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Research Article

The effect of Solvent on the Phytochemical and Antimicrobial activity of *Arachis hypogaea* Leaf

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Abstract

Arachis hypogaea is one of the most common widely consumed leguminous crops in Africa. The leaf is widely used because of its antioxidant, anti-inflammatory and antimicrobial tendencies. The effect of solvent on the Phytochemical and antimicrobial activity of *Arachis hypogaea L* was carefully examined in this research work using ethanol, n-Hexane, ethyl-acetate and aqueous as extracting solvent. Standard methods were adopted for the Phytochemical and antimicrobial activity. The Phytochemical results shows the presence of almost all the secondary metabolite tested for such as Alkaloids, Flavonoids, Saponins, Carbohydrate and Tannins in all the four crude extract except for ethanolic extract where Flavonoids was found to be absent, so also Glycoside, Phytosterols, Steroids, Phenols, and Anthraquinones were also found to be absent in the Aqueous extract. The antimicrobial activity of the plant ethanolic, n-Hexane, etyl-acetate and Aqueous crude extract was carried out against *Escherichia coli, Shigella dysenteriae, Salmonella typhi, Klebsiella pneumoniae, Helicobactea pylori, Pseudomonas aeruginosa, Streptococcus aureus* and were all found to be reasonably active and effective against the tested isolate, however ethanol and aqueous solvents are better extracting medium for the Phytochemical and antimicrobial activity of *Arachis hypogaea* than the rest of the solvents used as this gives reason for the continual consumption of *Arachis hypogaea* as food and for medicinal purposes.

Keywords: *Arachis hypogaea*; Antimicrobial activity; Phytochemical analysis; Microorganism.

Introduction

Medicinal plants are useful for healing and curing of human diseases due to the presence of phytochemical constituents [1]. About 25% of prescribed drugs in the world today are sourced from plants [2]. About 75- 80% of people in the developing countries rely on traditional plants based medicines for their primary health care needs [3]. There is abundant number of medicinal plants and only small amounts of them have been investigated for their biological and pharmacological activity. Phytochemicals occur naturally in the medicinal plants such as leaves, vegetables, barks and roots that have defense mechanism and protect from various diseases.

Groundnut [Peanut] (*Arachis hypogaea L.*) is one of the world's most popular oil seed crops which is grown as an annual plant but perennial growth is possible in climates which are warm until harvest. Peanut belongs to the family Fabaceae (commonly known as the bean, pea or legume family. Peanuts and its products have been a component of the world's diet for years. It is the fourth important oilseed crop of the world in production after soybean, cottonseed and rapeseed. Oil yield of peanut is ranged from 18.6- 20.8 %. Peanut contained about 16.2 – 36 % protein. These proteins are classified into albumin (water soluble), globulins (salt soluble) and glutelins (acid/alkaline soluble); the globulins constitute about 87 % and consists of arachin and conarachin.

Peanuts are known to be rich in acidic amino acid, but however deficient in essential amino acids, lysine, methionine and threonine 2-7. Peanut contains 18 % carbohydrates with a

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starch content of 0.5 – 5 %, and sucrose content of 4 – 7 %. Peanuts also contained 3 % ash which is composed of 26 inorganic constituents of which phosphorus, potassium, magnesium and sulfur are high and virtually unaffected by heat. It's also contains vitamins, minerals, flavonoids and many other biologically active constituents 9-16. The pharmaceutical and medical industries use peanut oil as a vehicle for medication in external, enteral or parenteral preparations; the cosmetics industry uses it in skin, sun and massage oil [1]. Many pharmacological effects such as antioxidant, hypolipidemic, antiinflammatory, analgesia mediated by opioid receptor affinity, sympathomimetic, endocrine, antimicrobial, antiparasitic, sedative, hypotensive and haemostatic were attributed to the constituents of *Arachis hypogaea* [4]. The present work is aimed to find the effect of solvents on the Phytochemicals and antimicrobial activity of *A. hypogaea* leaf using ethanol, n-hexane, ethyl-acetate and aqueous as extracting solvents.

Materials and methods

Apparatus and reagents

Incubator at 35 and 37^{o}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 Stony Lab (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. hypogaea* was collected from Tunfure area council of Akko Local Government, Gombe State of Nigeria. The leaf sample was identified by a botanist/taxonomist at Department of Biological Science Gombe State University, Gombe City in Nigeria. The leaf obtained 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 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 procedures was repeated to obtain the remaining solvents of the leaf sample. The fractional aqueous and solvent extracts obtained was concentrated to dryness on the rotary evaporator and then screened for their antimicrobial activity [5].

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 [5-8].

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 [5].

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 [6].

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 [7].

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 will indicate the presence of tannins [8].

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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 [6].

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 [5].

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 [8].

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 [7].

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 [8].

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 indicates the presence of anthraquinones. [6].

Determination of Antimicrobial Activity

The antimicrobial activity of the crude ethanolic, n-Hexane, ethyl- acetate and aqueous extract of *Arachis hypogaea* were determined using Agar well diffusion method [9]. 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 pneumoniae, Helicobactea pylori, Pseudomonas 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 [10-12].

Result and discussion

Phytochemical analysis results

The results obtained from the Phytochemical investigation revealed that most of the bioactive compounds tested for, were present in the ethanolic, N-hexane, ethyl-acetate and Aqueous crude leaf extract of *Arachis hypogaea* except for Aqueous extract where Glycoside, Phytosterols, Steroids, Phenols, Anthraquinones and Flavonoids of the ethanolic extract of *A. hypogaea* were all found to be absent. The results of phytochemical investigation of this study is in line with that of [13], where also, almost all of the Phytochemical screened were present and varies from that of the other researchers, which reasons could be due to the part of the plant used, age of the plant, percentage humidity, climatic condition, soil

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condition, geographical location, time of harvesting or method of extraction [14].

Results of the antimicrobial activity of leaf extract of A. hypogaea

The antibacterial activity of ethanolic, n-Hexane, ethyl acetate and Aqueous crude leaf extract of *Arachis hypogaea* all shows a reasonable zone of inhibition against tested Microorganism as showed in table 2-8. The highest zone of inhibition demonstrated by the plant leaf was produced by ethanol on *Pseudomonos aeruginosa*, Aqueous on *Escherichea coli*, Aqueous on Shigella dysentriae and ethanol on *Helicobacta pylori* as compared to control (Agumentin 30 µg/l) with the highest zone of inhibition (17 mm, 17 mm, 17 mm and 16 mm) respectively at concentration of 1000 µg/l. Followed by n-Hexane with zone of inhibition 13 mm on E.coli and 12 mm each on *Salmonella dysenteriae*, *Pseudomonos* and *Klebsilla pneumonia* at 1000 µg/l concentration and lastly ethyl-acetate with inhibition zone of mostly 10 mm each at 1000 µg/l. Generally the results reveal that the plant is active and have high antibacterial activity as showed by the activity 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 comparism 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 showed by the results, therefore ethanolic and Aqueous as an extracting solvent shows more activity with the highest inhibition zone, followed by n-Hexane, followed by Ethyl acetate which has the least activity. The highest zone of inhibition produced by the ethanol and Aquous extract demonstrated that ethanol and aqueous are better extracting medium for the Phytochemical and antimicrobial activity of *Arachis hypogaea* than the rest of the solvent [15].

Table 1. Phytochemical results for *Arachis hypogaea* Leaf extracts

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 the solvent used at various concentrations

Solvent	Concentration, µg/l				
	1000	500	250	125	Control Agumentin, 300 µg /l
Ethanol (Z.I)	12	10	10	10	18
n-Hexane (Z.I)	10	10	10	8	19
Ethyl-Acetate (Z.I)	10	10	10	10	15
Aqueous (Z.I)	17	15	14	14	14

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

Solvent	Concentration, µg/l				
	1000	500	250	125	Control Agumentin, 300 µg /l
Ethanol (Z.I)	12	11	10	10	23
n-Hexane (Z.I)	13	12	10	10	19
Ethyl-Acetate (Z.I)	15	12	10	10	19
Aqueous (Z.I)	17	15	13	12	17

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

Solvent	Concentration, µg/l				
	1000	500	250	125	Control Agumentin, 300 µg/l /l
Ethanol (Z.I)	13	12	12	12	20
n-Hexane (Z.I)	12	10	10	10	36
Ethyl-Acetate (Z.I)	10	10	10	10	20
Aqueous (Z.I)	10	10	10	10	23

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 Agumentin, 300 µg /l
Ethanol (Z.I)	11	10	10	10	18
n-Hexane (Z.I)	12	10	10	9	19
Ethyl-Acetate (Z.I)	12	10	9	8	15
Aqueous (Z.I)	12	11	10	9	18

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

Solvent	Concentration, µg/l				
	1000	500	250	125	Control Agumentin, 300 µg /l
Ethanol (Z.I)	16	15	14	10	26
n-Hexane (Z.I)	10	10	10	10	25
Ethyl-Acetate (Z.I)	10	10	10	10	19
Aqueous (Z.I)	10	10	10	10	16

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

Solvent	Concentration, µg/l				
	1000	500	250	125	Control Agumentin, 300 µg /l
Ethanol (Z.I)	17	15	13	11	27
n-Hexane (Z.I)	12	10	10	10	17
Ethyl-Acetate (Z.I)	12	10	10	10	17
Aqueous (Z.I)	10	10	10	10	13

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

Solvent	Concentration, µg/l				
	1000	500	250	125	Control Agumentin, 300 µg /l
Ethanol (Z.I)	15	13	13	10	23
n-Hexane (Z.I)	11	10	10	10	23
Ethyl-Acetate (Z.I)	12	11	10	10	18
Aqueous (Z.I)	8	8	8	8	20

(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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