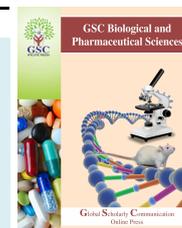


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(RESEARCH ARTICLE)



Antibacterial and Antifungal activities of aqueous leaves extract of some medicinal plants

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Abstract

The aqueous leaves extracts of *Psidium guajava*, *Vernonia amygdalina* and *Azadirachta indica* were evaluated for phytochemical compositions, antibacterial and anti-fungi activities against microbial isolates. Ampicillin and vastatin were used as standards for antibacterial and antifungal assays respectively. All phytochemicals tested were present in the aqueous extract of the plants. Glycoside and anthraquinone were absent in methanol extract of *P. guajava* and *A. indica* respectively. *Psidium guajava* recorded the highest alkaloids (6.40 ± 0.35 mg/g) and phenol (18.63 ± 0.29 mg/g) while *Azadirachta indica* recoded the least. *Vernonia amygdalina* had the highest saponins (6.63 ± 0.34 mg/g) while *Azadirachta indica* recoded the least (2.40 ± 0.34 mg/g). However, *Azadirachta indica* had the highest tannin contents of (23.52 ± 0.25 mg/100g). Aqueous extract of *Azadirachta indica* had a greater zone of inhibition range between 10.00 ± 0.10 to 14.00 ± 0.05 mm and 13.00 ± 0.05 to 14.00 ± 0.05 mm against bacteria and fungi respectively. *Psidium guajava* show a lower activity against bacteria (12.00 ± 0.05 mm) and higher antifungal activities (11.00 ± 0.05 mm and 19.00 ± 0.05 mm). *Vernonia amygdalina* on the other hand had 13.00 ± 1.45 mm zone of inhibition against *Streptococcus faecalis* and *Pseudomonas aeruginosa*, and antifungal activities range between 9.00 ± 0.35 mm and 15.00 ± 0.45 mm. In conclusion *Azadirachta indica* extract exhibited more antibacterial activities while *Psidium guajava* exhibited more antifungal activities than the other plant extracts. Therefore, more research should be carried out to enable the purification of the specific biopotential chemicals from these plants and their subsequent processing into antimicrobial agents in food industries.

Keywords: *Azadirachta indica*; *Psidium guajava*; *Vernonia amygdalina*; Antimicrobial

1.0 Introduction

Food safety is a global issue with significant implications to human health. The world health organization reports that, annually, unsafe food results in the illnesses of at least 2 billion people worldwide [1]. Some countries have made progress in controlling foodborne disease but the number of those affected by foodborne diseases is growing globally [2]. Fruits and vegetables are prone to several post-harvest losses due to diseases like bacterial rot, smut etc which in turn reduces the shelf-life of the fruits. Estimated post-harvest losses are about 25-30 % due to microbial spoilage [3]. Moreover, wastage of spoilt fruits and vegetables can lead to scarcity and famine

From ancient times, a large number of plants and herbs have been used in traditional medicines against bacterial infection [4]. The uses of plant and herb extracts as antimicrobial agents in food and soft drinks have also been reported for centuries [5]. Due to potential toxicity of chemical food preservatives, there has been increased demand for food preservatives from natural sources [3]. This has led researchers and food processors to come across natural food additives with a wide range of antimicrobial activities. As a result today plant antimicrobial products have acquired importance in food system to retard bacterial and fungal growth [6]. Consumer interest is also increasing in

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consumption of food products having natural ingredients. Several studies have reported the use of natural molecules i.e. organic acids, peptides and essential oils in past several years [7].

Azadiracta indica (Neem or dongoyaro) leaves has antibacterial properties and could be used for controlling airborne bacterial contamination of food substances in the residential premises [8]. The Neem seed has also been used as traditional medicine to treat microbial infections. The aqueous extract of Neem has a powerful chemotherapeutic and viral agent [9]

Psidium guajava (Guava) belongs to the family Myrtaceae and is considered to have originated from tropical South America. Guava tree grow in tropical and sub-tropical area of the world like Asia, Africa and Hawaii [10] *Psidium guajava* is a phytotherapeutic plant used in folk medicine that is believed to have active components that help to treat and manage various infectious diseases [11]. The many parts of the plant have 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 [12]. Its leaf contains copious amounts of phenolic phytochemicals which inhibit peroxidation reaction in the living body, and therefore can be expected to prevent various chronic diseases such as diabetes, cancer, heart-disease [13]. Guava is rich in tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, vitamins, fiber and fatty acids. The leaves of guava are rich in flavonoids and phenols.

Vernonia amygdalina commonly called bitter leaf is a perennial shrub belonging to the family Asteraceae [14]. Traditionally, this plant is used for treatment of stomach disorder, skin wound, swelling, diarrhea, scabies, hepatitis, ascariasis, tonsillitis, fever, mastitis, tapeworm and worms infection [15] Considering the antimicrobial reputations of these plants, the present study was aimed to evaluate antibacterial and anti-fungi activities of aqueous leaves extracts of these medicinal plants against microbial isolates from spoiled fruit.

2.0 Material and methods

2.1 Collection of plant

Spoilt tomato fruits (*Lycopersicum esculentum*) were gotten from Kure market in Minna, Niger. The leaves of *Veronia amygladina* (bitter leaf), *Azadirachta indica* (Neem plant), *Psidium Guajava* (Guava leaves) were collected from Green farms in Niger state and identified by a plant taxonomist in Federal University of Technology, Minna. The plants were brought to the laboratory and rinsed with water to remove the soil particles. Then air dried at room temperature.

2.2 Sources of microorganisms

Bacteria isolates including; *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Micrococcus roseus* and *Streptococcus faecalis*, and fungi including; *Trichophyton tonsurans*, *Aspergillus niger* and *Candida tropicalis* were cultured and isolated from spoilt tomatoes and bell pepper fruit according to the methods of Egofure et al. [16].

2.3 Extraction of crude extract

A 50 g of accurately weighed plant powder was put into a round bottom flask containing 250 mls of distilled water. This was then boiled for 2 hours at a temperature. It was then cooled and filtered using a whatman filter paper. The filtrate was then concentrated in a water bath and stored for further use.

2.4 Qualitative and quantitative screening for secondary metabolites

The plant extract was analyzed for the presence of some secondary metabolite including alkaloids, terpenes, tannins, saponins, phenols, steroids, phlobatannins and flavonoids using standard procedures [17-19]. Quantitative analysis was conducted for flavonoid, alkaloids, total phenol, tannin and saponins using standard procedures [20]

2.5 Antibacterial and anti-fungal Activity

The plate-hole diffusion assay as described by Ieven et al. [21] was used to determine the zone of inhibition of bacteria (antibacterial activity) by plant crude aqueous extracts. The selected microorganisms obtained was maintained at 4°C on nutrient agar plates before use. Using a sterile cork-borer of 5 mm diameter, five holes per plate were made into the set agar containing the bacteria culture. A total of 7 drops (40 mg/ml, 80 mg/ml and 120 mg/ml) of the different plant extracts were poured into the wells and one contained distilled water and ampicillin (50mg/ml). The plates were placed in the incubator at 37 °C for 12 hrs. Antibacterial activity was recorded in millimeters [22, 23]. This was also done for

isolated fungi to know the antifungal activity of the extracts. Ampicillin was used as standards for antibacterial assay, while vastatin was used as standards for antifungal assay.

2.6 Statistical analysis

Values were analyzed using statistical package for social science (SPSS) version 21 and presented as means \pm SE of the mean. Comparisons between different groups were carried out by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The level of significance was set at $P < 0.05$.

3.0 Results

3.1 Qualitative phytochemical composition

The phytochemical composition of the aqueous and methanol leaves extracts of *Psidium guajava*, *Vernonia amygdalina* and *Azadirachta indica* presented in Table 1. All phytochemicals tested were present in the aqueous extract of the plants. Glycoside and anthraquinone were absent in methanol extract of *P. guajava* and *A. indica* respectively. All other phytochemicals tested were present in the methanol extract of the three (3) plants.

Table 1 Qualitative phytochemical compositions of Aqueous and methanol Extracts of *Psidium guajava*, *Vernonia amygdalina* and *Azadirachta indica*

Plants	Aqueous extract			Methanol extract		
	<i>P. guajava</i>	<i>V. amygdalina</i>	<i>A. indica</i>	<i>P. guajava</i>	<i>V. amygdalina</i>	<i>A. indica</i>
Flavonoid	+	+	+	+	+	+
Saponin	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Phenols	+	+	+	+	+	+
Glycosides	+	+	+	-	+	+
Anthraquinone	+	+	+	+	+	-

Key: Present (+); Absent (-)

3.2 Quantitative phytochemical compositions

The quantitative phytochemical composition of aqueous extract of leaves of *Psidium guajava*, *Vernonia amygdalina*, and *Azadirachta indica* presented in Table 2. *Psidium Guajaza* recorded the highest alkaloids (6.40 ± 0.35 mg/g) and phenol (18.63 ± 0.29 mg/g) while *Azadirachta indica* recoded the least. *Vernonia amygdalina* had the highest saponins (6.63 ± 0.34 mg/g) while *Azadirachta indica* recoded the least (2.40 ± 0.34 mg/g). However, *Azadirachta indica* had the highest tannin contents of (23.52 ± 0.25 mg/g).

Table 2 Quantitative constituents of *Psidium guajava*, *Vernonia amygdalina*, and *Azadirachta indica*

Plants	Saponin (mg/g)	Alkaloids (mg/g)	Tannins (mg/g)	Flavonoids (mg/g)	Phenols (mg/g)
<i>Psidium guajava</i>	3.38 ± 0.78^b	6.40 ± 0.35^c	11.84 ± 0.23^b	11.75 ± 0.21^a	18.63 ± 0.29^b
<i>Vernonia amygdalina</i>	6.63 ± 0.34^c	2.44 ± 0.34^b	8.19 ± 0.32^a	11.29 ± 0.05^a	17.14 ± 0.38^{ab}
<i>Azadirachta indica</i>	2.40 ± 0.34^a	1.68 ± 0.22^a	23.52 ± 0.25^c	17.19 ± 0.29^b	16.00 ± 0.27^a

Data are Mean SEM of triplicate determination. Data followed by different superscript alphabets are significantly different $P < 0.05$.

3.3 Antibacterial and antifungal activities of aqueous extract of *Azadirachta indica* (Dogonyaro) leaves

Antibacterial and antifungal activities of aqueous extract of *Azadirachta indica* (Neem) leaves are presented in Table 3. Aqueous extracts of *Azadirachta indica* had a greater zone of inhibition on *Pseudomonas aeruginosa* (12.00 ± 0.40 to 14.00 ± 0.15 mm), *Klebsiella pneumoniae* (12.00 ± 0.05 to 14.00 ± 0.05 mm) and *Micrococcus roseus* (10.00 ± 0.10 to 14.00 ± 0.05 mm). But when compared to ampicillin (positive control), there was an appreciable zone of inhibition

(15.00±0.25 to 25.00±0.45mm) than the aqueous extracts. Aqueous extract of *Azadirachta indica* however, had no effect on *Trichophyton tonsurans* and *Aspergillus niger* but inhibited the growth of *Candida tropicalis* (13.00±0.05 and 14.00±0.05 mm).

Table 3 Antibacterial and Antifungal Activities of Aqueous Extract *Azadirachta indica* (Neem) Leaves

Concentration of extracts	40 mg/ml	80 mg/ml	120 mg/ml	Ampicillin (50 mg/ml)	DMSO
BACTERIA					
<i>Klebsiella pneumoniae</i>	13.00±0.05 ^b	12.00±0.05 ^{ab}	14.00±0.05 ^b	25.00±0.45 ^b	-
<i>Micrococcus roseus</i>	10.00±0.10 ^a	13.00±0.25 ^b	14.00±0.05 ^b	15.00±0.25 ^a	-
<i>Streptococcus faecalis</i>	12.00±0.09 ^{ab}	11.00±0.55 ^a	12.00±0.10 ^a	-	-
<i>Pseudomonas aeruginosa</i>	13.00±0.50 ^b	14.00±0.15 ^b	12.00±0.40 ^a	-	-
				Vastatin (+) 67 mg/ml	DMSO (-)
FUNGI					
<i>Trichophyton tonsurans</i>	-	-	-	20.00±0.45	-
<i>Aspergillus niger</i>	-	-	-	15.00 ±0.25	-
<i>Candida tropicalis</i>	14.00±0.05	13.00±0.05	-	-	-

Values are mean ± SEM of 3 determinations. Values greater than 5 mm shows sensitivity

3.4 Antibacterial and antifungal activities of aqueous extract of *Psidium guajava* (Guava) leaves

Antibacterial and antifungal activities of crude aqueous extract *Psidium guajava* (Guava) leaves as presented in Table 4. Aqueous Extract of *Psidium guajava* (Guava) Leaves were not active against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* but show little activity against *Micrococcus roseus* (12.00±0.05 mm) and *Streptococcus faecalis* (12.00±0.05 mm). The extract demonstrated anti-fungal activity range between 11.00±0.05 mm and 19.00±0.05 mm against *Trichophyton tonsurans*, 10.00±0.05 to 16.00±0.05 mm against *Aspergillus niger* and 7.00±0.05 mm to 15.00±0.05 mm against *Candida tropicalis*.

Table 4 Antibacterial and Antifungal Activities of Aqueous Extract of *Psidium guajava* (Guava) Leaves

Concentration of extracts	40 mg/ml	80 mg/ml	120 mg/ml	Ampicillin (50 mg/ml)	DMSO
BACTERIA					
<i>Klebsiella pneumoniae</i>	-	-	-	25.00±0.45 ^b	-
<i>Micrococcus roseus</i>	-	-	13.00±0.05 ^a	15.00±0.25 ^a	-
<i>Streptococcus faecalis</i>	-	-	12.00±0.05 ^a	-	-
<i>Pseudomonas aeruginosa</i>	-	-	-	-	-
				Vastatin (+) 67 mg/ml	DMSO (-)
FUNGI					
<i>Trichophyton tonsurans</i>	-	11.00±0.05 ^b	19.00±0.05 ^b	20.00±0.05	-
<i>Aspergillus niger</i>	10.00±0.05	11.00±0.05 ^b	16.00±0.05 ^a	15.00±0.05	-
<i>Candida tropicalis</i>	-	7.00±0.05 ^a	15.00±0.05 ^a	-	-

Values are mean ± SEM of 3 determinations

3.5 Antibacterial and antifungal activities of aqueous extract of *Vernonia amygdalina* leaves

Antibacterial and antifungal activities of crude aqueous extract of *Vernonia amygdalina* leaves as presented in Table 5. Aqueous Extract of *Vernonia amygdalina* leaves were not active against *Micrococcus roseus* but show little activity

against *Klebsiella pneumoniae* (13.00±1.45 mm) *Streptococcus Faecalis* and *Pseudomonas aeruginosa*. The extract also demonstrated antifungal activities against *Trichophyton tonsurans* (12.00±0.00 mm), *Aspergillus niger* (9.00±0.35mm to 13.00±0.10 mm) and *Candida tropicalis* (13.00±0.05 mm and 15.00±0.45 mm).

Table 5 Antibacterial and antifungal activities of crude aqueous extract of *vernonia amygdalina* (bitter leaf) leaves

Concentration	40 mg/ml	80 mg/ml	120 mg/ml	Ampicillin 50 mg/ml	DMSO
BACTERIA					
<i>Klebsiella pneumoniae</i>	-	13.00±1.45 ^b	-	25.00±0.15 ^b	-
<i>Micrococcus roseus</i>	-	-	-	15.00±0.55 ^a	-
<i>Streptococcus Faecalis</i>	-	10.00±0.55 ^a	10.00±0.55 ^a	-	-
<i>Pseudomonas Aeruginosa</i>	-	12.00±0.10 ^{ab}	12.00±0.24 ^b	-	-
FUNGI				Vastatin (Positive) 67mg/ml	DMSO (Negative)
<i>Trichophyton tonsurans</i>	-	-	12.00±0.00 ^a	20.00 ±0.15	-
<i>Aspergillus niger</i>	-	9.00±0.35 ^a	13.00±0.10 ^a	15.00 ±0.55	-
<i>Candida tropicalis</i>	-	13.00±0.05 ^b	15.00±0.45 ^b	-	-

Values are mean ± SEM of 3 determinations. Values greater than 5 mm shows sensitivity

4.0 Discussion

Plants serves as vegetables and are used the preparation of food nutritive seasoning used in the preparation of food. Apart from its nutritive value, plants have been found to contains bioactive metabolites with potentials to inhibit the growth of microorganisms [24]. In this study, *Psidium guajava*, *Vernonia amygdalina* and *Azadirachta indica* was found to contain high amounts of flavonoids in addition to other phytochemicals like tanins, saponins, phenols and alkaloids (Table 4.1), these findings agree with the workers [25-27], on phytochemical constituents of *Psidium guajava*, *Vernonia amygdalina* and *Azadirachta indica*. The bioactivity of medicinal plants has been linked to the presence of phytochemicals [28]

Researchers have also studied the phytochemical and antimicrobial properties of leaf of *Vernonia amgdalina*. Phytochemicals present in *Vernonia amygdalina* included flavonoids, cardiac glycosides, reducing sugar, terpenoids, saponins, anthraquinones, alkaloids and steroids as shown in table 1. These phytochemicals were present in both aqueous and methanol extract. Akinjogunla et al. [29] reported the presence of Carbohydrates, saponins, flavonoid and phlobatannin in *V. amygdalina* extracts.

Aqueous Extract of *Vernonia amygdalina* leaves were not active against *Micrococcus roseus* but show little activity against *Klebsiella pneumoniae* (13.00±1.45 mm) *Streptococcus Faecalis* and *Pseudomonas aeruginosa*. The extract also demonstrated antifungal activities against *Trichophyton tonsurans* (12.00±0.00 mm), *Aspergillus niger* (9.00±0.35mm to 13.00±0.10 mm) and *Candida tropicalis* (13.00±0.05 mm and 15.00±0.45 mm). This finding is in agreement with earlier work by Udochukwu et al. [30], who reported the phytochemical and antibacterial activity of *Vernonia amygdalina*. However, Evbuomwan et al. [31] reported that the ethanol extract of *V amygdalina* had higher zones of inhibition ranged from 8.0±2.0mm at 25mg/ml to 12.5±1.5 at 200mg/ml against *P. aeruginosa*; 9.0±1.0mm at 50mg/ml to 15.0±1.5mm at 200mg/ml against *S aureus*. The discrepancy observed in the activities could be attributed to the variations in the dissolution capacity of the different solvents which in turn affected the degree of phytochemicals extracted [32]. The resistance of *V amygdalina* to *Micrococcus roseus* may have arisen from drug/phytochemical inactivating enzymes present in the bacteria. Also, variations observed in the susceptibility of Gram positive and negative bacteria could have resulted from their relative composition of cell wall components [33]. Hence, the activities of the *Vernonia amygdalina* extract, especially the could confirm the traditional use of the plants against Eczema [34], wounds [35] and typhoid fever [36]. The previous studies also verified the antimicrobial activities of the plant extracts against *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Salmonella typhi* and *Pseudomonas aeruginosa* [37].

Several authors have established the potential of neem extracts and their components as antifungal agents. Govindachari et al. [38] The present study revealed that *Azadirachta indica* leaves possessed good anti-bacterial and anti-fungal activity better than the two other plant tested, confirming the great potential of bioactive compounds and rationalizing the use of this plant in primary health care [39]. The extracts of Neem when used as medicinal plant, could be useful for the growth inhibition of the harmful fungus. Investigating the antimicrobial effect of the plant extract in this study involved a comparison of their effect with commercially developed antibiotics and by comparing the inhibition it was observed that the commercial antibiotics had a larger inhibitory effect than the plant extracts. This is not surprising and reinforces the position that commercially perfected and tested antibiotics should be used in treatments whenever available [40]

The results of the present study shows similarities to the findings of Nwanneka et al. [41] which investigated the antimicrobial activity of *Psidium guajava* leaf extract, the results showed that both aqueous and ethanol extracts of guava leaf inhibited the growth of the bacteria and fungi tested but the ethanolic extract showed stronger inhibition than the aqueous extract against the organisms. The finding of this study was also inconformity with that of Pandey and Shweta [42], where the results of antibacterial activity of *Psidium guajava* leaf and stem reveals that methanol extract showed stronger anti-bacterial activity than aqueous extract [43].

Conclusion

This research work has shown that *Vernonia amygdalina*, *Psidium guajava* and *Azadirachta indica* has potential bioactive phytochemicals that are responsible for their antibacterial and antifungal activities. It has also proven that *Azadirachta indica* leaf was more potent against bacteria while *Psidium guajava* was more active against fungi than the other plants tested. Therefore, more research should be carried out to enable the purification of the specific biopotential chemicals and their subsequent processing into chemotherapeutic agents.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest exists.

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