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Antioxidant and Antimicrobial Properties of Selected Plant Leaves

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Authors' contributions

This work was carried out in collaboration between all authors. Author BOTI designed the study, managed the literature searches and wrote the first draft of the manuscript. Authors JFF and FE carried out the research in the laboratory. Author ASO performed the statistical analysis. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: To determine the antimicrobial and antioxidant potentials of some readily available plant leaves in order to source for alternate antioxidants and antibiotics.
Study Design: Randomized complete block design.
Place and Duration of Study: Department of Food Science and Technology, Federal University of Technology Akure Nigeria between Feb 2010 and Jan 2011.
Methodology: Ethanol, hexane and water extracts from leaves of *Anacardium occidentale* (cashew), *Cocos nucifera* (coconut), *Citrus sinesis* (sweet orange), *Citrus limon* (lemon) *and Carica papaya* (pawpaw) were prepared and screened for their antioxidant and antimicrobial properties. The total phenolic content (TPC) was determined by folin-Ciocalteu assay. Antioxidant property of the plant extracts were evaluated using inhibition of free radical 2, 2- diphenyl-2-picryl hydrazyl (DPPH). The antimicrobial activity of the extract against microorganisms (*Acinetobacter* spp., *Bacillus cereus*, *Escherichia coli*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Salmonella typhi*, *Aspergillus niger* and *Aspergillus flavus*) was determined using modified agar-well diffusion method.
Results: Total phenol content (TPC) of leaf extracts based on tannic acid equivalent revealed that the TPC of cashew leaf ranged from 2.21 to 7.49 mg TAE/g, coconut leaf

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extract (0.59-2.22 mg TAE/g), lemon leaf extract (0.97-3.9 mg TAE/g), sweet orange (0.54-0.69 mg TAE/g) and pawpaw leaf extract (0.22- 0.36mgTAE/g). At 0.2mg/ml concentration, the highest antioxidant activity was observed from hexane extracts (45.03%-76.05%) followed by water extracts (45.82% -71.7%) and ethanol extracts (32.75%-56.79%). Ethanol extract (0.2mg/ml) from *A. occidentale* and *C. papaya* showed antimicrobial activity against all the eight microorganisms tested with inhibition zones ranging from 2-12 mm. The highest inhibition zone of 12mm was observed in *A. occidentale* leaf against *Shigella dysenteriae* while *C. limon* leaf had the lowest inhibition zone of 2 mm against *B. cereus.*

Conclusion: We may conclude that *A. occidentale* and *C. papaya* leaves demonstrated broad spectrum activities. The results provided evidence that the plant leaves investigated in this study might indeed be potential sources of natural antioxidant and antimicrobial agents if further investigated.

Keywords: Antioxidant; antimicrobial; Anacardium occidentale; Cocos nucifera; Citrus sinesis; Citrus limon and Carica papaya.

1. INTRODUCTION

The medicinal value of plants have assumed important dimension in the past few decades owing mainly to the discovery that extracts from plants contain not only minerals and primary metabolites but also a diverse array of secondary metabolites with antioxidant potential [1]. The leaves of certain crops such as okra, black pepper, cassava, pawpaw, garden eggs, cashew, cocoyam and cotton are grown for their fruits, roots or tubers but the leaves are also harvested and consumed as vegetables.The active components are normally extracted from all plant structures, but the concentrations of these components vary depending on genetic, environmental, pre-and post-harvest treatments and analytical methodologies. However, parts which include, leaves, stems, barks, roots, bulks, corms, rhizomes, woods, flowers, fruits or the seeds known to contain the highest concentration of the bioactive compounds are preferred for therapeutic purposes [2].

Plant-based antioxidants are now preferred to the synthetic ones because of safety concerns [1]. These factors have inspired the widespread screening of plants for possible medicinal, antimicrobial and antioxidant properties [3]. Antioxidant substances block the action of free radicals which have been implicated in the pathogenesis of many diseases including atherosclerosis, ischemic heart disease, cancer, Alzheimer's disease and in the aging process [4]. Antioxidants are also used to preserve food quality mainly because they arrest oxidative deterioration of lipids.

Phenolic compounds, tannins and alkaloids are the most important antimicrobial agent and bioactive constituents in plant [5]. The leaf of cashew (*Anacardium occidentale*) contains high concentrations of vitamin C, carotenoids, phenolic compounds and minerals [6]. Lemon leaf (*Citrus limon)* possesses certain therapeutic properties and has been used for a variety of ailments including relief of digestive tract spasms, reduction of fever and strengthening of the nervous system [7]. *Carica papaya* extracts possess antibacterial, anti-inflammatory activity, antifertility, anti-hypertensive and anti-cancer properties. In addition, it demonstrated anti-ulcer and diuretic activities [8]. The essential oils neroli, from flowers and petitgrain from leaves of sweet orange (*Citrus sinesis*) are used in perfumery. In addition, orange leaves can be boiled to make tea [9].

Several researchers reported the extensive use of additive in agro allied industry, however, restrictions on the use of these compounds are being imposed because of their carcinogenicity [10]. Furthermore, multiple drug resistance in human pathogenic microorganism has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases [5]. Therefore, we carried out this work to determine the antimicrobial and antioxidant potentials of some readily available plant leaves that produce edible fruits.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fresh leaves from orange, lemon, cashew, pawpaw and coconut trees were collected from local farms in Akure town in Ondo State. They were taken to the Forestry Department of Federal University of Technology Akure Nigeria for identification.

2.2 Sample Preparation

The leaves were dried at room temperature, ground into uniform powder to increase the surface area of the sample for extraction. Sample (500g) of each leaf was weighed, poured into three sterile glass beakers each containing 1000 ml of 95% ethanol, hexane and water. Each beaker was tightly covered, shaken vigorously and kept for 3 days to enhance proper dissolution of the bioactive compounds in the samples [11]. Each sample solution was shaken vigorously and filtered with Whatmann filter paper 125mm (Whatmann Int. Ltd., Maidstone, U.K) at room temperature. Each filtrate (ethanol and hexane extracts) was then evaporated in a rotary evaporator (BUCHI Rotavapor R-114, Switzerland) at 45ºC until the extracts became completely dry. The extracts were stored at 4ºC in a refrigerator until required for further analyses.

2.3 Determination of Total Phenolic Content

The total phenolic content (TPC) was determined by Folin-Ciocalteu assay using tannic acid as standard [12]. One hundred microliter of extract concentration (using extract solvent) containing 0.2mg extract was dispensed into a test tube, 100µl of distilled water and 2.5ml of folin-ciocalteu reagent (Merck, Darmstadt, Germany) was added respectively and shaken thoroughly, after 3 minutes, 2.0 ml of 7.5% sodium carbonate solution was added and the mixture was incubated at 45^0C in a water bath for 40 minutes. Absorbance was measured at 760nm against a blank. The same procedure was repeated to all standard tannic acid solution. The blank is a mixture of 0.2ml of distilled water, 2.5ml of folin- ciocalteu reagent and 2.0ml of 75% sodium carbonate. The total phenolic content was expressed as tannic acid equivalent (mg of TAE/g sample) through the calibration curve of tannic acid. All tests were carried out in triplicate.

2.4 Free Radical Scavenging Activity of Leaves

The hydrogen atom or electrons donating ability of the corresponding extract were measured from the bleaching of purple colour methanol solution of DPPH (Sigma-Aldrich, St. Louis, Mo., U.S.A). The spectrometric assay uses stable radical 2, 2-diphenyl picryhydraxzyl (DPPH) reagent [13]. Six hundred microliter of extract concentration (using extract solvent)

containing 0.02mg of extract was added to 0.6ml of 0.0185% methanoic solution of DPPH. After a 30 minute incubation period at room temperature the absorbance was read against distilled water as blank at 517nm. Controls with 1% solvent (ethanol, hexane and water) and without the extract were also set up under the same condition for all the experiments. The percentage antioxidant activity:

$$(AA\ \%) = \frac{absorbance\ of\ control - absorbance\ of\ sample}{Absorbance\ of\ control} \quad x \quad 100$$

2.5 Antimicrobial Activity of the Crude Leaf Extracts

The extracts were tested for activity against bacteria and fungi using modified agar-well diffusion method procedures described by Clinical and Laboratory Standard Institute [14]. The ethanol, hexane and water extracts of the leaves were individually tested against a range of eight microorganisms. The microorganisms stock cultures which include *Acinetobacter* spp., *Bacillus cereus, Escherichia coli, Salmonella typhi, Shigella dysenteriae, Staphylococcus aureus*. *Aspergillus flavus* and *Aspergillus niger* were provided by the Microbiology unit of Department of Food Science and Technology, Federal University of Technology, Akure. Five hour broth cultures of the test bacteria and fungi adjusted to $10^8 CFU^{-1}$ respectively were applied on the surface of Nutrient agar (HiMedia Laboratories Limited, Mumbai, India). A sterile flamed cork borer of 8 mm diameter size was used to punch four wells into each of the seeded plates and 0.5 ml of each extract was dispensed in each well. For fungus, the density of spore suspension was determined using haemocytometer and adjusted to 4×10^5 spores/ml. One millilitre of the suspension was added to 20ml PDA, shaken gently and allowed to solidify before boring wells into the agar and applying the extract. Controls were set up by filling wells with 1% of various solvents used. The plates were then incubated at 35ºC for 24hr for bacteria and at 30ºC for 48-72hr for yeasts and fungi. The experiments were performed in duplicate and the means of the diameters of the inhibition zones were calculated.

3. RESULTS AND DISCUSSION

3.1 Phenolic Content

Total phenolic content (TPC) of ethanol extract and hexane extract from plant leaves are presented in Fig. 1, while water extracts from the leaves yielded no positive activity. The TPC of *A. occidentale* leaf extracted by different solvent ranged from 2.21 to 7.49mgTAE/g, *C. nucifera* leaf (0.59 to 2.22mgTAE/g), *C. limon* leaf (0.97 to 3.9mgTAE/g), *C. sinesis* leaf (0.54-0.69 mgTAE/g) and *C. papaya* leaf ranged from 0.22 to 0.36mgTAE/g. Comparing the solvent used, TPC of 70% ethanol extract ranged from 0.36 – 7.49mgTAE/g and that of hexane extract ranged from 0.69 to 3.97mgTAE/g. We observed that bioactive substances demonstrated varying degree of solubility in different solvents. The medicinal effects of plants are often attributed to the antioxidant activity of the phytochemical constituents, mostly the phenolics. Plants having significant medicinal values have often been found to be rich in phenolics and to have high antioxidant potentials [1]. The antioxidant activity of phenolics is due to their redox properties which allow them to act as reducing agents, metal chelators and free radical quenchers [15]. The secondary metabolites such as phenolics and flavonoids from plants have been reported to be potent free radical scavengers. They are found in all parts of plants such as leaves, fruits, seeds, roots and bark [16].

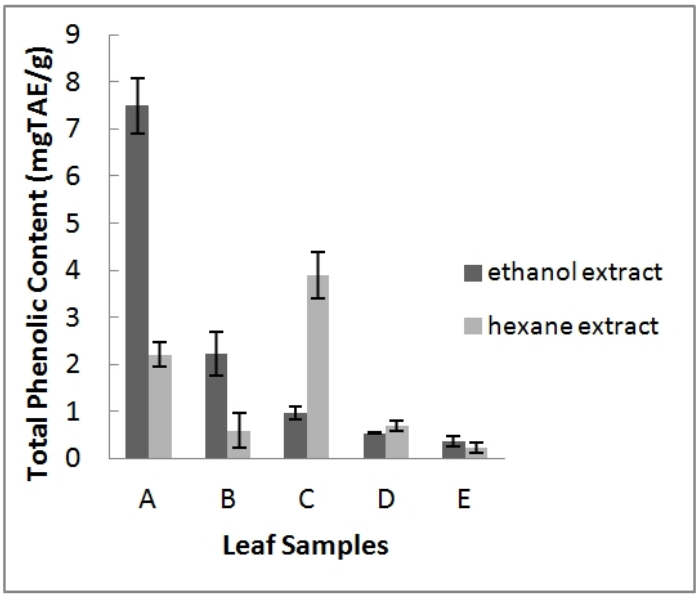

Fig. 1. Total Phenolic Content of ethanol and hexane extracts from leaf samples A *(Anacardium occidentale), B (Cocos nucifera), C (Citrus sinesis), D (Citrus limon) and E (Carica papaya)*

3.2 Free Radical Scavenging Activity

The reagent 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a free radical and reduction of this chemical by probable antioxidants result in loss of absorbance. Thus, the degree of discolouration of the solution indicates the scavenging efficiency of the added substance. The results of free radical scavenging properties of the extracts expressed in percentage DPPH activities are shown in Fig. 2. All the plant extracts exhibited moderate to high antioxidant activities. From ethanolic extract highest antioxidant activity was observed in *C.sinesis* leaf (56.79%) followed by *C. nucifera* leaf (45.28%), *C. papaya* leaf (42.59%), *A. occidentale* leaf (39.55%) and *C. limon* (32.75%). For hexane extract the highest antioxidant activity was obtained from *C. papaya* leaf (76.05%) while the least scavenging property (45.03%) was from *C. sinesis* leaf. Ability of water extracts from the tested leaves showed that the radical scavenging property ranged from 45.82% from *C. papaya* to 71.7% in *A. occidentale* leaf. However, the values obtained for phenol content and radical scavenging activity of leaves investigated in this study were lower when compared to that reported for some green leafy vegetables commonly consumed in Nigeria. Sun drying of the green leafy vegetables led to a significant increase in the total phenol content (6.45-223.08% gain), and free radical scavenging ability (126.00-5757.00% gain) [17].

Natural phenolic exert beneficial effects mainly through their antioxidant activity. These compounds are capable of decreasing oxygen concentration, intercepting singlet oxygen, preventing first chain initiation by scavenging initial radical, such as hydroxyl radicals, chelating metal ion catalyst, decomposing primary product of oxidation to non radical specie and breaking chains to prevent continued hydrogen abstraction from substance [18]. In addition, polyphenolic compounds are primarily responsible for the antioxidant activity of natural extract due to their redox properties and chemical structures [19]. Several

researches have demonstrated that there can be a correlation between phenolic content and antioxidant capacity of plant extracts or their essential oils [20,21]. However, other bioactive compounds such as naphtoquinones [22], carnosic acid and carnosol [23], capsaicinoids compounds [24] and allicin from garlic [25] are considered as free-radical scavengers.

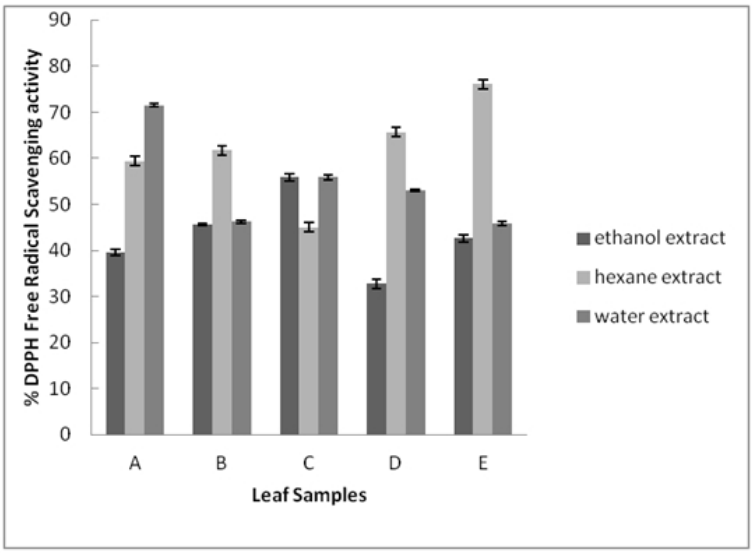

Fig. 2. Free Radical Scavenging activity of ethanol, hexane and water extracts from leaf samples A (*Anacardium occidentale*), B (*Cocos nucifera*), C (*Citrus sinesis*), D (*Citrus limon*) and E *(Carica papaya*)

3.3 Antimicrobial Activities

The antimicrobial activity of the plant extract against microorganism examined was assessed by the presence or absence of inhibition zones. The result showed that the ethanol extract of the plant leaves (Table 1) demonstrated better antimicrobial activity compared to water extract which produced inhibition against three of the tested bacteria (*B. cereus, S. dysenteriae and Salmonella typhi*) within the range of 4.0mm-10.0mm. On the other hand, the hexane extract of the plants produced no antimicrobial activities. Table 1 revealed that the ethanol extract of *C. papaya* leaf and *A. occidentale* leaf showed antimicrobial activity against the eight microorganisms tested, *C. nucifera* leaf inhibited six out of eight microorganisms while *C. sinesis* and *C. limon* leaf exhibited antimicrobial activity against five microorganisms. The highest inhibition zone of 12mm was observed in *A. occidentale* leaf against *Shigella dysenteriae* while *C. limon* leaf had the lowest inhibition zone of 2mm against *B. cereus*. Plants having significant medicinal values have often been found to be rich in phenolics and to have high antioxidant potentials [1]. Based on the results it can be concluded that the antimicrobial nature of ethanol extract of *A. occidentale* leaf is apparently related to its high phenolic content. The antioxidant and antimicrobial activities recorded from cashew leaf may be as a result of the earlier report which documented a wide range of chemicals such as anacardic acids, anacardol, hydroxybenzoic acid, kaempferol, salicylic acid and tannins isolated and identified from this plant [26]. Furthermore, the isolation of antibacterial phenolic compounds such as anacardic acids, cardols, methylcardols and cardanols from cashew nut shell oil [27]. The antifungal, antiaflatoxigenic and antioxidant

activity of *C. sinensis* is equally reported [28]. Flavonoid, phenolic compound, tannins and alkaloid are the most important antimicrobial agent and bioactive constituents in plant [5]. Some of these bioactive compounds singly or in combination inhibit the life processes of microorganisms by binding their protein molecules, acting as chelating agents, altering their biochemical systems, or causing inflammation of the cells [29]. Furthermore, the bitter taste, pungent and repulsive smell in some plants have been found to have repressive ability over the metabolic activities of microorganisms.

Table 1. Antimicrobial activity of ethanol extract (0.1 mg/ml) from the leaf samples

Method: modified agar-well diffusion			**Zones of inhibition (mm)**			
Microorganisms	***Anacardium occidentale***	***Cocos nucifera***	***Citrus sinesis***	***Citrus limon***	***Carica papaya***	**Control (ethanol)**
Acinetobacter spp.	10.0	8.0	-	3.6	6.0	-
Bacillus cereus	10.0	7.0	2.0	4.0	5.0	-
Escherichia coli	10.0	3.0	4.0	2.0	4.0	-
Shigella dysenteriae	12.0	5.0	5.0	5.0	6.0	-
Staphylococcus aureus	10.0	-	-	-	5.0	-
Salmonella typhi	7.0	3.0	4.0	2.0	4.0	-
Aspergillus niger	6.0	3.0	9.0	-	10.0	-
Aspergillus flavus	2.0	-	-	-	10.0	-

(-)No inhibition

4. CONCLUSION

Antioxidant and antimicrobial properties of various extracts from plants have recently been of great interest in both research and food industry, because of their possible use as natural additives which emerged from a growing tendency to replace synthetic antioxidants with natural ones. Owing to the antioxidant and antibacterial activities exhibited by the leaf extracts investigated in this study, some of them could be considered a natural herbal source that can be used in food and pharmaceutical industries. However, further studies are needed to obtain purified compounds that may be responsible for the activities observed from the tested leaves.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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