Tertiary Education Trust Fund (TET-Fund) for providing financial support for the production of this journal.

Prof. Rahamon A. Bello
Vice-Chancellor
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Akoka, Lagos.

ARTICLES

SAPONIN, OTHER PHYTOCHEMICAL CONSTITUENTS AND ANTIOXIDANT PROPERTY OF *CARICA PAPAYA* LEAF EXTRACT

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Abstract

This study examined the preponderant phytochemical and antioxidant potential in the extract fractions of Carica papaya leaf to ascertain possible mechanism of action of the plant's earlier reported antioxidant and antisickling properties. Soxhlet extraction of the C. papaya leaves gave rise to the initial crude methanolic extract of the papaya leaf and subsequent exhaustive fractionation using a separatory funnel with chloroform, ethyl acetate and butanol organic solvents were done to obtain the desired extract fractions. Determination of quantitative and qualitative phytochemical constituents of C. papaya leaf extract and determination of antioxidant property of the different fractions were done using standard protocols. The results showed that saponin was the most abundant phytochemical in the chloroform, butanol and water-soluble fractions but not the ethyl acetate fraction. While the antioxidant assay showed that the chloroform fraction had the highest antioxidant property. The result of this study supports earlier findings on the rich antioxidant property of the C. papaya leaf crude extract and specifically pinpoints the type of phytochemical and fraction that may possess more of this antioxidant property.

Keywords: Saponins, Phytochemicals, Antioxidant, Antisickling, *Carica papaya*

INTRODUCTION

Medicinal plants consist of secondary metabolites which are evenly distributed throughout the plant root, bark, stem, leaf, seed or fruit. These tannins, saponins, steroids, flavonoids, alkaloids, phenols, terpenoids, etc. make up the active principles of the plants and are responsible for their medicinal properties (Edeoga *et al.*, 2005). Antioxidants may be defined as radical scavengers which protect the human body against free radicals that may cause various pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neurodegeneration.

Several herbs and spices have been reported to exhibit antioxidant activity, including rosemary, sage, thyme, nutmeg, turmeric, white pepper, chilli pepper, ginger, and several Chinese medicinal plant extracts (Okwu, 2001). The majority of the active antioxidant compounds are flavonoids, isoflavones, flavones, Anthocyanins, coumarins, lignans, Catechins, and Isocatechins (Cushnie and Lamb, 2005). In addition to the above, compounds found in

natural foods, vitamins C and E, betacarotene, tocopherol, melatonin, uric acid (found in animal blood stream) and glutathione are known to possess antioxidant properties. Phenol and phenolic compounds like flavonoids have been reported to possess significant antioxidant and free radical scavenging activity (Aiyegoro and Okoh, 2009). These compounds are known to be biologically active through different mechanisms and may be the possible explanation of the reported antisickling effects of some plants.

Carica papaya is a medicinal plant which in the plant kingdom belongs to Caricaceae family. It is sometimes called "tree melon" it is a large tree–like plant with a single stem growing from 5 to 10 meters tall and spirally arranged leaves confined to the top of the trunk (Iwu 1993). All parts of *Carica papaya* are used to cure different diseases. For example it is used ethnomedically as a heart tonic analgesic, an anti- inflammatory and anthelminthic remedy and also to treat stomach ache. The crude extract and fractions of *C. papaya* leaf have been reported to possess possible antisickling properties (Imaga *et al.*, 2009); analysis of two different concentrations of *C. papaya* crude extract (10 and 5 mg/mL) showed the 10 mg/mL extract as the concentration with highest antisickling effect. Butanol extract showed the highest antisickling activity at 10 mg/mL concentration, while the ethyl acetate extract had the highest antisickling activity at 5 mg/ml concentration. These reported findings indicate the possibility of *C. papaya* leaf extract as potential phytotherapy for sickle cell anemia.

Previous studies have identified the rich antioxidant property present in the crude aqueous extract of *Carica papaya* leaves (Imaga *et al.*, 2010) but the exact phytochemical and fraction responsible for the antioxidant property have not been reported. Thus this research was undertaken to investigate this.

The objectives of this study therefore are:

- Extraction and Fractionation of dried *C. papaya* leaves using four different solvents namely chloroform, ethyl acetate, butanol and water
- Determination of the antioxidant activity of the extract fractions using different *in vitro* methods

MATERIALS AND METHODS

Chemicals

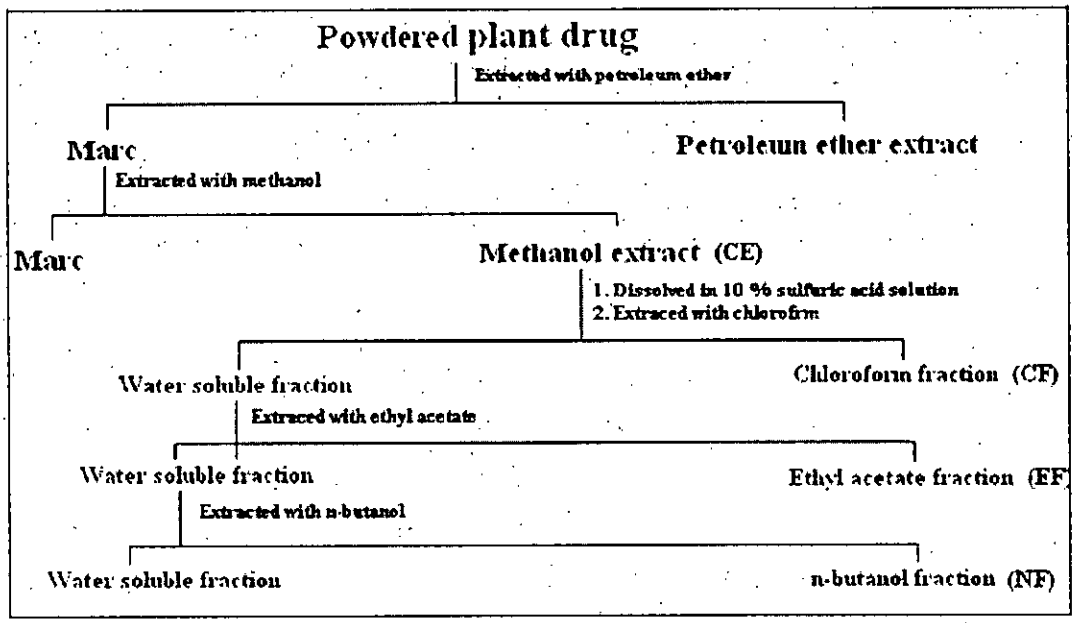
Petroleum Ether (60-80°C), chloroform, ethyl acetate, n-butanol and methanol solvents used were of analytical grade and were purchased from Sigma Chemical Company, USA.

Collection of Plant Material

Dried leaves of *C. papaya* were collected from a reputable herbal market in Mushin, Lagos State, Nigeria and authenticated at the Department of Botany, University of Lagos, Akoka, Nigeria.

Extraction and Fractionation of Plant Material

The dried leaf of *C. papaya* was blended into powdered form using a mechanical blender. Soxhlet extraction was done using methanol which was fractionationed into different solvent fractions via a separatory funnel using the method of Parida *et al.*, (2010) as depicted in the chart below.



Phytochemical screening and Quantitative estimation of chemical constituency

Chemical tests were carried out on the aqueous extract and on the powdered specimens to identify and quantify the constituents which include tannins, flavonoids, alkaloids, saponins, and steroids using standard procedures as described by Edeoga *et al.*, (2005).

Antioxidant assay on *C. papaya* fractions

The total antioxidant activity was carried out via the ferric reducing antioxidant power (FRAP) assay, using standard methods described by Aiyegoro and Okoh (2009). 0.02g of each fraction was dissolved in 20mL of its solvent to make 100ig solution. 0.3mL of each solution was measured into a test tube and 3mL of phosphomolybdate reagent added and the solution was boiled for 1 hour 30 minutes. It was cooled and the absorbance measured at 760nm.

Determination of Reducing Power

The reducing power of the extract was evaluated according to the methods described by Aiyegoro and Okoh, (2009). A mixture containing 2.5 mL of 0.2M phosphate buffer (pH 6.6) and 2.5 mL of $K_3Fe(CN)_6$ (1% w/v) was added to 1.0 mL of each extract fraction. The resulting mixture was incubated at 50 °C for 20 min, followed by the addition of 2.5 mL of trichloroacetic acid (TCA, 10% w/v). The mixture was centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution (2.5 mL), mixed with distilled water (2.5 mL) and 0.5 mL of $FeCl_3$ (0.1%, w/v). The absorbance was measured at 700 nm against blank sample.

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay

Standard methods as described by Aiyegoro and Okoh, (2009) were used for the determination of scavenging activity of DPPH free radical. 1mL of each extract

fraction was added 3mL of methanol and 2mL of DPPH and the mixture was placed in the dark for 30minutes at room temperature after which the absorbance was measured at 514nm. A mixture of 4mL of methanol and 2mL of DPPH was used as negative control and ascorbic acid as the positive control. 2mL of methanol was used as blank.

RESULTS

Phytochemical Constituent of *C. papaya* Leaf Extract

The results of phytochemical screening of the crude extract of *C. papaya* showed that saponins, alkaloids tannins, phlobatanins, flavonoids, cardiac glycosides and steroids were present (Table 1) and quantitative estimation gave the yield for alkaloids as 26.67μg/mL, saponin 300μg/mL, phenol 6.4356μg/mL, tannin 14.8552μg/mL and flavonoids 2.1589μg/mL.

The concentration of the phytochemicals determined in crude extract fractions are in this order, saponin>alkaloids>tannin>phenol>flavonoid, saponin with the highest yield. The butanol fraction was found to contain more phytochemicals than the chloroform, ethyl acetate and water-soluble (aqueous) fractions of *C. papaya* leaf extract.

Total Antioxidant Activity of Different Fractions

The results obtained for the antioxidant activity of the chloroform, butanol, ethyl acetate and water-soluble fractions are as shown in Fig. 1. The chloroform fraction had the highest activity, followed by the butanol>water-soluble>ethyl acetate fractions in that order.

Reducing Power of Different Fractions

The reducing power of each of the chloroform, butanol, ethyl acetate and water-soluble fractions were obtained (Fig. 2). The chloroform fraction also had the highest activity, followed by the water-soluble>ethyl acetate>butanol fractions.

DPPH Radical Scavenging Percentage Inhibition of Different Fractions

The results of the DPPH assay of the chloroform, butanol, ethyl acetate and water-soluble fractions also showed (Fig. 3) that the chloroform fraction had the highest scavenging inhibition activity followed by the ethyl acetate>butanol>water soluble fractions, at all concentration ranges tested (25, 50, 75,100mg/mL).

DISCUSSION

Antioxidants are present at a wide range of concentrations in body fluids and tissues, with some such as glutathione or ubiquinone mostly present within cells, while others such as uric acid are more evenly distributed (Aiyegoro and Okoh, 2009). The action of one antioxidant may therefore depend on the proper function of other members of the antioxidant system. The amount of protection provided by any one antioxidant will also depend on its concentration, its reactivity towards the particular reactive oxygen species being considered, and the status of the antioxidants with which it interacts (Aiyegoro and Okoh, 2009).

In this study, the phytochemical, saponin was present in the chloroform, butanol and aqueous fractions, but not in the ethyl acetate fraction, while steroid was the least in quantity and found only in the chloroform fraction. Quantitative phytochemical screening on the crude *C. papaya* showed that saponin had the highest

concentration(296μg/mL), thus indicating that it is the most abundant phytochemical present in *C. papaya* crude extract and solvent fractions. This confirms earlier findings that crude *C. papaya* leaf can have inhibitory effect on inflammation, can be used to develop immunity and protect the body against infection (Imaga *et al.*, 2010). This is because saponins, a group of phytochemicals that can be found in a variety of plant foods, have been shown to have a number of protective effects on the human body, including warding off cancer, lowering cholesterol, and preventing heart disease (Skene and Philip, 2006). Saponins are potent antioxidants, substances that neutralize free radicals to prevent disease. Saponins stimulate the immune system by increasing the production of antibodies, which help fight off bacterial and fungal infections (Skene and Philip, 2006). Other health benefits of saponins include reducing inflammation by producing an inhibitory effect on inflammation, lowering blood glucose responses, preventing dental caries, protecting against bone loss and increasing the effectiveness of certain vaccines as adjuvant (Skene and Philip, 2006). Saponin has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness. These immense benefits of saponin make its presence in large quantities in *C. papaya* leaf extract a very palpable reason for the plants reported antioxidant and antisickling activities.

It is also probable that the flavonoids, tannins, steroids and glycosides present in the extract fractions all act in synergy to produce the medicinal benefits inherent in the *C. papaya* leaf extract.

Antioxidant property carried out on the fractions *C. papaya* fractions showed that chloroform fraction had the highest total antioxidant capacity compared to other fractions, with a $p<0.005$ significance. In the reducing power assay, Choloform fraction had a more significant result than all the other fractions, suggesting that the chloroform fraction may contain the antioxidant property of *C. papaya* leaf extract. Further research is however required, to elucidate the structures of the chemical constituents present in the chloroform fraction of *C. papaya* leaf extract.

This current report of the natural antioxidant compounds of *C. papaya* may help to develop new herbal formulations for antioxidant therapy and the plant extract fraction can be considered as a good source of natural antioxidants for medicinal uses such as against aging and other diseases related to radical mechanisms.

CONCLUSION

In this study, the phytochemical screening of *C. papaya* fractions showed a high concentration of saponin in almost all the fractions while antioxidant assay of the fractions of *C. papaya* leaf showed that the chloroform fraction had the highest antioxidant property, thus suggest the exact phytochemical constituents and fraction possessing the plant's inherent anitoxidant and antisickling activities.

ILLUSTRATION

Table 1: Phytochemicals present in the different fractions of *C. papaya* leaf extract

TEST	WSF	CF	BF	EAF
TANNIN	–	–	+	+
SAPONIN	+	+	+	–
CARDIAC GLYCOSIDES	–	–	+	+
STEROIDS	–	+	–	–
PHLOBATANIN	+	–	+	–
FLAVONOIDS	–	+	–	+

Legend: + stands for present and – stands for absent. WSF-water soluble fraction, CF- chloroform fraction, BF – butanol fraction, EAF- ethyl acetate fraction.

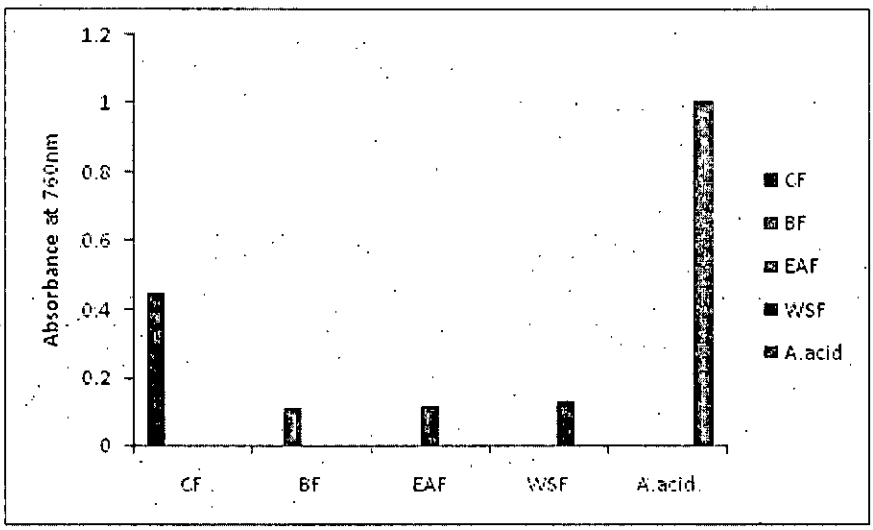


Fig. 1: Total antioxidant capacity of different fractions of *C. papaya* .
Values are mean ± SD of triplicate results. WSF – Water Soluble Fraction, CF – Chloroform Fraction, EAF- Ethyl Acetate Fraction, BF – Butanol Fraction and A. acid – Ascorbic acid as standard.

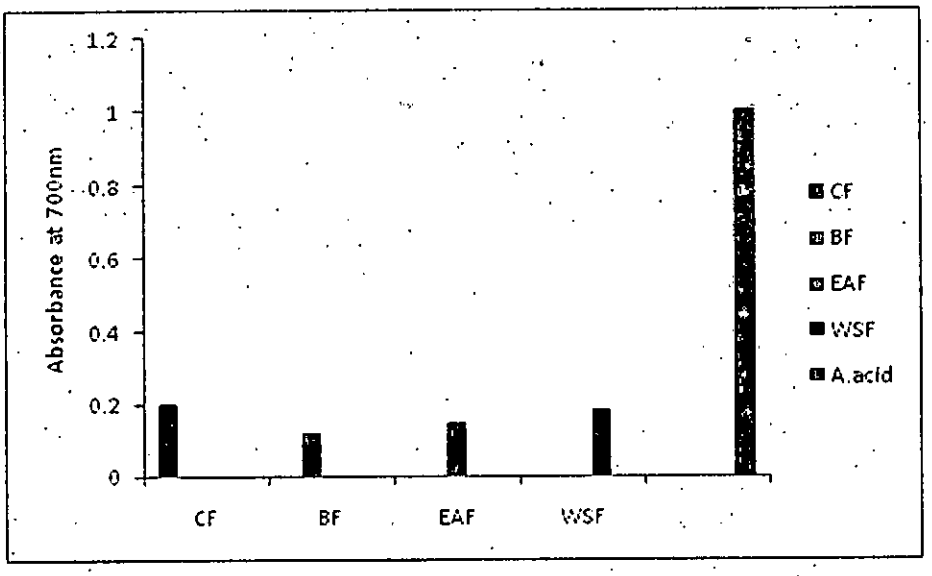


Fig. 2: Reducing power of different fractions of *Carica papaya*.
Values are mean ± SD of triplicate results. CF- Chloroform fraction, BF – Butanol fraction, EAF – Ethyl acetate, WSF – Water soluble fraction and A. acid - Ascorbic acid as standard.

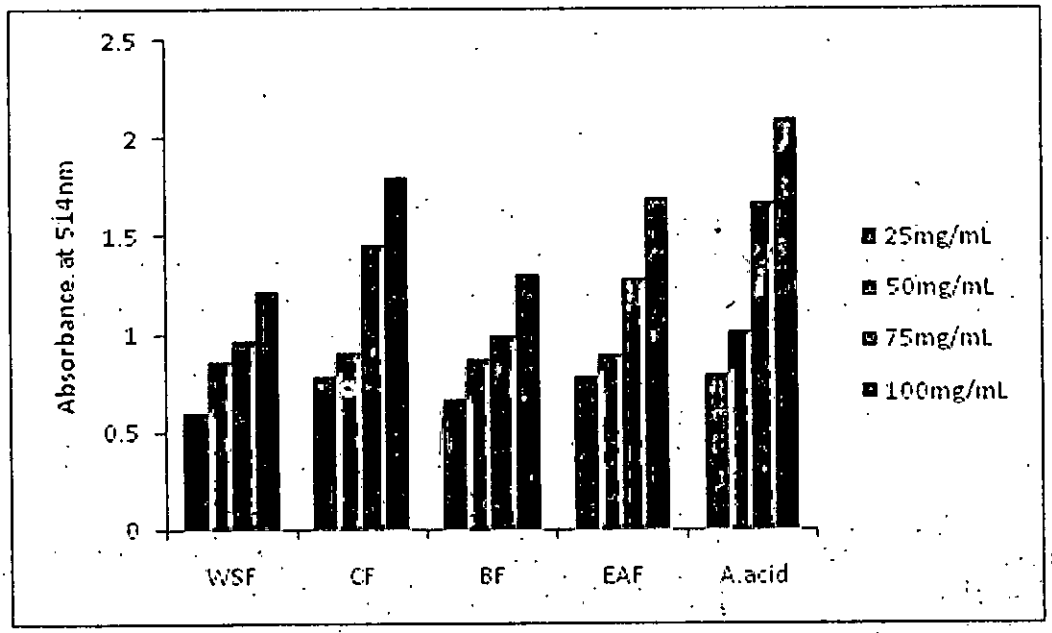


Fig. 3: DPPH scavenging inhibition by *C. papaya* leaf fractions at different concentrations (25 - 100mg/mL).
Values are mean ± SD of triplicate results. WSF – water soluble fraction, EAF- ethyl acetate, CF – chloroform fraction, BF – butanol fraction and A. acid – Ascorbic acid as control.

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