Phytochemical Profile and Cytotoxicity of *Adansonia digitata* and *Borerria verticillata* Root Extracts

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Abstract: *Adansonia digitata* and *Borerria verticillata* are medicinal plants used to treat different types of diseases. The aim of this study was to extract and screen the roots of both plants for phytochemical constituents, to access their cytotoxicity using Brine Shrimp Lethality Assay and to determine the functional groups present in them by Fourier Transform Infrared Spectroscopy (FTIR) technique. The method of cold maceration was used for the extraction. The extracts were subjected to phytochemical screening to determine the classes of secondary metabolites present in the plant materials. The Brine Shrimp Lethality Assay (BSLA) was carried out to observe the cytotoxic effect of the extracts and were analyzed for characteristic functional groups using Fourier Transmission Infra-red (FTIR) spectrophotometry. Both root extracts revealed the presence of alkaloids, tannins, phenols, glycosides, saponins, steroids and reducing sugars while there was absence of flavonoids in both and terpenoids in *B. verticillata* root extract. The result of BSLA revealed LC_{50} values of 94.984µg/ml and 0.246µg/ml for ADR and BVR respectively. FTIR spectroscopic investigation showed the presence of 13 and 15 characteristic peak values with different useful mixtures of 12 functional groups. An intense peak of 2002cm^{-1} and 2005 cm^{-1} for BVR and ADR respectively were observed in the FTIR spectra which corresponds to carboxylic acid groups. The results indicated the presence of potent cytotoxic, bioactive and probably anti-tumor components of these plants. Thus, the results of the study highlight the ethno-therapeutic importance of *Adansonia digitata* and *Borerria verticillata* roots.

Keywords: *Adansonia digitata, Borerria verticillata*, phytochemical constituents, Brine Shrimp Lethality Assay and Fourier Transform Infrared Spectroscopy.

INTRODUCTION

Adansonia digitata (L.) (English name: Monkey-bread tree; Bengali name: Baobab) belongs to the family Malvaceae. It is an emblematic, culturally important and physically majestic sub-tropical tree indigenous to Africa. It is the most widespread species of the genus Adansonia and is native to the African continent. Its parts are used as food, medicine, shelter (Yusha'u *et al.,* 2010 and Yagoub, 2008). Various parts of the plant are used as immuno-stimulant, anti-inflammatory, analgesic, insect repellent and pesticide, it is also used in the treatment of diarrhea and dysentery (Gupta and Saxena, 2015). Previous phytochemical investigations of *A. digitata* led to the isolation of terpenoids, flavonoids, sterols, vitamins, amino acids, carbohydrates and lipids (Tahia *et al*., 2015).

Borreria verticillata (*B. verticillata*) or *Spermacoce verticillata* is also known as broom vassourinha plant. It is used as a medicinal source and has been used as a traditional herb (Ushie *et al*., 2013; Abdullahi-Gero, 2014). Studies have confirmed that extracts from *Borreria species* as well as their isolated compounds possessed diverse biological activities, including antibacterial, analgesic, anti-inflammatory, antitumor, antidiarrheal, larvicidal, antioxidant, gastrointestinal, antimalarial, anti-ulcer, and hepatoprotective with alkaloids and iridoids as the major active compounds (Abdullahi-Gero, 2014).

Fourier Transform Infrared Spectroscopy (FTIR) offers a fast, cost-effective, non-destructive way of obtaining a biochemical fingerprint of samples, herbal extracts or powders thus giving structural information on the chemical compounds present rather than just elemental information (Altemimi *et al.,* 2017 and Palacio *et al*., 2014). FTIR technique is very useful to reveal different types of organic and inorganic compounds present in plant samples based on the peak values in the region of infrared radiation (Chandra *et al*., 2019). It is a widely used tool in plant biological studies, allowing not only identification of the main organic constituents of plant material but also

characterization of the forms of inorganic compounds present in the plants (Palacio *et al*., 2014; Roberto *et al.,* 2017).

The plant kingdom still holds many species of plants containing substances of medicinal value which are yet to be discovered, *A. digitata* and *B. verticillata* are plants which have been used in traditional medicine for many years. Therefore, the aim of this study was to extract and screen *B. verticillata* and *A. digitata* roots for phytochemical studies, to access their cytotoxicity by Brine Shrimp Lethality Assay and to determine the functional groups present using Fourier Transform Infrared Spectroscopy (FTIR) technique.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Root of *B. verticillata* (BUKHAN 133) and *A. digitata* (BUKHAN 36) were collected from Munture Agro-allied farms, Rano LGA of Kano State and identified at Herbarium section of the Department of Plant Biology, Bayero University Kano.

Extraction of Plant Materials

The plant materials were dried under the shade and ground well using mechanical blender into fine powder and transferred into labelled airtight containers. The plant materials were extracted as described by Salim and Imam, (2016). A quantity, 500g of each part was percolated with 96% ethanol (2.5L). The percolates were then evaporated to dryness using a rotary evaporator (R110) at 40^0C.

Qualitative Phytochemical Screening

The extracts were subjected to phytochemical screening to determine the classes of secondary metabolites present in the plant materials following the protocol described by Arunkumar and Muthuselvam, (2009).

Brine Shrimp Lethality Bioassay (Cytotoxicity assay)

Brine shrimp lethality bioassay is a rapid and comprehensive bioassay for testing the bioactivity of compounds from natural and synthetic origin (Asaduzzaman *et al.,* 2015). The eggs of the brine shrimp (*Artemia salina)* were collected from Center for Dryland Agriculture (CDA), Bayero University Kano. Fifty milligram (50mg) of the eggs were hatched in a hatching chamber containing sea water and kept under a fluorescence bulb for 24hrs. Twenty milligram (20mg) of each extract was dispensed into sterile vials and dissolved into 2ml DMSO. Exactly 500, 50 and 5µl each of this solution was transferred into empty vials corresponding to 1000, 100 and 10µg/ml concentrations respectively and diluted to 5ml using sea water and each test was prepared in triplicate. The vial containing 50µl DMSO diluted to 5ml was used as negative control while Potassium per manganate (LC_{50} =5µg/ml) was used as positive control. Ten *Artemia salina* larvae were transferred to each of the test vials using micro-pipette. All the tubes were incubated for 24hrs and number of surviving shrimp larvae were counted and recorded from each vial. The lethal concentration required to kill 50% (LC_{50}) were determined with 95% confidence intervals using regression analysis (Moshi *et al.*, 2010).

Isolation of bioactive compounds using Fourier Transform Infra-red (FTIR) Spectroscopy

Air-dried sample of ethanol extract of *A. digitata* and *Borerria verticillata* roots (ADR and BVR) were analyzed for identification of characteristic functional groups using Fourier Transmission Infra-red (FTIR) spectrophotometer (Shimadzu 8400). A small quantity (0.1g) of each extract was placed on the spectrophotometer in which the spectra was measured (Chandra *et al*., 2019).

RESULTS

The physical properties, percentage yield of ethanolic extracts of *A. digitata* and *B. verticillata* root are presented in Table 1. The extract of *A. digitata* root was gummy and light brown while *B. verticillata* root have powdery texture and dark brown in color. The percentage yields from *B.*

verticillata root extract was 3.6% and that of *A. digitata* root is 1.2%.

Table 1: Physical Properties and Percentage Yield of Ethanolic Extracts of *A. digitata* and *B. verticillata* Roots

Extracts	Texture	Color	Yield	% Yield
ADR	Gummy	Light brown	2.4	1.2
BVR	Powdery	Dark brown	7.2	3.6

Key: ADR- *Adansonia digitata* Root*., B*VR- *Borerria verticillata* Root

Tannins, Alkaloids, Saponins, reducing sugar, Steroids, Phenolic compounds and Glycosides were found in both the extracts, Flavonoids was not found in either while Terpenoids were absent in BVR but present in ADR extracts as presented in Table 2.

Table 2: Phytochemical Constituents of Ethanolic Extracts of *A. digitata* and *B. verticillata* Roots

Extracts	TNS	ALK	FLD	RS	STS	SPN	PCD	GLS	TPN
ADR	+	+	-	+	+	+	+	+	+
BVR	+	+	-	+	+	+	+	+	-

Key: ADR- *Adansonia digitata* Root*., B*VR- *Borerria verticillata* Root*,* TNS-Tannins, ALK- Alkaloids, FLDS- Flavonoids, RS- Reducing sugars, STS- Steroids, SPN- Saponins, PCD- Phenolic compounds, GLS- Glycosides and TPN- Terpenoids.

The result of the Brine Shrimp Lethality Test revealed that both ADR and BVR had a low LC_{50} of 94.984 and 0.246µg/ml with percentage mortality of 93.33% and 100% at 1000µg/ml concentration respectively

Table 3: Brine shrimp lethality of Ethanolic Extracts of *A. digitata* and *B. verticillata* Roots

Concentrations	Total	ADR	% Mortality	BVR	% Mortality
1000 µg/ml	30	2	93.33	0	100
100 µg/ml	30	19	36.67	5	83.33
10 µg/ml	30	24	20.00	12	60
LC_{50}		94.984		0.246	

Key: ADR- *A. digitata* Root, BVR- *B. verticillata* roots, Lethal Concentration $_{50}$ -LC_{50}

The result of Infra-Red (IR) spectroscopy of plant extracts showed the Frequency peak values, Stretching vibration and specific functional groups. FTIR spectroscopic investigation of *B. verticillata* Root (BVR) and *Adansonia digitata* root (ADR) showed the presence of 13 and 15 characteristic peak values with different useful mixtures of 12 functional groups such as hydroxy group, Saturated aliphatic (Lipids), carbonyl, alcohols, nitrile, phenols, alkynes, ketones, Phosphate ion, Alkyl/ Aryl Halides, carboxylic acids, amides, aromatics Fatty acids and Proteins. This showed the presence of these functional groups in both the extracts except for PO3 stretch in BVR and Double bond, =C-H bonding which is Alkenes/aromatic (benzene) group in ADR. An intense peak of 2002cm^{-1} and 2005 cm^{-1} for BVR and ADR were observed respectively which corresponds to carboxylic acid groups.

Table 4: Bioactive Constituents Isolated from *A. digitata* and *B. verticillata* Root Extracts

S/no.	Frequency ranges (cm^{-1})	Plant extracts/ Frequency peak values		Functional Group Assignment	Expected Phytochemical Compounds
		BVR	ADR		
1	3600-3200	3286	3260	O-H stretching vibration	presence of phenolic/ hydroxy / alcohols group
2	2935-2915	2921	2921	Asymmetric stretch of -CH(CH2) vibration	Saturated aliphatic compounds - Lipids
3	2865-2845	2854	2851	symmetric stretch of -CH(CH2) vibration	Fatty acids, Lipids and Proteins
4	2260-2100	2117	2110	Multiple bond	alkynes, Nitriles
5	2100-1800	2002	2005	Carboxyl compound	carboxylic acid
6	1820-1670	1737/1711	1737/1711	C=O stretching vibration, carbonyl compounds	presence of Ketone, aldehyde group
7	1669-1510	1611	1607/1521	C=C stretching vibration	alkenes
8	1510-1361	1443/1369	1443/1369	C=C-C, Aromatic ring	Aromatic compound
9	1360-1210	1249	1283	C-N stretching vibration, NO2 stretch	Aliphatic amines, Nitro compounds
10	1210-1030	1201	1205/1164	C-H bond (-C-H_2X)/C-F stretch	Alkyl Halides
11	1100-1000	-	1148/1100	PO3 stretch	Phosphate ion
12	1000-840	866	-	Double bond, =C-H bonding	Alkenes, aromatic (benzene)
13	840-600	803/773	821/769	C-Cl stretch	Alkyl/ Aryl Halides

DISCUSSION

The result of this study revealed the presence of different classes of secondary metabolites from both extracts which are known to show medicinal activity as well as exhibiting physiological activity against several bacteria. It is not surprising that these plant extracts are used traditionally by herbalist to cure bacteria related ill-health (Njoku and Obi, 2009 and Ushie *et al*., 2014). The result is in conformity with previous findings where phytochemical screening of the leaf extracts of *B. verticillata* has shown the presence of these bioactive constituents and indole alkaloids such as borrevine and borreverine (Rufa'i *et al.* 2020) while that of *A. digitata* extracts is in line with the findings of Masola *et al*., (2009) who reported the phytochemical profile of *A. digitata* root bark and leaves extract to show the presence of the same bioactive agents with this study but reported absence of steroids in the two extracts. Several researchers (Masola *et al*., 2009; Ushie and Adamu, 2010; Yusha'u *et al*., 2010; Ushie *et al.,* 2014; Iwu, 2014; Abdullahi-Gero *et al.,* 2014; Jackson, 2015; Altemimi *et al.,* 2017) reported the chemical compounds that are found abundantly in both extracts and their different uses.

The result of the Brine Shrimp Lethality Test revealed that both ADR and BVR had a low LC_{50} of 94.984 and 0.246µg/ml with percentage mortality of 93.33% and 100% respectively at 1000µg/ml concentration which indicated high cytotoxic effect and therefore the death of nauplii was high. ADR and BVR extracts possessed cytotoxic activity against the brine shrimp as considered to have active or potent components. The percentage mortality (%M) was calculated by dividing the number of dead nauplii by the total number and then multiplying by 100%. This is to ensure that

the death (mortality) of the nauplii was attributed to the bioactive compounds The observed lethality of the extracts to brine shrimps indicated the presence of potent cytotoxic, bioactive and probably anti-tumor components of these plants. The cytotoxic action of a drug was believed to be due to the disturbing of fundamental mechanisms associated with the cell growth, mitotic activity, differentiation and function (Chukwudi and Yusha'u, 2016) hence, the cytotoxic activity of the plants might be due to one of these mechanisms.

This is in agreement to the study by Tahia *et al.* (2015), where the LC_{50} value of pet ether pertitionate of *A. digitata* leaves was 0.284 µg/ml and that of methanol extract of barks of *A. digitata* was 6.99 µg/ml. According to Owolarafe *et al.,* (2014) and Asaduzzaman *et al.* (2015), crude plant extract is bioactive if it has an LC_{50} less than 1000 µg/ml while it is considered inactive if its LC_{50} value is greater than 1000 µg/ml.

FTIR spectroscopic investigation of *B. verticillata* Root (BVR) and *Adansonia digitata* root (ADR) showed the presence different useful mixtures of functional groups which belongs to different types of compounds. The functional groups isolated are known to be parts of compounds which are bioactive and have been confirmed to have medicinal as well as physiological activity and therefore could be said to be responsible for the efficacy of the roots of the plants studied in treatment of different ailments. This is in conformity with a study present in the plant extracts (Muhammad *et al*., 2016). by Hemmalakshmi *et al.* (2017), which confirmed the presence of these functional groups. Also, in a study by Chandra *et al.* (2019), the FTIR analysis of leaf powder of *Nicotiana plumbaginifolia* revealed the presence of functional groups in which the frequency ranges were 3600-665cm^{-1}. Several researchers used FTIR to screen the bioactive group of chemicals from plant extracts and reported different functional groups and compounds (Kareru *et al*., 2008; Starlin *et al*., 2012; Ashokkumar and Ramaswamy, 2014).

CONCLUSION

From this study, the phytochemical compounds identified in both the extracts are known to be bioactive and have been confirmed to have medicinal as well as physiological activity and therefore could be said to be responsible for the efficacy of the roots of the plants studied in treatment of different ailments. The observed lethality of the extracts to brine shrimps indicated the presence of potent cytotoxic, bioactive and probably anti-tumor components of these plants. The FTIR spectroscopy revealed different useful mixtures of functional groups which belongs to different types of compounds of medicinal importance. Thus, might be the reason why *A. digitata* and *B. verticillata* plants have been used in traditional medicine for many years.

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