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PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITIES OF *VERNONIA AMYGDALINA* (BITTER LEAF), *TELFAIRIA OCCIDENTALIS* (PUMPKIN LEAF) AND *OCIMUM GRATISSIMUM* (SCENT LEAF)

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Abstract: Phytochemical and antimicrobial screening is the extraction, analysis and identification of the bio-active components found in plants and the effects of such bio-active components against bacterial species. The analysis were conducted on three plant samples: *Vernonia amygdalina* (Bitter leaf), *Telfairia occidentalis* (Pumpkin leaf) and *Ocimum gratissimum* (Scent leaf). The leaf samples were collected from the Botanical Garden of the department of Biological Sciences Ibrahim Badamasi Babangida University Lapai, Niger State, Nigeria. The bacterial isolate; *Staphylococcus aureus* was collected from the department of Microbiology, Ibrahim Badamasi Babangida University, Lapai, Niger State, Nigeria. The phytochemical screening of the plant samples was conducted using standard methods, where bio-active components such as saponins, tannins, flavonoids, alkaloids, phenols, glycosides, phlobatannins and steroids were screened. The antimicrobial analysis was conducted using the Nutrient agar diffusion method. The bio-active components found from the phytochemical screening were saponins, tannins, glycosides, alkaloids and flavonoids. The antimicrobial activities showed the highest zones of inhibition against *Staphylococcus aureus* in *Vernonia amygdalina* (7.5 mm) and lowest in *Ocimum gratissimum* (6.0 mm). These leaf samples are good source of bio-active components which are capable of suppressing bacterial infection that cause diseases.

Keywords: Antimicrobial screening, bio-active components, *Ocimum gratissimum*, *Telfairia occidentalis*, *Vernonia amygdalina.*

Introduction

Phytochemical screening is the extraction, qualitative analysis and identification of the medicinally bio-active substances found in plants [YESSUF, 2015]. Many reports by [ONIFADE & AGUNLOYE, 2019; OBOH, 2006; EVBUOMWAN & al. 2018] have reported that the bioactive substances found in *Vernonia amygdalina* Delile (Family Compositae), *Telfairia occidentalis* Hook.f. (Family Cucurbitaceae) and *Ocimum gratissimum* L. (Family Lamiaceae) are flavonoids, alkaloids, tannin, saponin, phenolics and antioxidants. Thus, it can be therefore concluded that *V. amygdalina*, *T. occidentalis* and *O. gratissimum* leaves can greatly contribute to the nutrient requirements of man and should be consumed as they great sources of minerals and vitamins to supplement other major sources.

Although the knowledge of how these bio-active substances provide medicinal benefits to humans reflects the scientific understanding, the exploitation of plants and plant extracts to treat, relieve pain and improve good health dates back to before the invention of medical science [SOFOWORA, 1999; UDOCHUKWU & al. 2015]. Reports has shown that there are about 4000 phytochemicals that are found in plants, and these phytochemicals are

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capable of providing medical remedies such as strokes, cancer and microbial infections [YEDJOU & al. 2008; EVBUOMWAN & al. 2018].

Many of the local vegetable are widely consumed not just for their nutritional or phytochemical potentials, but for their ability to fight against diseases or infections that are caused by certain microorganisms. Many studies [SAXENA & SAXENA, 2002; IBRAHIM & al. 2009] have reported that leaves of *V. amygdalina*, *T. occidentalis* and *O. gratissimum* has an effective antimicrobial activities on bacteria such as *Escherichia coli* and *Staphylococcus aureus*.

Many plants have been used because of their antimicrobial properties; this is due to the bio-active compounds present which are synthesized in the secondary metabolism of the plants. These plants are known by their bio-active substances, for example; phenols are compounds which are components of the essential oils, as well as in tannin [DAS & al. 2010].

Vernonia amygdalina, Telfairia occidentalis and *Ocimum gratissimum* are vegetable crops are considered as weeds on fields and farm lands [OGWU & al. 2016]. *V. amygdalina, T. occidentalis* and *O. gratissimum,* have various phytochemical constituents and are capable of inhabiting against microorganisms such as bacterial species. This study was based on the phytochemical screening of *V. amygdalina, T. occidentalis and O. gratissimum,* and their anti-microbial activities against *Staphylococcus aureus*.

Materials and method

Samples collection

Leave samples of *V. amygdalina, T. occidentalis* and *O. gratissimum* used were collected from the Agricultural Research Farm of the Faculty of Agricultural Lapai, Niger State. The collected samples were sealed and labeled separately in sterile polythene bags and were taken to the Laboratory of the Departments of Microbiology and Biochemistry of Ibrahim Badamasi Babangida University, Lapai.

Phytochemical screening of the plant samples

Collected samples were rinsed with distilled water and their stalks were removed and air-dried for 2-3 days at room temperature (25 °C) on a clean or laboratory bench. The air-dried samples were grounded into powdered form using pestle and mortar. The powdered samples were sieved with a 2.0 mm mesh sieve to obtain fine powdered sample. 30 ml of ethanol was added to the dried powdered samples and were properly mixed. The mixtures were filtered and the filtrates were kept for 2 hours for the ethanol to volatilize to obtain ethanolic extracts [YADAV & al. 2014].

The recommended method of Pharmaceutical and Allied Sciences [UGWOKE & EZUGWE, 2010] was used for the determination of saponins, alkaloids, tannins, phenols, flavonoids, steroid, glycosides and phlobatannins.

Antimicrobial screening of the bacterial isolates

The bacterial isolates used for the screening was *Staphylococcus aureus.* It was collected from the Department of Microbiology of Ibrahim Badamasi Babangida University, Lapai. The bacterial specie used was cultured on nutrients agar slant and was kept in the refrigerator at a temperature of 4 °C from which they were sub-cultured unto freshly prepared media at regular intervals.

Pure culture of *Staphylococcus aureus* was obtained by sub-culturing discrete colonies into freshly prepared nutrient broth and was incubated at 37 °C for 18-24 hours. The isolates developed were pure cultures which were stored in the refrigerator as stock culture for subsequent characterization [EVBUOMWAN & al. 2018].

Reconstitution and sterilization of extracts

The dried fine powdered leaves samples were measured into McCartney bottles and appropriate volumes of the extractants were added to obtain a stock solution of 200 mg/ml. Sterilization of the stock solution was carried out using 0.65 membrane filter by suction pump. The sterilized extract from the leaves were kept inside sterile McCartney bottle and stored in the refrigerator at 7 °C which was used for the antibacterial test. Test for sterility of the extracts investigated by placing it on nutrient agar and incubated for 24 hours at 37 °C [EVBUOMWAN & al. 2018].

Agar diffusion analysis of antimicrobial activity of the plant extracts

Sterilized nutrient agar were prepared, poured in a sterile culture plate and was kept to solidify. *Staphylococcus aureus* isolate of 0.1 ml of a day old was introduced into the plate and sterile cotton swab was used to spread the inoculant evenly on the surface of the agar and the excess was drained off. The plates were left on the bench for 1 hour for proper diffusion of the inoculant into the agar. Five dishes was bore on the plates using 5 mm sterile cork borer. Varying concentrations of the extracts which include 200 mg/ml, 100 mg/ml, 50 mg/ml and 25 mg/ml were prepared and 0.5 ml of the extracts was dropped in each of the appropriately labeled ditch in the agar plate.

Control was set up for each plate and this was done by adding 0.5 ml of the appropriate extract into the 5^{th} ditch. The plates were triplicated and kept on the bench for few minutes for proper diffusion of the extracts into the agar and later were incubated at 37 °C for 24 hours. After incubation, the zone of clearance around each ditch was carefully measured using a metric ruler. This was done by taking measurement from the edge of the plate to the point where the growth of the organisms started. Measurement in diameter (mm) of the zone of inhibition against the tested microorganisms [GUPTA & al. 2016].

Results

Phytochemical analysis of the ethanolic and aqueous extracts of *Vernonia amygdalina* (Bitter leaf), *Telfairia occidentalis* (Pumpkin leaf) and *Ocimum gratissimum* (Scent leaf) leaf.

Phytochemicals such as saponins, glycosides, tannins, alkaloids, and flavonoids were all present in the ethanolic extracts of *V. amygdalina*, *T. occidentalis* and *O. gratissimum*. Phlobatannins was absent in all the three plant extracts. Steroids and phenolics was either present or absent in the three plant extracts (Table 1).

Saponins, tannins and alkaloids were all present in the aqueous extracts of *V. amygdalina*, *T. occidentalis* and *O. gratissimum*. Glycosides, flavonoids, steroid and phenolics were either present or absent in the three plant extracts. Phlobatannins was absent in the three extracts (Table 2).

Table 1. Phytochemical analysis of the ethanolic extract of *V. amygdalina*, *T. occidentalis* and *O. gratissimum.*

PHYTOCHEMICALS	*V. amygdalina*	*O. gratissimum*	*T. occidentalis*
Saponins	+	+	+
Glycosides	+	+	+
Tannins	+	+	+
Alkaloids	+	+	+
Flavonoids	+	+	+
Phlobatannins	–	–	–
Steroids	–	–	+
Phenolics	+	–	+

Key: + = present; – = absent

Table 2. Phytochemical analysis of the aqueous extract of *V. amygdalina*, *T. occidentalis* and *O. gratissimum.*

PHYTOCHEMICALS	*V. amygdalina*	*T. occidentalis*	*O. gratissimum*
Saponins	+	+	+
Glycosides	+	–	–
Tannins	+	+	+
Alkaloids	+	+	+
Flavonoids	–	+	+
Phlobatannins	–	–	–
Steroids	–	–	+
Phenolics	–	–	+

Key: + = present; – = absent

Antimicrobial activities of the ethanolic and aqueous plant extracts against *Staphylococcus aureus*

The antimicrobial activity of *Vernonia amygdalina*, *Telfairia occidentalis* and *Ocimum gratissimum* (Table 3). The result revealed that the ethanolic extract of *V. amygdalina* at the concentration of 200 ml had the highest zone of inhibition of 7.5 mm while the least was observed at 6.0 m for *O. gratissimum*.

Aqueous extract of *V. amygdalina* had the highest zone of inhibition 6.0 mm at the concentration of 200 ml, while the least was observed at 4.0 mm for *T. occidentalis* (Table 3).

Table 3. Antimicrobial activity of *V. amygdalina*, *T. occidentalis* and *O. gratissimum* against *Staphylococcus aureus.*

Plant samples	Concentrations							
	Ethanolic extract (ml/mm)				Aqueous extract (ml/mm)			
	25	50	100	200	25	50	100	200
V. amygdalina	3.0	5.0	6.5	7.5	3.0	4.0	5.0	6.0
T. occidentalis	2.5	4.5	6.0	6.5	1.0	2.0	3.0	4.0
O. gratissimum	3.5	4.0	5.0	6.0	3.5	3.0	3.5	4.5

Discussion

Phytochemical analysis revealed the leaf extracts (aqueous and ethanolic) of *Vernonia amygdalina* (Bitter leaf), *Telfairia occidentalis* (Pumpkin leaf), and *Ocimum gratissimum* (Scent leaf) have the presence of saponins, tannins, alkaloids, flavonoids and glycosides. This findings correlate with the reports of [IZEVBIGIE & al. 2004, EVBUOMWAN & al. 2018] that leafy vegetables such as *V. amygdalina* had the presence of alkaloids, saponins, tannins and glycosides, antihelmitic, antimalarial, antitumourigenic, hypoglycemic and hypolipidaemic properties. Medicinal plants contain substances which could be used for treatment purposes and for drugs productions. Secondary active compounds such as tannins, saponins, glycosides and alkaloids were reported by UDOCHUKWU & al. (2015) to be present in leafy vegetables.

Many of these plants are known to be useful to alleviate symptoms of illnesses, and have been screened to have medicinal benefits, some of which are; *Azadirachta indica* (neem), *V. amygdalina* (Bitter leaf), *Allium sativum* (Garlic), *O. gratissimum* (Scent leaf), and *Zingiber officinale* (Ginger). These plants have been reportedly used in the treatment of ailments such as stomach disorder, fever symptoms and cough traditionally [YEDJOU & al. 2008, EVBUOMWAN & al. 2018].

The antimicrobial analysis of the leaf extracts of *Vernonia amygdalina*, *Telfairia occidentalis*, and *Ocimum gratissimum* showed profound antimicrobial activities against *Staphylococcus aureus*. The result revealed that there were differences in the degree of antimicrobial activities of the extracts. This is in accordance to the report of PELCZAR & al. (1993) the reported that the differences in the susceptibility of bacteria to various antimicrobial agents may be as a result of their structural differences in their cell wall. The obvious difference of the leaf extracts on *Staphylococcus aureus* therefore, is suggestive of the activities against the cell wall components of the bacteria isolate. The antimicrobial substances appear to exert antimicrobial activity by inhibiting the growth of and by killing the sensitive bacteria. The ethanolic extract of the plants have the highest zone of inhibition in all, this is definitely due to the higher concentrations of the bio-active components (saponins, tannins, alkaloids, flavonoids, and glycosides). This is in correlation to the work of IJEH & ADEDOKUN (2006). One of the factors that affect antimicrobial screening is the concentration of the bio-active components. The higher the concentration of the bio-active components, the higher the activities of the bio-active components.

The phytochemical and antimicrobial activities of *V. amygdalina*, *T. occidentalis* and *O. gratissimum* been demonstrated by OGUNDARE & ADEMOLA (2011) that the efficacy of these plants is as a result of the age of the plant, solvent use for the extraction, method used for extraction and the season of harvest of the plant materials.

Conclusion

The extracts of *Vernonia amygdalina*, *Telfairia occidentalis* and *Ocimum gratissimum* plants contain bio-active phytochemical substances and antimicrobial properties which are capable of inhibiting microorganisms to caused diseases in plants and animals.

Notes on contributors

Dr. Habiba Maikudi MUHAMMED is a plant biologist with special interest in plant pathology and plant physiology. Her focuses in the plant diseases and management methods of vegetable crops North central Nigeria.

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