



Phytochemical Screening and Evaluation of the Antibacterial Properties of Five Plant Species on *Aeromonas hydrophila*

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Abstract

Phytochemicals are bioactive compounds that have protective or disease preventive properties which are considered to be beneficial to human health. Plants have been used to cure various diseases of humans for ages. Five different plants; *Mangifera indica*, *Psidium guajava*, *Persea americana*, *Allium cepa* and *Tithonia diversifolia* were screened for their phytochemical properties. The plants were obtained from Jos North LGA of Plateau state. The leaves were destalked, washed and dried under shade and then grounded into powder, 250g of each plant powder was soaked in 500ml of 95% ethanol and water in beakers. After 48 hours, the fluid was sieved and filtered. The filtrate was then concentrated with the aid of an evaporator at 65°C. The plant extracts were screened for the presence of bio-agents and antibacterial activity using standard procedures. The phytochemical profile of the extracts of the five plants showed that flavonoids, alkaloids, tannins, phenols, terpenes and cardiac glycosides were present. The results revealed that all the plant species showed antibacterial activity on *Aeromonas hydrophila*, with the ethanolic extracts of *P. guajava* exhibiting the highest inhibitory effect on the organism with inhibition mean zone of 21.05mm at a concentration of 250mg/ml. These were followed by the ethanolic extracts of *M. indica* and *P. americana* with mean zones of 18.16 and 13.39 mm respectively. Antibiotic resistance by microorganisms is a serious public health concern. This study has demonstrated that some plant extracts can be a means of treating *Aeromonas* infections which is cost effective with minimal side effects.

Introduction

Nature has given humanity the gift of an immense therapeutic wealth due to its great diversity in medicinal plants [1]. The consumption of natural substances to cure and prevent diseases is old and universal and plays an important role in access to basic health care for populations [2]. Research on the therapeutic potential of plants has flown over the years with a wealth of scientifically proven information, showing the considerable power of plants in the treatment of a wide range of diseases [3]. The leaf of the five plant species that is the subject of this study are traditionally recognized in the treatment of diarrhea,

dysentery, gastrointestinal problems, respiratory problems, obesity and hypertension [4,5]. Plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions and to defend against attack from predators [6,7]. Many of the herbs and spices used by humans to season food also yield useful medicinal compounds. The plant based traditional medical systems continue to provide the primary health care to more than three-quarters of the world's populace [8]. Antibiotic resistance has become a serious and widespread problem in developing countries, causing high mortality each year [9]. Anti-bacterial compounds

More Information

How to cite this article: Eluma M, Itelima JU, Onwuliri FC. Phytochemical Screening and Evaluation of the Antibacterial Properties of Five Plant Species on *Aeromonas hydrophila*. Eur J Med Health Res, 2023;1(2):19-26.

DOI: 10.59324/ejmhr.2023.1(2).03

Keywords:

Phytochemicals,
Aeromonas hydrophila,
diseases antibiotics,
resistance,
bioactive,
diarrhoea



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with herbal sources such as alkaloids, flavonoids, tannins, saponin and glycosides have a wide range of therapeutic use. These compounds are not only efficient for the treatment of infectious diseases, but also concurrently diminish existing side effects [10]. This implies that the search for alternative antimicrobial compounds is an urgent area of biomedical research and extracts derived from plants have long held interest as potential sources of new therapeutic agents [11]. Several scientific studies carried out on plant species such as (*Psidium guajava*), (*Mangifera indica*), (*Tithonia diversifolia*), (*Persea americana*) and (*Allium cepa*) have confirmed the traditional claims of their effectiveness in treating diarrhoea related infections.

Guava (*Psidium guajava*) belongs to the myrtle family (*Myrtaceae*) and is an excellent source of vitamin C, Flavonoids, tannins, saponins, phenol and essential oil. Many parts of the plant have been used in traditional medicine to manage conditions like malaria, gastroenteritis, vomiting, diarrhoea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums and a host of other conditions [12,13]. According to Abdelrahim et al. [12] and Begum et al. [14], guava leaf has also been used for controlling life-changing conditions such as diabetes, hypertension and obesity. Native to tropical areas from Southern Mexico to North South America, guava trees have been grown by many other countries having tropical and subtropical climates, thus allowing production around the world [15]. The consumption of decoction, infusion, and boiled preparations is the most common way to overcome several disorders in India, China, Pakistan and Bangladesh [16,17].

Mango (*Mangifera indica*) belongs to the family of *Anacardiaceae* which consist of about sixty genera and six hundred species which are mainly tropical trees and shrubs [18]. Mango are native to South Asia and possess numerous phytochemicals in their leaves and fruits such as polyphenols, carotenoids, tannins and xanthonoids (mangiferin) [19]. The aqueous leaf extract of mango has been shown to inhibit the growth of *Aeromonas* species [20]. Mango leaves contain flavonoid, alkaloid, saponin and phenol which have antimicrobial properties use in the treatment of bacillary, parasitic and viral infections [20].

Sunflower (*Tithonia diversifolia*) (Hemsl), commonly referred to as wild sunflower or Mexican sunflower of the family *Asteraceae*, is a robust herbaceous perennial plant that grows up to 3 meters tall. They are originally domiciled in North America. The leaf extract of sunflower has been reported to have antimalarial, anti-inflammatory, anti-diarrhoeal, anti-diabetic, anti-spasmodic and phytoremediative properties [21]. It is known to be used in the treatment of constipation,

snake bites, diarrhoea, abscesses, sore throat and diabetics [10].

Pear (*Persea americana*) belongs to the family *Rosaceae*. About 3000 known varieties of pears are grown worldwide [22]. The pear tree is native to the coastal regions of Western Europe, North Africa and Asia [23]. Most pears are deciduous but one or two species in Southeast Asia are evergreen. Pears are consumed fresh, canned as juice or dried. They can also be made into jellies and jam in combination with other fruits [24]. *Persea americana* are full of vitamins, antioxidants and fiber, the leaves are used for treating diarrhoea, cancer, asthma, kidney stone, insomnia and diabetes [19]. The antimicrobial property of pear leaf extract is due to the presence of bioactive compounds such as Flavonoids, Alkaloids, Tannins, Saponin, Phenols and Glycosides [19].

Onion (*Allium cepa* L.) is from the family *Amaryllidaceae*. Onion is one of the oldest cultivated vegetables and most widely cultivated species of the genus *Allium* [25]. Onions are an important source of several phyto-nutrients such as flavonoids, fructo-oligosaccharides (FOS), thiosulfinates and other sulfur compounds, recognized as important elements of the Mediterranean diet [26]. They contain high levels of phenolic compounds, which have antioxidant properties on different degenerative pathologies (cardiovascular and neurological diseases as well as dysfunctions based on oxidative stress) [27]. Flavonoids are the major phenolics in onions and sulfur compounds are responsible for its typical odour and flavor, hence, onions may be used as natural preservatives to control microbial growth [28].

Aeromonas are gram negative, non-spore forming facultative anaerobes responsible for gastrointestinal and extra-intestinal infections such as septicemia and wound infections in both healthy and immunocompromised individuals [29]. New syndromes attributed to this genus include hemolytic uremic syndrome, burns associated sepsis and a variety of respiratory tract infections [29]. *Aeromonas hydrophila* has been associated with opportunistic infections of humans such as food-borne gastroenteritis, diarrhea and wound infections [30]. *Aeromonas* infections are usually treated with antimicrobial agents, although increasing frequency of occurrences of antibiotic resistance had been reported [29]. The present study was undertaken to determine the presence of bioactive compounds and antibacterial properties of extracts of 5 plant species on *Aeromonas*.

Materials And Method

Source of Plant Materials

All the plants used for the study were obtained from Jos North Local Government Area of Plateau state, Nigeria. The plants were identified and authenticated by Dr. O. E. Agyeno, coordinator of the herbarium unit of the



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Preparation of Plant Extracts

Plant extracts were prepared according to the method described by Bipul et al. [4] The leaves of the various test plants were destalked, washed and dried under shade after which they were grounded into fine powder using pestle and mortar, 250g of each plant powder was soaked in 500ml of 95% ethanol and 500ml of water in beakers. The beakers were kept at room temperature for 48 hours. The fluid was sieved with muslin cloths and filtered using Whatman no.1 filter paper (0.45µm). The filtrate was then collected and concentrated with the aid of rotary evaporator (Resona, Germany) at 65°C. The extract obtained was dried in the oven at 40°C to completely evaporate the residual solvent. After complete drying the extract was weighed and stored in the refrigerator at 4°C.

Phytochemical Screening of Plant Extracts

The plant extracts were screened for the presence of bio-agents using the standard procedure described by Ajuru et al. [8].

Test for flavonoids

A few drops of 10% lead acetate solution were added to 2.0ml of each of the plant extracts in a test tube. The observation of either cream or light yellow colouration confirms the presence of flavonoids.

Test for tannins

Preliminary test

One (1ml) of the extract was diluted with 4.0ml of distilled water (in a ratio of 1:4) and a few drops of 10% very dilute ferric chloride solution were gradually added to the aqueous extract. The presence of blue or green precipitate shows the presence of tannins.

Confirmatory test

A few drops of lead acetate solution were added to 2.0ml of the extract. The resulting solution was observed for brown precipitate which indicates the presence of tannins.

Test for phenols

A few drops of ferric chloride solution were added to 2ml of the extract in a watch glass; the appearance of bluish green colour indicated the presence of phenol.

Test for alkaloids

Preliminary test (Dragendoff reagent test)

A few drops of Dragendoff's reagent were added to 2.0ml of the extract and the solution of potassium bismuth iodide was added and observed for orange coloration.

Confirmatory test (Wagner reagent test)

A few drops of the Wagner reagent were added to 2 ml of the extract and a solution of iodine in potassium iodide was also added and the formation of deep brown precipitate would indicate the presence of alkaloid.

Test for saponins

Five (5) ml of distilled water added to 2.0ml of the extract in a test tube. This was shaken vigorously after which a few drops of olive oil was added. Formation of an emulsion will indicate the presence of saponins. When foams are formed during plant extractions it is an indication that saponins are present.

Test for terpenes and steroids

Two drops of acetic anhydride were added to 2.0ml of the extract and then concentrated sulphuric acid was carefully added down the side of the test tube. An observation of reddish brown colour at the inter phase indicates the presence of terpenes and steroids.

Test for glycoside

Preliminary test (Lieberman's test)

Two milliliters of the plant extracts were added to 2.0ml of acetic anhydride and cooled in ice. Sulphuric acid was added carefully along the side of the test tube. A colour change from violet to blue green indicates the presence of glycoside.

Confirmatory test (Salkowski test)

Two milliliters of the plant extracts were dissolved in 2.0ml of chloroform. Sulphuric acid was carefully added to form a lower layer. A reddish-brown colour at the interphase indicates the presence of glycoside.

In-Vitro Testing of the Plant Extracts on *Aeromonas* Species

Aeromonas culture was maintained on Nutrient agar slant and kept in the refrigerator prior to use. The various plants extracts were prepared using doubling dilution method described by Taura and Oyeyi to obtain 250mg/ml, 125mg/ml, 62.5mg/ml, 31.3mg/ml and 15.63mg/ml [31]. The antimicrobial activity of each of the plants extracts was determined using agar well diffusion method [8]. *Aeromonas* isolates were sub-cultured three times in fresh peptone water in order to obtain a more vigorous population. A standardized culture was obtained using the McFarland standard. The stocks were incubated at 37°C for 24 hours. A 0.5ml of the standardized culture was aseptically transferred into Petri dishes containing nutrient agar and left for about 20 minutes to allow the microorganism diffuse into the media. A cork borer was used to bore wells on the media and 1ml of each extracts of various concentrations (250 mg/ml, 125 mg/ml etc.) was added into the wells. A well was also made at the central portion of the agar media and 0.5ml of 250ml of ciprofloxacin was placed therein to serve as control. The plates were incubated at 37°C and the zones of inhibition measured after 24 hours. The presence of zones of inhibition was regarded as evidence of antimicrobial action. The zones of inhibition were measured with a ruler at right angles across the zone to find the average diameters in millimeters.

Results



The phytochemical profile of the ethanolic extracts of the five (5) plants species are shown in Table 1. The results showed that flavonoids, alkaloids, tannins, phenols, terpenes and cardiac glycosides were presents in the plant species used in this study. Some of the plants like mango (*Mangifera indica*), guava (*Psidium guajava*) and pear (*Persea americana*) have bioactive compounds in appreciable amount while the sunflower and onions leaves have them in trace quantities. Flavonoid was present in large quantities in Mango, guava and pear leaf while sunflower and onion leaf do not contain flavonoids. The antibacterial activities of ethanolic extracts of the five plants on *Aeromonas hydrophila* are presented in Table 2. Out of the 5 plant species tested 3 (*P. guajava*, *M. indica* and *P. americana*) showed inhibitory effects on the growth of *Aeromonas* at varying degrees. The other 2 plants (*T. diversifolia* and *A. cepa*) showed no inhibitory effect when extracted with ethanol but have little activity using aqueous extraction (figure 1).

The results revealed that among the 3 plant species that showed antibacterial activity, the ethanolic

extracts of *P. guajava* exhibited the highest inhibitory effect on the bacterial isolates with growth inhibition mean zone diameters of 21.05mm at a concentration of 250mg/ml. These were closely followed by the ethanolic extracts of *M. indica* and *P. americana* with mean zones of inhibition of 18.16 and 13.39 mm respectively at 250mg/ml. The results also showed that the ethanolic and aqueous extracts of *P. guajava* and *M. indica* inhibited the growth of *Aeromonas* at all concentrations employed in the study. When the results of the activity of aqueous and ethanolic extracts of the plant species on the test organism were statistically compared (Table 3), it was observed that there was a significant difference ($p < 0.05$) between the aqueous and ethanolic extracts with respect to the degree of their inhibitory effects with the latter having higher inhibition than the former. The result also indicated that the plant extracts that inhibited the growth of the test organism decreased in effectiveness with decrease in concentration of extracts. Thus at the lowest concentration of 15.63mg/ml most of the plant extracts showed little or no activity on *Aeromonas*.

Table 1: Phytochemical Analysis of Plant Extracts

Biochemical component	Plant species				
	1	2	3	4	5
Flavonoids	++	++	++	-	-
Alkaloids	++	++	++	+	+
Tannins	++	++	++	+	+
Saponins	+	+	++	-	+
Terpenes and steroids	+	+	+	-	-
Phenols	+	+	+	+	-
Resins	++	++	+	+	+
Cardiac glucoside	+	+	+	-	+

Key: 1 = *Mangifera indica* (Mango leaf); 2 = *Psidium guajava* (Guava leaf); 3 = *Persea americana* (Pear leaf); 4 = *Tithonia diversifolia* (Hemsl) (sunflower leaf); 5 = *Allium cepa* (Onion leaf); ++ = Appreciable quantity, + = Trace quantity, - = Not detected

Table 2: Antibacterial Activities of Ethanolic Extracts of Test Plants on *Aeromonas hydrophila*

Plants	250mg/ml	125mg/ml	62.5mg/ml	31.25mg/ml	15.63mg/ml
<i>Psidium guajava</i>	21±0.50 ^a	17±0.35 ^a	13±0.39 ^a	9±0.51 ^a	6±0.69 ^a
<i>Mangifera indica</i>	18±1.16 ^a	12±0.84 ^b	10±0.84 ^b	7±0.34 ^b	4±0.39 ^b
<i>Persea americana</i>	13±0.39 ^b	10±0.34 ^c	8±0.35 ^c	6±0.34 ^c	2.33±0.34 ^c
<i>Allium cepa</i>	0.00±0.00 ^c	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d
<i>Tithonia diversifolia</i>	0.00±0.00 ^c	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d	0.00±0.00 ^d

Means having different superscripts (a, b, c, d) along the rows are statistically significant ($p < 0.05$) using Duncan multiple range test (DMRT). L.S. D= 0.47



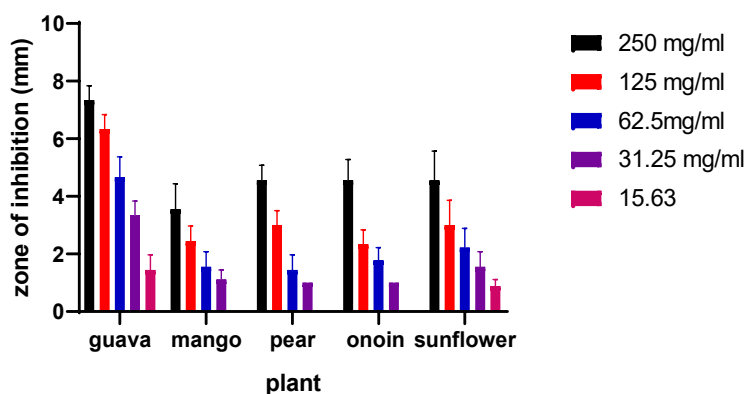


Figure 1: Zones of Inhibition of the Different Medicinal Plants on *Aeromonas hydrophila* using Aqueous Extraction

Table 3: Comparison of the Effect of the Antibacterial Activity of Different Concentrations of Ethanolic and Aqueous Extracts of Test Plants on *Aeromonas hydrophila*

Plant species	Solvent	Diameter of Zones of Inhibition (mm) at Various Concentrations mg/ml				
		250	125	62.50	31.25	15.60
<i>Psidium guajava</i>	E	21.05	17.30	13.30	9.05	4.00
	W	14.50	12.50	9.70	5.00	2.53
<i>Mangifera indica</i>	E	18.16	12.60	10.10	7.03	3.00
	W	8.88	4.53	2.53	1.54	1.00
<i>Persea americana</i>	E	13.39	10.03	8.03	6.04	1.60
	W	10.53	6.00	2.30	1.00	0.00
<i>Allium cepa</i>	E	0.00	0.00	0.00	0.00	0.00
	W	10.73	4.50	2.40	1.00	0.00
<i>Tithonia diversifolia</i>	E	0.00	0.00	0.00	0.00	0.00
	W	5.10	3.87	2.67	1.50	0.00

E= Ethanolic; W= Cold Water

Discussion

Plants produce phytochemicals to protect themselves but recent research demonstrates that they can also protect against diseases [32]. Qualitative screening of the plants in this study using ethanolic extraction indicated the presence of bioactive compounds in varying degrees. The presence of bioactive constituents of the different plant species showed that the leaves are rich in alkaloids, tannins, flavonoids, saponins and phenols. Flavonoid is present in large quantity in *P. guajava*, *M. indica* and *P. americana* while *T. diversifolia* and *A. cepa* do not contain them. *Psidium guajava* and *M. indica* have been shown from previous studies to have antioxidant, anti-inflammatory, anti-allergic, anti-carcinogenic, antimicrobial and antiviral properties due to the presence of flavonoids [33]. The study revealed the presence of alkaloid and saponin in pear and onion leaves which is in agreement with the report of Casley-Smith [34]. Alkaloids have a wide range of pharmacological properties including antimalarial, anti-asthma and anti-cancer properties as reported by Kittakoop et al. [35].

They were reported to have cholinimetric, vasodilatory, anti-arrhythmic and anti-hyperglycemic activities [36]. Alkaloids are also known to possess antimicrobial properties thus their presence in these plants suggest that their use in treating gastrointestinal diseases caused by pathogenic organism such as *Aeromonas hydrophila* will go a long way in preventing the enteric infections that manifest in form of diarrhoea. Secondary metabolites such as phenolic compounds found in these plants are known to protect against pathogens, prevent chronic illnesses such as cardiovascular disease, certain type of cancers, neurodegenerative disease, and diabetes [37]. Plants that contain phenol could be used as anti-inflammatory, immune enhancers and hormone modulators [38].

Tannins were found in appreciable amount in *M. indica*, *P. guajava* and *P. americana*, since they have been shown to possess antimicrobial activity against gram negative bacteria, these plants will be useful in the treatment of wound infection caused by *Aeromonas*. It has been reported that tannins possess physiological astringent and haemostatic properties, which hasten



wound healing and ameliorated inflamed mucus membrane. Amelio reported that tannins can also form complexes with digestive enzymes thus reducing the digestibility of proteins in foods [39]. The result of this study also showed that saponins occurred in all the five plants. However, they are more in *P. americana* and *A. cepa* than the other plants. Saponins are active as expectorant and is very useful in the treatment of upper respiratory tract infections; Saponins present in plants are cardiotoxic in nature and are reported to have anti-diabetic and anti-fungal properties [18,40]. Saponins, often referred to as natural detergent due to their foamy nature, also possess anticarcinogenic properties, immune modulation activities and regulation of cells proliferation as well as inhibition of the growth of cancer cells and cholesterol lowering activity [41].

Glycosides were found in varying degrees in all the plants analyzed in this study. Glycosides are reported to contain antimicrobial activity and as such can be used to treat infection caused by *Aeromonas* [8]. The use of plants containing glycoside should be with caution because they are known to release cyanic acid on hydrolysis. The presence of terpenes and steroids in the plants though in trace quantities is of medicinal importance as these compounds possess biological activities. Although terpenoids are mainly used for their aromatic qualities, they have also been found to be potential agents against inhibiting bacteria [42]. Resins were found in appreciable quantities in *M. indica*, *P. guajava* and *P. americana*, but in traces in *T. diversifolia* and *A. cepa*.

Resins have been shown to have both antibacterial and antidiarrheal properties [8]. It has also been reported to have the ability to bind enterobacteria such as *Aeromonas* and *E. coli* which are diarrhea causing organisms preventing their adhesion to the intestinal wall [32]. The ethanolic and aqueous extraction methods used in this study revealed that ethanolic extraction is more effective and reliable as it enabled easy filtration and contained more phytochemicals than the aqueous extraction which was difficult to filter due to inability of the plant materials to completely dissolve in it. Also, the aqueous extract had the tendency to easily go bad which was suspected to have led to the loss of some of the phytochemicals which were present in the ethanolic extract.

The emergence of drug-resistant bacteria appears to be a major limitation of the use of antibiotics [43]. In order to resolve these problems, there has been more exploration of medicinal plants to tackle the problem [44]. Anti-bacterial compounds with herbal sources are not only efficient for the treatment of infectious diseases, but also concurrently diminish existing side effects caused by the use of conventional antibiotics [10]. The significance of the inhibitory effects of the

extracts of various plant species against diseases caused by various organisms have been documented but there is still a dearth of information as regards the antimicrobial activities of these plant against *Aeromonas* [45].

Among the 5 plant species tested 3 (*P. guajava*, *M. indica* and *P. americana*) showed inhibitory effects on the growth of *Aeromonas* using both ethanolic and aqueous extractions. The other 2 (*T. diversifolia* and *A. cepa*) showed no inhibitory effect when extracted with ethanol but have little activity using aqueous extraction. The results also revealed that of the 3 plant species that showed antimicrobial activity, the ethanolic extracts of *P. guajava* exhibited the highest inhibitory effect on *Aeromonas*. The larger zone of inhibition induced by the extracts of *P. guajava* against the organism further supported the effective use of *P. guajava* leaves in the control of diarrhoea and gastroenteritis as reported by Bipul et al. [4].

Conclusion

From the statistical analysis, it was observed that there was a significant difference ($p < 0.05$) between the aqueous and ethanolic extracts with respect to the degree of their inhibitory effects suggesting that it is better to use the ethanolic extracts when inhibiting the growth of *Aeromonas*. The present findings and the reports of previous workers have confirmed the fact that some of the herbal preparations used by the traditional healers actually possess medicinal potency better than or similar to standard antibiotics. Doughari et al. reported that patients of rural communities who rely mostly on traditional medicine claimed to have a reduced risk of getting infectious diseases from resistant pathogen than people in urban areas treated with synthetic antibiotics. Among the plant species studied, *P. guajava*, *M. indica* and *P. americana* could be regarded as the plants of choice in treating *Aeromonas* infections since they gave larger zones of inhibitions. This *in vitro* study has demonstrated that certain folk medicine can be as effective as modern medicine in combating pathogenic microorganisms such as *Aeromonas*.

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