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Phytochemical Screening, Antioxidant and Antimicrobial Activities of the Essential Oils and Ethanol Extract of *Psidium guajava* Leaf

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Authors' contributions

This work was carried out in collaboration among all authors. Author AE designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors GYS and AE carried out all laboratory work, analysis of the study. Authors AW, GYS and DK managed the literature searches and edited the manuscript. All authors read and approved the final manuscript

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ABSTRACT

Objective: To investigate the Phytochemical screening, antioxidant and antimicrobial activities of the essential oil and ethanol extract of *Psidium guajava*.
Methods: The leaf of *Psidium guajava*belongs to the myrtle family (Myrtacease) which is used as herbal remedies for the cure of many ailments by natives in northern part of Nigeria, was collected in June, 2018 from the Professor's Quarters of Modibbo Adama University of Technology (MAUTECH) Yola. The leaf was air dried, pulverized and extracted by simple overnight maceration technique and then analyzed. Fresh leaf of the aforementioned was extracted using modified steam distillation. The phytochemical screening of the ethanol extract was carried out using standard method.

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Results: The result revealed the present of Tannin, Flavanoid, Alkaloid, Volatile oil, Triterpene, Saponin, Glycoside while phenolic compound was absent in the ethanol extract of *Psidium guajava*. The result of the antioxidant activity of the essential oil was screened using DPPH method and the IC_{25} values of ascorbic acid (standard drug) was 57.92 µl/m and *Psidium guajava* of the essential oil was 46.55 µl/ml respectively. Antibacterial activity was carried out using dics diffusion method and the results showed reasonable zone of inhibition against tasted organisms, with *Staphylococcus epidemidis* being the most inhibited (23 mm) and *Proteus vulgari* being the least inhibited (2 mm) with the ethanol extract of *Psidium guajava*. In contrast, *Staphylococcus aureus* was the most inhibited (13 mm) and *Salmonella typhi* showed the least inhibition (9mm) in the essential oil of *Psidium guajava*.

Conclusion: The result, thus support the use of the plants traditionally to treat chronic diarrhea, fever, diabetes, malaria and suggest its usage in the formulation of new antioxidant and antibacterial drugs.

Keywords: Phytochemical screening; antioxidant; antimicrobial and Psidium guajava.

1. INTRODUCTION

Medicinal plants are useful for healing and curing of human disease due to the presence of phytochemical constituents [1]. About 25% of prescribed drugs in the world today are source from plants [2]. About 75- 80% of people in the developing countries rely on traditional plants based medicines for their primary health care needs [3]. There is abundant number of medicinal plants and only small amounts of them were investigated for its biological and pharmacological activities. Phytochemicals occurred naturally in the medicinal plants such as leaves, vegetables and roots that have defense mechanism and protect from various disease. Phytochemicals are primary and secondary compounds. The primary compounds include Proteins, Chlorophyll and common sugars while the secondary compounds have terpenoids, alkaloids and phenolic compounds [4]. Terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anti-cancer, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities. Terpenoids are very important in attracting useful mites and consume the herbivorous insects. Alkaloids are used as anesthetic agents and are found in medicinal plants [4].

In some years back, there is a little advancement in the development of antimicrobial compounds in an effort to check the harmful effects of microorganisms [5]. Bacterial disease results when the harmful bacteria enter the organism then multiply and invade the body’s defense mechanism. These pathogenic bacteria enter the body through inhalation, ingestion or damaged skin tissue. The inability of the immune system to stop the bacteria from reproducing and spreading consequently results in the symptoms of bacterial disease [6]. The antimicrobial resistance is the foremost problem all over the world with present antibiotic therapy in treating infectious diseases [7]. The development of drug resistance by microorganisms reduces the effectiveness of modern drugs [8]. Thus, resistance to antibacterial agents poses threat in many areas of the world especially in the developing countries [9]. The integration of traditional and modern medicine is gaining increase recognition globally [10,8].

Psidium guajava **(**guava) belongs to the myrtle family (*Myrtacease*). Common names are guava (English), gioba (Hausa), goifa (Yoruba), gova (Igbo). A native of tropical America, it is now planted as a fruit tree in West Africa. A small tree of about 6 – 8 m high, bark is grayish brown, hard or very rough and resistant to termites. The fruits are up to 4 inches long, fleshy, globosely, ovoid or pear-shaped; generally yellowish or white when ripe. They contain a mass of small seeds embedded in the endocarp, though some are seedless or nearly so. It is said to be higher in vitamin C than citrus – 80 mg of vitamin C in 100 g of fruit and also contains an appreciable amount of vitamin A.

The leaves of guava tree in decoction are recommended for gastroenteritis, chronic diarrhea etc. the young leaves and shoots are used for dysentery and diarrhea [11]. It has been reported that the quercetin present can inhibit the intestinal movement and reduce capillary permeability in the abdominal cavity and this may explain the antidiarrheal mechanism of *Psidium guajava* extract [11]. Guava leaves also have antioxidant properties which are attributed to the polyphenols in the leaves.

Tender leaves are chewed to bring relief in tooth ache (analgesic) in Ghana [12]. They are chewed for stomach ache and piles [12]. When boiled with lemon grass (Cymbopogon citrates), an adjuvant, the decoction is used to cure cough. This decoction is also taken in Senegal for tracheobronchitis.Various parts of this plant has been used in traditional medicine to manage conditions like malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and a number of other conditions [13,14].

We as a Chemists are interested in identifying the chemical structures of the isolated essential oils. Having known the chemical structure of the oils, chemists can synthesize the essential oils in the laboratories, so that the sources of the essential oils are no longer confined by the availability of the plants [15].

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Fresh leaf of guava (*Psidium guajava*) was collected in June 2018 from Professor's Quarters of Modibbo Adama University of Technology (MAUTECH) Yola and the plant's leaf was used for the purpose of their phytochemical analysis. The leaf of *P.guajava* was dismembered from the stalk of the plant, washed and air dried under a room temperature, pulverized, crushed into fine powder and weighed. Aliquot portion of the powdered leaves was weighed and used for phytochemical analysis.

2.2 Sample Extraction

Powdered *P.guajava* leaf was weighed 100 g into container, about 400 ml of ethanol was added to sample of *P.guajava* leaf. The mixture was then left for 72 hours (for thorough extraction). The extract were then filtered first through a whatmann filter paper No. 42 (125 mm) and then through cotton wool, the extract is there after concentrated using a rotary evaporator with the water bath set at 60ºC to one-tenth its original volume and then finally freeze dried. The dried residue (crude extract) was then stored at 4ºC. Aliquot portion of the crude plant extract residue were weighed and used for phytochemical screening.

2.3 Phytochemical Screening

The phytochemical screening was performed using a standard procedure according to [16]. Assessing the presence of the following classes of compounds: tannin, alkaloid, saponin, flavonoid, triterpenoid, volatile oil,glycoside and phenol.

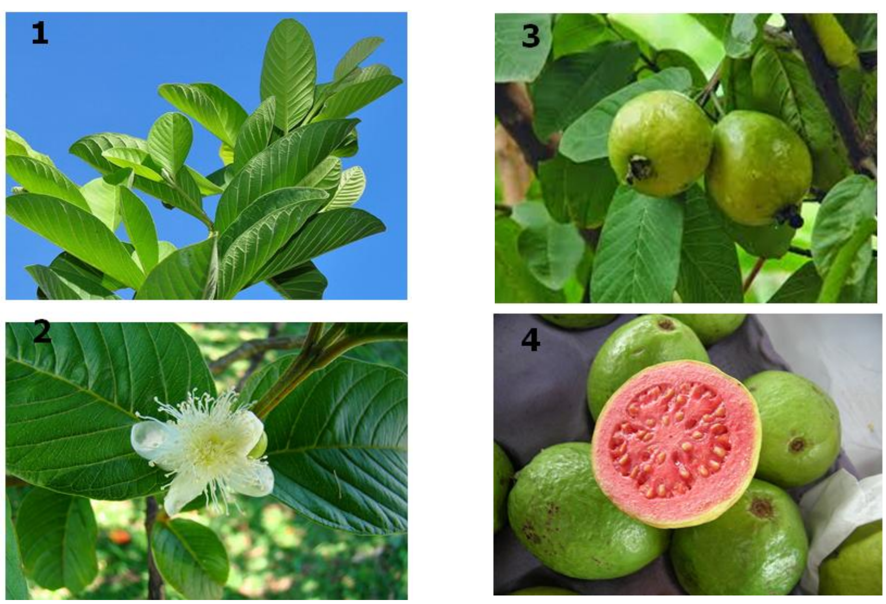

Fig. 1. Different parts of *Psidiumguajava*
1. Leaves 2.Flowering branch and Flower 3, Leaves and Fruits 4. Dorsal view of the fruit

2.4 Extraction of the Essential Oil

1 kg of the pulverized form of each of the samples was subjected to steam distillation in a steam distiller, according to the British pharmacopoeia (BP) method. The time taken for the Isolation of the oil is $2^{1/}2$ hours.

2.5 Antimicrobial Investigation for Essential Oils and Ethanol Extract of the Plants

2.5.1 Determination of antimicrobial activity

The antimicrobial activities of both the essential oil and the ethanol extract were determined using disc diffusion method [17]. Petri dish containing 10 mL of Mueller Hinton agar medium were seeded with 24 hours old culture of selected bacterial and fungal strains. Sterile filter paper discs (9 mm in diameter) containing 1000-5000 ppm of an essential oil and ethanol extract dissolved in DMSO and was placed on the surface of the medium. DMSO and water alone served as negative controls. A standard disc containing Amoxicillin antibiotic drug (30µg/disc) was used as a positive control. Incubation was carried out for 24 hours at 37ºC. The assessment of antimicrobial activity was based on the measurement of diameter of inhibition zone formed around the disc (Diameter of inhibition zone minus diameter of the disc). An average zone of inhibition was calculated in triplicate and an inhibition zone of 8mm or greater was considered sensitive [18]. According to [19], a cleared zone bigger than 10 mm was interpreted as sensitive while smaller than 9mm was interpreted as resistance.

2.5.2 Microorganisms

The bacterial used include: *Escherichia coli, Staphylococcus aureus, Staphylococcus epidemidis, Proteus vulgari, Salmonella typhi*andMethicillin-resistant *Staphylococcus aureus* (MRSA). All the microorganisms used were obtained from the stock culture of the Federal Teaching Hospital (FTH), Gombe state. Cultures were brought to the Department of Microbiology laboratory conditions and subjecting the organisms in peptone water and thereafter, sub cultured into nutrient agar medium and incubated for 24 hours at 37ºC.

2.6 Determination of Anti-oxidant Activity of Essential Oils

2.6.1 DPPH free radical scavenging assay

The 2, 2- diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was carried out for the evaluation of the antioxidants activity. The method was carried out as described previously by [20,21]. The essential oil was dissolved in methanol and various concentrations (5, 10, 25 and 100 µL/ml) were used. The assay mixture contained in a total volume of 1 ml, 500 µL, of the oil, 125 µL prepared DPPH (1ml in methanol), and 375 µL solvent (methanol). After 30 minutes incubation at 25ºC, the decrease in absorbance was measured at λ = 517nm. The radical scavenging activity then calculated from the equation:

% of radical scavenging activity = (Abs control – Abs sample)/Abs control Ẋ 100.

The concentration of sample required to scavenge 50% of DPPH free radical (IC_{50}) was determined from the curve of percent inhibition plotted against their respective concentration.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening of Ethanol Extract of *Psidium guajava*

From the results obtained (Table 1), the preliminary phytochemicals investigation revealed that most of the bioactive compounds tested of, were present in the ethanolic extract of *P. guajava* leaf of the plant. Saponins, glycosides, tannins, flavonoids, alkaloids, volatile oils and triterpenes were found to be present in the ethanol extract of the leaf of *P.guajava*, while phenolic compound happen to be the only bioactive compound absent in the ethanol extract of the leaf of *P.guajava*. The results of phytochemicals investigation of this study is in line with that of [22] and varies from that of the other researchers and it could be due to the part of the plant used, age of the plant, percentage humidity, climatic condition, soil condition, geographical location, time of harvesting or method of extraction [23].

The chemical constituents present in the extracts have some therapeutic values. Tannins are plant metabolites well known for their antimicrobial properties [24]. Flavonoids have both antifungal and antibacterial activities. They possess anti-inflammatory activity [25,26]. Flavonoids, terpenes and alkaloid are known to have antimicrobial and bactericidal properties against several [27]. Saponins, flavonoids, tannins and others have antibacterial activity [28] to act as plant protectants against pathogens in the wild, whereas phenol, flavonoids, tannins, quinines have anticarcinogenic and antioxident activities [29].

The active principles of many drugs found in plants are secondary metabolites [30]. Therefore, basic phytochemical investigation of its extracts for major phyto-constituents is also vital. In the present study the extract of *P.guajava* was screened for phytochemical constituents.

3.2 Percentage Yield of the Essential oil of *Psidium guajava*

696.71 g of the fresh plant leaf was subjected to steam distillation for extraction of essential oil. The results obtained show that *P.guajava* (guava leaves) has percentage yield of 0.26%. The variation in the yield of the essential oils compared to other research may be due to factors like site of collection, time of collection, part and form of the plant used and the extraction methods employed [31].

3.3 Percentage Inhibition of DPPH free Radical by Essential oils of *Psidium guajava* Ascorbic Acid at 517 nm

The essential oils and the ethanolic extract from the leaf of *P.guajava* showed a reasonable zone of inhibition in the antimicrobial activity. Literature reports showed a high correlation between antioxidant activity and phenolic compounds [32]. This implies that compounds that have tannin in nature are expected to exhibit antioxidant activity even though other phenolic compounds like flavonoid also possess antioxidant activity and they are known to be in synergistic relationship with tannin in plants [33].

The scavenging effects of leaf essential oil on the DPPH were expressed as percentage inhibition and they were compared with the standard antioxidant, ascorbic acid. The lowest concentration of the essential oil 5µl/ml showed the highest percentage inhibition value for the plant leaf *P.guajava* of 56.81%, which is very close to the standard, ascorbic acid 64.37%. There was a distinguishing increase in inhibition as the essential oil concentration decreased.

Antimicrobial activity of the essential oil and ethanol extract of *P.guajava*, revealed that the ethanol extract inhabited the growth of the entire microorganisms tested for while the essential oil could only inhibited the growth of two microorganisms (*S. aureus* and MRSA) Fig. 2.

Fig. 3 shows that, ethanol extract gave the highest zone of inhibition (23 mm) on *S. epidemidis* which was even higher or greater than the control of Amoxicillin (22 mm) as compared to the essential oil of the same plant which was resistant, followed by *E. coli* with zone of inhibition (20 mm) with the ethanol extract while the essential oil was also resistant. Essential oil of *P.guajava* showed a higher zone of inhibition (13 mm) on *S. aureus* as compared to ethanol extract which gave (11 mm) on the same microorganism, of the same plant (*P.guajava*). The least inhibition zone was shown by the ethanol extract (2mm) against or on P. vulgari while the essential oil of the same plant showed resistant on the same microorganism (Fig. 3). An inhibition zone of 8mm or greater was considered as a good antimicrobial activity [18]. According to [19], a cleared zone bigger than 10 mm was interpreted as sensitive while smaller than 9mm was interpreted as resistance extract.

Table 1. Phytochemical screening of ethanolic leaf extract of *Psidium guajava*

Bioactive compounds	*Psidium Gujava*
Saponins	+
Glycosides	+
Tannins	+
Flavonoids	+
Alkaloids	+
Volatile oils	+
Phenolic compound	–
Triterpene	+

(+) = Compound is Present, (–) = Compound is absent

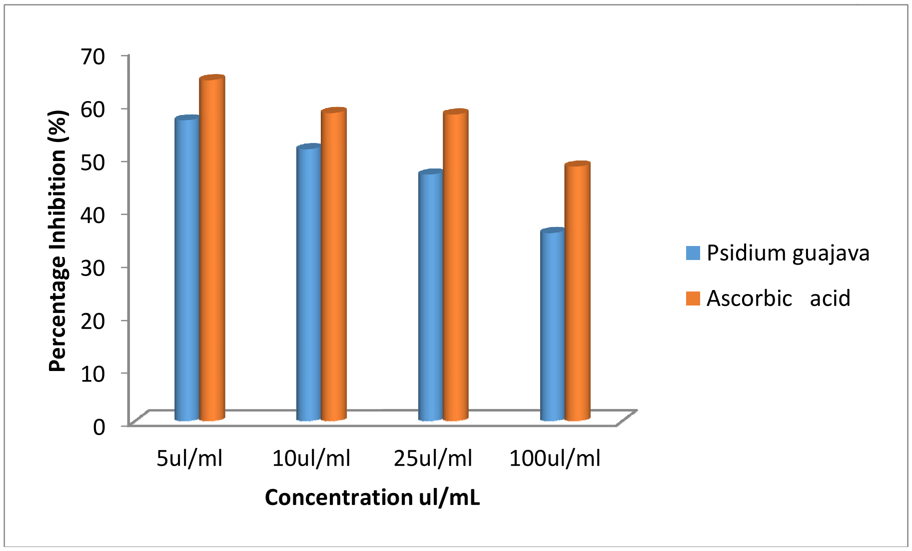

Fig. 2. Antimicrobial activity of the essential oil and ethanol extract of *Psidium guajava*

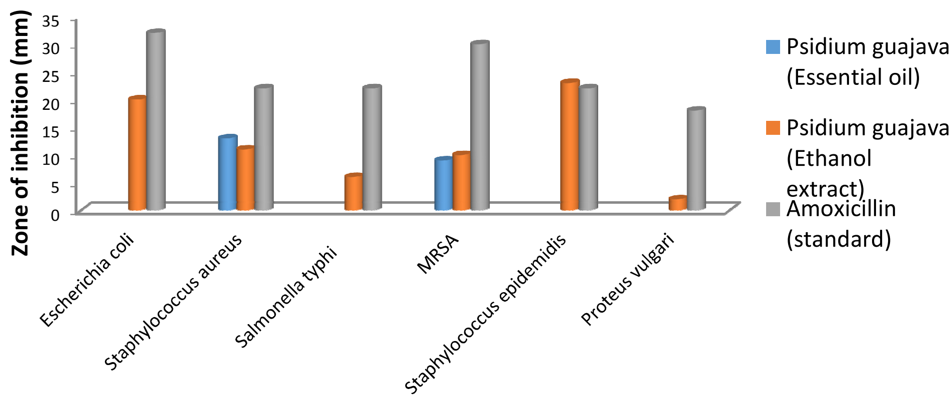

Fig. 3.The inhibition zone of essential oil and Ethanol extract of *Psidium guajava* against some selected bacteria

4. CONCLUSION

The result, thus support the use of the plants traditionally to treat chronic diarrhea, fever, diabetes, malaria and suggest its usage in the formulation of new antioxidant and antibacterial drugs.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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