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Research Article

The effect of Solvent on the Phytochemical and Antimicrobial activity of *Adansonia digitata* Leaf

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Abstract

Adansonia digitata leaf is widely spread in hot drier region of tropical Africa. It is used mostly in the northern part of Nigeria as soup and to treat fatigue, dysentery, disease of urinary tract, snake bite etc. The effect of solvent on the Phytochemical and antimicrobial activity of *A. digitata* was carefully examined in this research work using ethanol, n-Hexane, ethyl-acetate and Aqueous as extracting solvent, using standard methods. The Phytochemical screening shows the presence of almost all the secondary metabolite tested for such as Glycoside, Saponins, Phytosterols, Phenols, Anthraquinones, Carbohydrate and Tannins in all the four crude extract, Alkaloids, Flavonoids were absent in Aqueous extract and Steroid was also found absent in n-Hexane extract. The antimicrobial activity of the plant ethanol, n-Hexane, ethyl-acetate and Aqueous crude extract was carried out using agar well diffusion method against *Escherichia coli, Shigella dysenteriae, Salmonella typhi, Klebsiella pneumoniae, Helicobacter pylori, Pseudomonas aeruginosa, Streptococcus aureus* and were all found to be reasonably active and effective against the tested isolates, however ethanol is a better extracting medium for the Phytochemical and antimicrobial activity of *A. digitata L* than the rest of the solvent used as this gives credence to the used of *A. digitata* as food and for medicinal purposes.

Keywords: *Adansonia digitata*; Antimicrobial activity; Phytochemical; Microorganism.

Introduction

Nature has been a major source of medicinal agent for thousands of years and an impressive number of modern drugs have been isolated from natural resource. The use of natural products with therapeutic properties is as ancient as human civilization and for a long time minerals, plants and animal products were the main source of drugs [1] as about 25 % of the drugs prescribed worldwide come from plant and 121 of such active compounds are in current use.

Baobab (*A. digitata*), a tree plant belonging to the Malvaceae family, is wide spread throughout the hot, drier regions of tropical Africa [2]. It is a deciduous, massive and majestic tree up to 25 m high, which may live for hundreds of years [3]. The leafs are also used traditionally to treat a wide variety of conditions including fatigue, as a tonic, and for insect bites, Guinea worm and internal pains, dysentery, diseases of the urinary tract, opthalmia and otitis in some parts of Africa [4] baobab leaf are potential protein source to be used to complement the amino acid profile to improve the overall protein quality of the local diet. Baobab leafs are also significant sources of minerals [4] .Some studies reported that baobab leaf is an important source of iron and have a higher content of iron compared to numerous other wild – gathered foods, and are a rich source of calcium [5]. Baobab leaf was also possess anti-oxidant properties [6].

Phytochemical are non-nutritive chemical compounds which occur naturally in plant. Scientists estimate that about 10,000 different Phytochemical have an effect on diseases like cancer and metabolic disorder. Antibacterial activity is a method use to assess the susceptibility of micro-organisms to Phytochemicals and other chemical agents from

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non-plant sources. Antibacterial agents are either drugs or any plant material that destroys or inhibits the growth of bacteria. The present work is aimed to determine the effect of solvent on the Phytochemicals and antimicrobial activity of *Adansonia digitata* using Ethanol, n-Hexane, ethyl-acetae and Aqueous as extracting solvent [1].

Materials and methods

Apparatus and reagents

Incubator at 35 and 37°C by Petersime incubators and machines (Olsene) Belgium. pipettes of various sizes, Conicalflasks, Beaker, Glass rod stirrer, Volumetric flasks, Test tube or Boiling tube, Wash bottles, Sample container, Measuring cylinde, Refrigerator by Thermo scientific High-Performance Laboratory Freezers (Powai) Mumbai India, Spatula, Rhetord stand and Clamp, Heating mantle, Watmann filter paper, Rotatory Evaporator by StonyLab (Nesconset, New York) United State of America, steam, Nutrient broth, Muller hinton Agar, weighing balance, alcohol; ethanol; n-Hexane; Ethyl acetate; Molish reagent; Mayer's reagent; Hydrochloric acid; Sodium Hydroxide; Conc Sulphuric acid; Fehling solution A & B; Ferric chloride; Conc Nitric Acid; Chloroform, Benzene, Ammonium solution, Wagner's reagent, Iodine solution, Diethyl ether, Ammonium hydroxide, Glacial aceted, Distle water and almost all laboratory equipment.

Plant collection and identification

The leaf extract of *A. digitata* was collected from Tunfure area council of Akko Local Government, Gombe state of Nigeria. The leafs sample was identified by a botanist/taxonomist at Department of Biological Science Gombe State University Gombe City in Gombe Nigeria. The leafs obtain and identified was allowed to dry sufficiently under shade at room temperature, after which it was finely powdered using pestle and mortar. The voucher specimen was deposited and kept in good condition for all subsequent analysis.

Preparation of Extracts

The powdered leaf extract was extracted using cold maceration exhaustively at room temperature with Ethanol for 72 h. The extract obtain was filtered using Whatman filter paper No 1 and then concentrated under reduced pressure with a rotary evaporator. The extraction procedure was repeated to obtain aqueous, n-Hexane, and ethyl acetate crude extract of the leaves sample. The fractional aqueous and solvent extracts obtained was concentrated to dryness on the rotary evaporator and then screened for their antimicrobial activity. [7].

Phytochemical Screening

Phytochemical examination was carried out for all the extract to determine the presence of Alkaloid, Glycoside, Saponin, Flavonoid, Phytosterol, Steroid, Phenol, Anthraquinones, Tannin and Carbohydrate according to the standard methods [7-10].

Test for Alkaloids (Mayer's Test)

0.2 g of 1 % hydrochloric acid was added to 2 ml of the extract in a test tube and 1 ml of mayer's reagent added. A yellowish buff precipitate will be indicative of the presence of alkaloid [7].

Test for Glycosides

1.0 ml of distilled water was added to 1.0 ml of alcoholic extract and a few drops of aqueous NaOH will be added. A yellow coloration obtain will indicates the presence of glycosides [8].

Test for Flavonoids

Few drops of concentrated hydrochloric acid will be added to 1 ml of an alcoholic extract of the plant material. Immediate development of a red color will indicates the presence of flavonoids [9].

Test for Tannins

2 ml of 5 % $FeCl_3$ solution will be added to 5 ml of the ethanol extract in a test tube. A greenish-black precipitate indicates the presence of tannins [10].

Test for Saponin

Extracts will be dissolved in water and treated with 20 ml of distilled water and shaken in a graduated cylinder for 15 Min. The formation of one centimeter layer of foam will indicates the presence of saponins [9].

Phenol Test (Ferric Chloride Test)

Extracts will be treated with 3-4 drops of ferric chloride solution. Formation of bluish black color will indicates the presence of phenols [8].

Test for Phytosterols (Salkowski's test)

Extracts will be treated with chloroform and filtered. The filtrates will also be treated with a few drops of concentrated sulphuric acid, shaken and allowed to stand. Appearance of golden yellow color will indicates the presence of phytosterols [7].

Test for Carbohydrates (Fehling's test)

Extract will be dissolved individually in 5 ml distilled water and filtered. The filtrates obtain will be used to test for the presence of carbohydrates. The Filtrates will be hydrolysed with dilute HCl, neutralized with alkali and fehling's solution A and B. The Formation of red precipitate on warming over water bath will indicates the presence of reducing sugars [8].

Test for Steroid

0.5 g of crude extract will be dissolved in 2 cm^3 of chloroform and concentrated H_2SO_4 will be added sidewise. Formation of Red color in the lower chloroform layer will indicates the presence of steroids [10].

Test for Anthraquinones

About 0.5 g of extracts will be boiled with 10 % HCL for few Min in a water bath. It will be filtered and allowed to cool; equal volume of chloroform will be added to the filtrates. Few drops of 10 % NH_3 will also be added to the mixture and heated. Formation of rose-pink color will indicate the presence of anthraquinones [7].

Determination of Antimicrobial Activity

The antimicrobial activity of the crude ethanol, n-Hexane, ethyl- acetate and aqueous extract of *Adansonia digitata* were determined using Agar well diffusion method [11]. Petri dish containing 10 ml of Mueller Hinton agar medium were seeded with 24 h old culture of selected bacterial. The bacterial used include *Escherichia coli, Shigella dysenteriae, Salmonella typhi, Klebsiella pneumonia, Helicobactea pylori, Pseudomonos aeruginosa, Streptococcus aureus.* 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 for identification and subjecting the organisms in peptone water and thereafter, sub cultured into nutrient agar medium and incubated for 24 h at 37ºC.

Sterile filter paper discs (7 mm in diameter) containing 1000-5000 ppm of the sample dissolved in DMSO, was placed on the surface of the medium. DMSO and water alone served as negative controls. A standard disc containing Agumentin antibiotic drug (300 µg/l) was used as a positive control. Incubation was carried out for 24 h at 37 °C. 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 for three replicates. An inhibition zone of 8 mm or greater of 6 sized cup borer was considered as a good antimicrobial activity and a cleared zone bigger than 8 mm was interpreted as sensitive while smaller than 7 mm was interpreted as resistance [12-14].

Result and discussion

Phytochemical screening results

The results obtained from the Phytochemical investigation in table 1, revealed that most of the bioactive compounds tested for, were present in the ethanol, N-hexane, ethyl-acetate and Aqueous crude leave extracts of *A. digitata*, except for aqueous and n-Hexane extract where alkaloids, flavonoids and steroids were all found to be absent. The results of Phytochemical investigation of this study is in line with that of [15] were also most of the Phytochemical screened were present and varies from that of the other researchers and reasons 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 [16].

Antimicrobial results

The antibacterial activity of the ethanol, n-Hexane, ethyl acetate and Aqueous crude leaf extract of *A. digitata* all shows a reasonable zone of inhibition against tested Microorganism as shown in table 2-8. The highest zone of inhibition demonstrated by the plant leaf was produced by ethanol extract as compared to control (Agumentin 300 µg/l). The ethanol extract showed the highest zone of inhibition (14.5 mm) on *Salmonella typhi* than all of the rest of the solvent, followed by n-Hexane with inhibition zone (13.5 mm) on *Helicobacter*

pylori. The next highest zone of inhibition is (11 mm) on *Escherichia coli* for ethyl acetate and the least zone of inhibition is (8 mm) on *Klebsiella pneumonia* for Aqueous crude extract. Generally the result reveals that the plant is active and has high antibacterial activity as shown by the activities of the plant against the tested microorganisms at various concentrations. The concentrations of the activity of the plant decreases as the concentration of the plant sample decreases i.e. as the concentration of the plant extract increases so also the concentration of the activity of the various solvents increases across all the solvents used in comparison to the control or standard (Agumentin 300 µg/l). Some activity of the plant sample tend to be even higher than that of the control as revealed by the results, therefore ethanol as a solvent shows more activity followed by n-Hexane, followed by Ethyl acetate and then lastly Aqueous extract has the least activity as most activity tend to be nil or not active may be because it is a neutral compound and therefore tend not to extract the active component of the plant as the rest of the other solvents. The highest zone of inhibition produced by the ethanol extract demonstrated that ethanol is a better extracting medium for the Phytochemical and antimicrobial activity of *A. digitata* than the rest of the solvent used [17].

Table 1. Phytochemical Results for *Adansonia digitata* (Kuka) Leaf extract

S. No.	Phytochemical constituent	Ethanolic extract	N-hexane extract	Ethyl-acetate extract	Aqueous extract
1	Alkaloids	+	+	+	-
2	Glycoside	+	+	+	+
3	Flavonoids	+	+	+	-
4	Saponins	+	+	+	+
5	Phtosterols	+	+	+	+
6	Steroids	+	-	+	+
7	Phenols	+	+	+	+
8	Anthraquinones	+	+	+	+
9	Carbohydrate	+	+	+	+
10	Tannins	+	+	+	+

+ (Plus) Indicate present; - (Minus) indicate absent.

Table 2. The activity of the microorganism – *Shigella dysenteriae* against solvent used at various concentrations

Solvent	Concentration, µg/l				
	1000	500	250	125	Control Agumentin 300 µg/l
Ethanol (Z.I)	13	12	11	10	10
n-Hexane (Z.I)	11	11	10	10	14
Ethyl-Acetate (Z.I)	11	10	9	8	16
Aqueous (Z.I)	9	8	6 Nil	6 Nil	16

Table 3. The activity of the microorganism – *Escherichea coli* against solvent used at various concentrations

Solvent	Concentration, µg/l				
	1000	500	250	125	Control Augmentin 300 µg/l
Ethanol (Z.I)	12	11	11	10	10
n-Hexane (Z.I)	13	12	12	10	14
Ethyl-Acetate (Z.I)	6 Nil	6Nil	6Nil	6Nil	11
Aqueous (Z.I)	6Nil	6Nil	6Nil	6Nil	16

Table 4. The activity of the microorganism – *Salmonella pneumonia* against solvent used at various concentrations

Solvent	Concentration, µg/l				
	1000	500	250	125	Control Augmentin 300 µg/l
Ethanol (Z.I)	14	13	13	12	10
n-Hexane (Z.I)	12	11	11	10	14
Ethyl-Acetate (Z.I)	8	7	6Nil	6Nil	16
Aqueous (Z.I)	6Nil	6Nil	6Nil	6Nil	12

Table 5. The activity of the microorganism – *Klebsiella pneumonia* against the solvent used at various concentrations

Solvent	Concentration, µg/l				
	1000	500	250	125	Control Augmentin 300 µg/l
Ethanol (Z.I)	13	12	13	9	15
n-Hexane (Z.I)	11	11	11	10	16
Ethyl-Acetate (Z.I)	9	8	8	6 Nil	10
Aqueous (Z.I)	8	8	6 Nil	6 Nil	16

Table 6. The activity of the microorganism – *Helicobactea pylori* against the solvent used at various concentrations

Solvent	Concentration, µg/l				
	1000	500	250	125	Control Augmentin 300 µg/l
Ethanol (Z.I)	14	13	12	11	16
n-Hexane (Z.I)	13	12	12	10	14
Ethyl-Acetate (Z.I)	7	6 Nil	6 Nil	6 Nil	16
Aqueous (Z.I)	6 Nil	6 Nil	6 Nil	6 Nil	12

Table 7. The activity of the microorganism – *Pseudomonas aeruginosa* against the solvent used at various concentrations

Solvent	Concentration, µg/l				
	1000	500	250	125	Control Augmentin 300 µg/l
Ethanol (Z.I)	13	11	11	10	14
n-Hexane (Z.I)	12	11	11	10	14
Ethyl-Acetate (Z.I)	7	6 Nil	6 Nil	6 Nil	13
Aqueous (Z.I)	6 Nil	6 Nil	6 Nil	6 Nil	16

Table 8. The activity of the microorganism – *Streptococcus aureus* against the solvent used at various concentrations

Solvent	Concentration, µg/l				
	1000	500	250	125	Control Augmentin 300 µg/l
Ethanol (Z.I)	13	13	12	11	13
n-Hexane (Z.I)	12	11	10	10	14
Ethyl-Acetate (Z.I)	7	7	7	7	16
Aqueous (Z.I)	6 Nil	6 Nil	6 Nil	6 Nil	13

(Z.I)= Zone of Inhibition. The zone of inhibition is considered from 7 mm above while the resistances are considered at 6 mm.

Conclusions

The effect of solvent on the phytochemical and antimicrobial activity of *Adansonia digitata* indicate the potential and potency of the plant against tested microorganisms however ethanol is a better extracting solvent for the Phytochemical and antimicrobial activity of *A. digitata* than the rest of the solvents used. Furthermore this justifies the usage of the plant both as medicine and as food even as this study further supports the continual usage of *Adansonia digitata*.

Conflict of interest

The authors of this work declare no conflict of interest.

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