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Evaluation of the antioxidant activities of *Psidium guajava* and *Aloe vera*

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ABSTRACT

This paper aimed to study the antioxidant properties of two Nigerian plants *Psidium guajava* (guava) Myrtaceae and *Aloe vera* Liliaceae plants which have a broad application in phytomedicine. The plants were assessed by quantifying their individual chemical contents and their 1:1 (mass/mass) homogenous combination (guava+*A. vera*) simultaneously. The non-antioxidant phytochemical quantified included total alkaloids. There was a significant difference in the total alkaloids content (measure on dry weight basis, mg/g) in the analysed plant materials in the order of guava (111.13±0.45)>guava+*A. guava* (65.99±0.37)>*A. vera* (22.86±0.15). The antioxidant properties measured were the levels of total phenol, tannin, total flavonoid, total saponin, vitamin C, 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging ability and trolox equivalent antioxidant capacity (TEAC). *P. guajava* recorded significantly higher ($p<0.05$) antioxidant phytochemicals contents than *A. vera* except for vitamin C where it recorded significantly lower ($p<0.05$) value. There was no significant difference ($p>0.05$) in the vitamin C contents of *A. vera* and the combined plant materials, guava+*A. vera*. Guava had also significantly higher ($p<0.05$) DPPH scavenging ability (0.056 mg/ml), and TEAC (12.51±0.40 mM/gdw) than *A. vera*. The combined plant materials guava+ *A. vera* showed synergistic properties in the DPPH free radical scavenging ability (0.15 mg/ml) and antagonistic activity in the TEAC (4.58±0.17 mM/gdw). This study suggests that while guava may be a better antioxidant than *A. vera* when used separately, the combined plant materials produces synergistic antioxidant interaction, which could be used to enhance their medicinal applications.

Keywords: Psidium guajava, Myrtaceae, Aloe vera, Liliaceae, phytochemical composition, antioxidant activities

1. INTRODUCTION

Plants have been known to contain components of phytomedicine since times immemorial [1]. Man is able to obtain from them a wondrous assortment of industrial chemicals [2]. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc. that is any part of the plant may contain active components. These plant-derived substances have recently become of great interest owing to their versatile applications [1]. Medicinal plants are the richest bioresource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, pharmaceutical intermediates and chemical entities for synthetic drugs [2].

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In living systems, free-radicals are generated as part of the body's normal metabolic process, and the free radical chain reactions are usually produced in the mitochondrial respiratory chain, liver mixed function oxidases, by bacterial leucocytes, through xanthine oxidase activity, atmospheric pollutants, and from transitional metal catalysts, drugs and xenobiotics [3]. The management of oxidative stress due to free radical-mediated pathophysiology has become a central focus for scientific efforts designed to prevent or ameliorate tissue injury. A lot of researches have been carried out with the aim of discovering antioxidant phytochemicals from natural products or medicinal plants for the prevention or treatment of free radical-induced diseases [4]. The most likely and practical way to ameliorate or even prevent such free radical induced disorders and associated diseases, is to improve the body antioxidant status. In this wise dietary bioactive compound from different functional foods, herbs and nutraceuticals can be used. In traditional medicine these include classes of preparations known as decoctions, infusions, fluid extracts, tinctures, pilular (semisolid) extracts or powdered extracts. Such preparations have been popularly called galenicals, named after Galen, the second century Greek physician [2]. These active principles differ from plant to plant due to their biodiversity and they produce a definite physiological action on the human body.

The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects and economic viability. The systematic screening of plant species with the purpose of discovering new bioactive compounds has been a routine activity in many laboratories [2,5].

Psidium (P.) guajava Linnaeus (guava), Myrtaceae, is used widely in traditional medicine throughout Latin America and the Caribbean for the treatment of diarrhoea, dysentery, gastroenteritis, stomachaches, and indigestion [5]. The main constituents of guava are vitamins, tannins, phenolic compounds, flavonoids, essential oils, sesquiterpene alcohols and triterpenoid acids. These and other compounds are related to many health effects of guava [6]. *P. guajava* extracts are also employed for antinociceptive, antimicrobial, hepatoprotective, antispasmodic [7-9] and anticancer properties [8]. Many other pharmacological activities of this plant have also been described: antioxidant, anti-allergy, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, antitussive, and antidiabetic activities, stimulating many studies with this plant [1]. In Egypt, guava leaf infusion and/or inhaled vapour are widely used for their antiinflammatory effect in soothing a persistent cough and alleviating the symptoms of the common cold [8]. In a study conducted in Nigeria guava stem bark extract was shown to exhibit high antioxidant and antibacterial activities [10]. The antioxidant activity of guava due to phenolic compounds, are of great importance in preventing the action of free radicals in the body and, therefore, able to prevent cancer and help to prevent premature skin aging [1].

Aloe (A.) vera (*Aloe (A.) barbadensis* Miller) of the family Liliaceae has been found to contain many active components some of which can individually or in combination with other compounds exert antimicrobial activities. Some of these active compounds are anthraquinones, polysaccharides, enzymes, salicylic acid, tannin, lignin, alkaloids, saponins, fatty acids, hormones and amino acids [11,12]. According to Meenatshi et al. [13], *Staphylococcus aureus* and *Escherichia coli* were inhibited extensively by ethyl acetate extract at the lowest concentration of 1.06 mg/ml which equals approximately 0.001% concentration. *A. vera* has also been shown through *in vitro* and *in vivo* studies to exhibit antiinflammatory, antibacterial, antioxidant, hypoglycaemic properties, immune-boosting hepatoprotective, antiproliferative, anticarcinogenic, antiaging, and laxative effects which are attributed to its radical scavenging and immunomodulatory mechanisms [14,15]. *A. vera* has been shown to have antidiabetic, antitumour, and antimutagenic properties [16].

The interest in using these organic compounds derived from plants arises from the fact that the consumption of fruits and vegetables is associated with reduced incidence of cancer and cardiovascular diseases, while many bioactive compounds are beneficial to human health. Recently, scientists have found evidences that specific combinations of phytochemicals are more effective in protecting against diseases than the isolated compounds, pointing to a need to study the synergy among active compounds in plants [1]. Wang et al. [17] in his work showed that medicinal plants in combination interact synergistically with high efficacy and have a broader spectrum of action. Plants combined are usually those known to exert similar curative effects. This study therefore was fashioned to compare the crude phytochemical composition of *P. guajava* (stem bark) and *A. vera* (leave) and evaluate their synergistic antioxidative potentials *in vitro*.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Fresh stem bark and leaf samples of *P. guajava* and *A. vera* respectively were collected from Uturu, Abia State, Nigeria. The plant materials were identified and authenticated in the taxonomy unit of Department of Plant Science and Biotechnology, Abia State University, Nigeria. The plant materials were after authentication dried and milled into powder. Exposure to direct sunlight was avoided to prevent the loss of active components. The different powdered plants materials were separately put in air-tight bottles and stored at 4^{o}C for further analyses. At the point of analyses, a 1:1 (mass/mass) homogenous mixture of the stem bark and leaf samples of *P. guajava* and *A. vera* respectively was prepared by homogenizing 5 g of each sample together.

All the chemicals used for analysis were of analytical grade. Reagents were freshly prepared using standard methods.

2.2 Phytochemical Analyses

These analyses determine the biologically active non-nutritive compounds that contribute to the flavour, colour, and other characteristics of plant parts. Quantitative analyses were done at the International Institute of Tropical Agriculture (IITA), Ibadan.

2.2.1 Preparation of Methanolic Extract

Methanolic extracts of the guava powder (stem bark) and *A. vera* (leaf) were separately prepared following the method of Chan et al. [18], by adding 25 ml of methanol to 0.5 g of samples contained in a covered 50 ml centrifuge tube, and shaking continuously for 1 hour at room temperature. The mixtures were centrifuged at 3,000 rpm for 10 minutes, and then the supernatant were collected and stored at -4^{o}C for further analysis.

2.2.2 Determination of Total Phenol Content (TPC)

The total phenol content of samples of methanolic extracts was determined according to the Folin–Ciocalteu method reported by Chan et al. [18]. Briefly, for each sample, 300 µl of extract was dispensed into test tube (in duplicates). To this was added 1.5 ml of Folin–Ciocalteu reagent (diluted 10 times with distilled water), followed by 1.2 ml of Na_2CO_3 solution (7.5% w/v). The reaction mixture was mixed, allowed to stand for 30 minutes at room temperature before the absorbance was measured at 765 nm against a blank prepared by dispensing 300 µl of distilled water instead of sample extract. TPC was expressed as gallic acid equivalent (GAE) in mg/g material.

2.2.3 Determination of Tannin Content

Tannin content of samples was determined according to the method of Padmaja [19] as follows. Sample (0.1 g) was extracted with 5 ml of acidified methanol (1% HCl in methanol)

at room temperature for 15 minutes. The mixture was centrifuged at 3,000 rpm for 20 minutes. About 0.1 ml of the supernatant was added with 7.5 ml of distilled water, 0.5 ml of Folin-Denis reagent, 1 ml of 35% sodium carbonate (Na_2CO_3) solution and diluted to 10 ml with distilled water. The mixture was shaken well, kept at room temperature for 30 minutes and absorbance was measured at 760 nm. Blank was prepared with water instead of the sample. Tannin content was expressed as tannic acid equivalent (TAE) in mg/g material.

2.2.4 Determination of Total Flavonoid Content (TFC)

Total flavonoid content was determined using aluminum chloride method as reported by Kale et al. [20]. About 0.5 ml of methanolic extract was dispensed into test tube, followed by 1.5 ml of methanol, 0.1 ml of aluminum chloride (10%), 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The reaction mixture was mixed, allowed to stand at room temperature for 30 minutes, before absorbance was read at 514 nm. TFC was expressed as quercetin equivalent (QE) in mg/g material.

2.2.5 Determination of Vitamin C Content

The vitamin C content of the aqueous extracts was determined using the method reported by Benderitter et al. [21]. Briefly, for each sample, 75 µl DNPH (2 g dinitrophenyl hydrazine, 230 mg thiourea and 270 mg $CuSO_4.5H_2O$ in 100 ml of 5 M H_2SO_4) was added to 500 µl reaction mixture (300 µL appropriate dilution of hydrophilic extract with 100 µl of 13.3% trichloroacetic acid and distilled water). The reaction mixture was subsequently incubated for 3 hours at 37^{o}C, then 0.5 ml of 65% H_2SO_4 (v/v) was added to the medium, and the absorbance was measured at 520 nm, and the vitamin C content of the sample was subsequently calculated from the calibration curve prepared with ascorbic acid standard.

2.2.6 Determination of Total Saponin Content

Total saponin was determined by the method described by Makkar et al. [22]. About 0.5 g of sample was extracted with 25 ml of 80% aqueous methanol by shaking on a mechanical shaker for 2 hours, after which contents of the tubes were centrifuged for 10 minutes at 3,000 rpm. In a test tube an aliquot (0.25 ml) of the supernatant was taken to which 0.25 ml vanillin reagent (8% vanillin in ethanol) and 2.5 ml of 72% aqueous H_2SO_4 were added. The reaction mixtures in the tubes were heated in a water bath at 60°C for 10 minutes. Then tubes were cooled in ice for 4 minutes and then allowed to acclimatize to room temperature. Subsequently, the absorbance was measured in a Uv/Visible spectrophotometer at 544 nm. Diosgenin was used as a standard and the results obtained were expressed as mg diosgenin equivalent per g of sample dry matter.

2.2.7 Determination of Total Alkaloid Content

The total alkaloid content of the samples were measured using 1,10-phenanthroline method described by Singh et al. [23] with slight modifications. About 100 mg sample powder was extracted in 10 ml 80% ethanol. This was centrifuged at 5000 rpm for 10 minutes. Supernatant obtained was used for the further estimation total alkaloids. The reaction mixture contained 1 ml plant extract, 1 ml of 0.025 M $FeCl_3$ in 0.5 M HCl and 1 ml of 0.05 M of 1,10-phenanthroline in ethanol. The mixture was incubated for 30 minutes in hot water bath with maintained temperature of 70 ± 2^{o}C. The absorbance of red coloured complex was measured at 510 nm against reagent blank. Alkaloid contents were estimated and it was calculated with the help of standard curve of quinine (0.1 mg/ml, 10 mg dissolved in 10 ml ethanol and diluted to 100 ml with distilled water). The values were expressed as mg/g of dry weight.

2.3 Antioxidant Activity Determination

2.3.1 Estimation of DPPH Free-Radical-Scavenging Ability

The free-radical-scavenging ability of the methanolic extracts against 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was evaluated as described by Cervato et al. [24] with slight modification. Briefly, for one sample, appropriate dilution of the extracts (1 ml) was mixed with 3 ml of 60 µM methanolic solution of DPPH radicals; the mixture was left in the dark for 30 minutes before the absorbance was taken at 517 nm. The decrease in absorbance of $DPPH^{\cdot}$ on addition of test samples in relation to the control was used to calculate the percentage inhibition (%Inh.) following the equation: $\%Inh. = [(A517_{control} - A517_{sample}) \div A517_{control}] \times 100$. The IC_{50}, which stands for the concentration of extract required for 50% scavenging activity, was calculated from the dose-inhibition linear regression curve of each extract.

2.3.2 Estimation of ABTS* Radical-Scavenging Ability

The ABTS* radical-scavenging ability of extract was determined according to the method described by Sellappan and Akoh [25]. The ABTS* radical was generated by incubating equal volume of a 7 mM ABTS aqueous solution with $K_2S_2O_8$ (2.45 mM) in the dark for 16 hours at room temperature and adjusting the absorbance at 734 nm to 0.7 ± 0.02 with 95% ethanol. Then 0.2 ml appropriate dilution of the extract was added to 2.0 ml ABTS* solution and the absorbance was measured at 734 nm after 15 minutes. The trolox equivalent antioxidant capacity (TEAC) was subsequently calculated. The calibration equation for TEAC was $Y = -0.0505x + 0.1954$ ($R^2 = 0.9902$).

3.0 RESULTS AND DISCUSSION

As at the time of this study, no reports exist for the comparison of phytochemical composition and synergistic antioxidant activities of *P. guajava* and *A. vera* though a lot of research has been done separately on both plants [5,26-30]. In the light of this observation, the study therefore investigated the quantitative comparative and synergistic antioxidant activities of *P. guajava* (stem bark) and *A. vera* (leaf) popularly used in traditional medicine as medicinal plants. This study was on the basis of similar medicinal effects of both plants.

Plant and plant products are being used as a source of medicine since long. The folkloric use of *P. guajava* and *A. vera* in the management of disease conditions has also been validated [30-32]. The antioxidant activity of plants is mainly contributed by the active compounds present in them, and may contribute to the improvement in quality of life by delaying onset and reducing the risk of degenerative disease associated with ageing [29]. Flavonoids and phenolic compounds widely distributed in plants have been reported to exert multiple biological effects, including, free radical scavenging abilities, antiinflammatory, and anticarcinogenic properties. They were also suggested to be potential iron chelators [4].

The phytochemicals reported in dry weight of the analysed plant materials are presented in table 1. The total phenol, tannin and total flavonoid contents of *A. vera*, guava and *A. vera*+guava were significantly different ($p<0.05$) in the order of guava>*A. vera*+guava>*A. vera*. The combined property of *A. vera* and guava (*A. vera*+guava) showed total phenolic, tannin, and total flavonoids contents of 51.39 ± 0.008 mg/g, 54.39 ± 0.28 mg/g and 2.51 ± 0.19 mg/g respectively.

Table 1: Phytochemical compositions of *A. vera*, guava bark and guava+*A. vera* in dry weight basis (mg/g)

Phytochemical	*A. vera*	Guava bark	Guava/*A. vera*
Total Phenol	4.064 ± 0.083^a	111.86 ± 0.47^b	51.39 ± 0.008^c
Tannin	4.65 ± 0.086^a	142.93 ± 0.51^b	54.39 ± 0.28^c
Total flavonoids	1.43 ± 0.031^a	3.37 ± 0.040^b	2.51 ± 0.19^c
Vitamin C	4.68 ± 0.81^a	1.53 ± 0.066^b	2.51 ± 0.063^a

Total saponin	60.85 ± 0.61[a]	285.76 ± 0.81[b]	123.070 ± 0.56[c]
Total alkaloids	22.86 ± 0.15[a]	111.13 ± 0.45[b]	65.99 ± 0.37[c]

Data represent mean ± standard deviation of duplicate reading obtained. Data in the same row, having different alphabet are statistically significant ($p<0.05$).

The antioxidant inhibitory ability of phenolic compounds has been reported to be due to their hydroxyl groups. Phenols have also been shown to exhibit antidiabetic properties by inhibiting alphaamylase, sodium glucose transporter 1 (SGLUT-1) of the intestinal brush border as well as sucrase [4]. The presence of tannin in the plant materials could be responsible for their astringent properties. Water soluble antioxidants like flavonoid prevent cell damage by oxidative stress which has been implicated in the pathogenesis of various neurodegenerative diseases. Flavonoids have also been shown to exhibit anticancer and antiinflammatory properties.

Conversely, *A. vera* had a high vitamin C content (4.68 ± 0.81 mg/g). While this value was not significantly different ($p>0.05$) from the value of the combined, guava+*A. vera*, guava had significantly lower ($p<0.05$) vitamin C content. The reason for the significantly lower ($p<0.05$) vitamin C content of guava could be due to the protective roles of the plant part (stem bark) thereby harbouring more of secondary metabolites. By virtue of solubility in water, Vitamin C as an antioxidant has the ability to scavenge aqueous peroxyl radical.

The total saponin and total alkaloid contents of the plant materials were significantly different ($P<0.05$) in the order of guava>guava+*A. vera*>*A.vera*. The review of the biological activities of saponins has shown that they exhibit antioxidant activities. In addition, saponins have haemolytic and hypolipidaemic activities as well as the ability to lower cancer risks and inhibit microscopic life forms [4]. Alkaloids have been reported to be the most efficient therapeutically active significant substances [10]. Pure alkaloids, though no report of antioxidant activity as yet, and their derivatives have their basic medicinal value due to the analgesic, antispasmodic and antibacterial properties they possess.
With the exception of vitamin C composition of guava, the phytochemical contents of the combined plant material guava+*A. vera* is higher than that of *A. vera.* This may be due to the chemical nature and reactivity of the compounds present in the mixture, which may have resulted to negative cooperativity. In other words components of the crude guava extract could be acting as an enhancer in the combined form.

DPPH radicals having been extensively used by many workers to investigate the scavenging activity of some natural components [29,33] due to the high sensitivity and was employed to investigate the free radical scavenging ability of the plant materials and their combined activity. As antioxidants donate protons to this radical, the absorption decreases. The extent of the decrease in absorption is taken as a measure of the extent of radical scavenging. The plant materials plus their combination all showed free radical scavenging ability in a dose-dependent manner in the order of guava>guava+*A.vera*>*A.vera*. The IC_{50} value for each extract defined as the concentration of the extract causing 50% inhibition of DPPH absorbance is presented in table 2. Since IC_{50} is a measure of inhibitory concentration, a lower IC_{50} value is a reflection of greater antioxidant activity of the sample. Guava (0.056 mg/ml) had higher antioxidant ability than *A. vera* (3.82 mg/ml). In the combined ability of both plants guava+*A. vera*, the value of 0.15 mg/ml is an indication of a synergistic interaction in the free radical scavenging ability. This interesting outcome could still be attributed to the crude nature of the extract which leaves the possibility of cooperative interactions to enhance effects.

Table 2: Antioxidant activities of *A. vera*, guava bark and guava+*A. vera*

Antioxidant activity	*A. vera*	Guava bark	Guava+*A. vera*
DPPH IC_{50} (mg/ml)	3.82	0.056	0.15
TEAC (mM/gdw)	0.050 ± 0.008^{a}	12.51 ± 0.40^{b}	4.58 ± 0.17^{c}

TEAC results represent mean ± standard deviation of duplicate reading obtained. Data in the same row having different alphabet are statistically significant ($p<0.05$).

Nitric oxide and superoxide radicals are involved in host defense, over production of these two radicals contributes to the pathogenesis of some inflammatory diseases. Moreover in the pathological condition nitric oxide reacts with superoxide anion and form potentially cytotoxic molecules, peroxynitrite. Nitric oxide inhibitors have been shown to have beneficial effects on some aspects of inflammatory diseases [29]. Balakrishnan et al. [29] in their study showed that stem bark extract of *P. guajava* significantly inhibited nitric oxide in a dose-dependent manner with IC_{50} value of 281.51 μg/ml.

ABTS* scavenging ability reported as the trolox equivalent antioxidant capacity (TEAC) is shown in table 2. The result showed significant difference ($P<0.05$) in the order of guava>guava+*A. vera*>*A. vera*. Interestingly, the combined plant material, guava+*A. vera* with TEAC of 4.58 ± 0.17 mM/gdw is an indication of antagonistic property. While *A. vera* had TEAC value of 0.050 ± 0.008 mM/gdw, guava with 12.51 ± 0.40 mM/gdw exhibited a better ABTS* scavenging ability.

The reason for the different interactions obtained in the combined plant material guava+*A. vera* using DPPH and ABTS* free radicals could in addition be associated with the differences in methods.

ABTS assay is based on the inhibition of the absorbance of the radical cation ABTS* which has a characteristic long wavelength adsorption spectrum. ABTS* radicals are more reactive than DPPH radicals and unlike the reactions with DPPH radical which involve H atom transfer; the reactions with ABTS* radicals involve electron transfer process [3]. The extensive use of DPPH for screening antioxidant activity assay may have been because it can accommodate many samples in a short period and is sensitive enough to detect active ingredients at low concentration [29].

4. CONCLUSION

The results of this study indicated that there is a strong linear correlation between the total phenol and total flavonoid contents of the extracts with the antioxidant activities of the plant extracts which is reflected in their high DPPH free radical scavenging ability and TEAC. *P. guajava* exhibited higher activities than *A. vera*. This proved that *P. guajava* could be more effective if used separately in managing oxidative stress condition and its complications.

The results of this study also suggest the possibility of combined use of medicinal plants to achieve synergy with efficacy and broader spectrum of action.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS CONTRIBUTIONS

This work was carried out in collaboration between all authors. Author CI designed the study, searched out the literature and carried the experiments. Authors CI did the analysis and

wrote the first draft of the manuscript. Authors CCJ, CI2 and KUO contributed to analysis of the results and writing the manuscript. All authors read and approved the final manuscript.

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