JOURNAL OF ADVANCED
BOTANY AND ZOOLOGY
Journal homepage: http://scienceq.org/Journals/JABZ.php
ISSN
2348 – 7313
V7I4.04
Research Article
Open Access

Phytochemical screening and medicinal attributes of crude extracts of soyabean (glycin max) and black-eyed bean (*phaseolus vulgaris*) leaves

Mustapha Aliru Olajide*,
1. Department of Chemical, Geological & Physical Sciences, Kwara State University Malete, PMB 1530, Ilorin, Kwara State, Nigeria.
*Corresponding author: Mustapha Aliru Olajide, E-mail: aliru.mustapha@kwasu.edu.ng
Received: April 20, 2020, Accepted: May 22, 2020, Published: May 22, 2020.

ABSTRACT

Soyabean (*Glycin max*) and black-eyed bean (*Phaseolus vulgaris*) belongs to the family of leguminosae and are grown in West Africa especially Nigeria for its edible seeds and leaves. Phytochemical screening of the leaves obtained by the cold maceration method using 95% ethanol, petroleum ether (60-80°C), and water extracts of leaves revealed the presence of alkaloids, flavonoids, glycosides and tannins. The growth of *Salmonella typhi; Klebsiella pneumoniaae; Escherichia coli, Aspergillus funugatus, Mucor mucedo and Rhizopus stolonifer* from the extracts and the growth was inhibited. Ethanol and aqueous extracts of *Glycin max* showed appreciable inhibitions to the growth of Mucor muced and minimum inhibitory concentration was 20% for all extracts.

Keyword: phytochemical; medicinal; legumes; bacteria; fungi; inhibition

INTRODUCTION

The *Glycin max and Phaseolus vulgaris* commonly called 'soyabean' and 'black-eyed bean' are grown in some parts Nigeria for their seeds. They are legumes of pod-bearing plants that have about 13,000 species belonging to the family of leguminosae. These plants are widely grown throughout the world. Due to high protein content of the plants are popularly grown globally making them important in human nutrition. The leaves of these plants are also eaten in some part of Nigeria particularly in the South Eastern area of the country [1]. Since many higher plants are known to produce antimicrobial agents and the use of these plant-preparations in tradition has been well documented [2-6]. The search for new antimicrobial is a continuous one as the target pathogens often evolve into new genetic variant, therefore many plant materials by instinct or trial and error had been used to combat various ailments [7, 8]. The pharmacological activities of *P. vugaris* seeds has been reported in the literature apart from its being known as a popular dietary source [9-13]. It is in this light, that the study of the phytochemical analysis and antimicrobial screening of the fresh leaves was carried out to scientifically document the medicinal importance of these plants.

Keywords: *Glycin max; Phaseolus vulgaris;* pharmacological activities; antimicrobial screening ; protein.

1. Materials and Methods

1.1. Sample Collection

The fresh leaves of' Soya beans' *(G, max)* and 'Black-eyed beans' *(P. vugaris)* used were obtained from a farm settlement in Malete, Kwara State, Nigeria. The plants were identified at the Department of Plant and Environmental Sciences of the Kwara State University, Malete, Nigeria.

1.2. Preparation of materials

Ten grams (10g) of the plant samples were separately incarcerated in sterile mortar and pestle and kept separately in three (clean) conical flasks.

1.3. Extraction

An aliquot (100cm^3) of petroleum ether (60° -80° C) was added to the l0g of the marcerated leave samples in a conical flask. The mixture was stirred and covered. It was allowed to stand for 24 hours and thereafter, filtered using sterile Whatman Filter paper. The extracts were concentrated to 10cm^3 on a water bath. It was cooled and stored in a refrigerator. The procedure was repeated with 95% ethanol and distilled water. The petroleum ether and ethanol extracts were light-yellow and water extracts were faint yellow.

1.4. Phytochemical Analysis

The extracts were evaluated for the presence of alkaloids, flavonoids, steroids, glycosides, saponin and tannins according to the standard methods reported in literature [14-19].

1.4.1.Alkaloids

One (1) cm^3 of 1% HC1 was added to 3cm3 of the extracts in a test tube. The mixture was heated for 20 minutes. It was cooled and filtered. The filtrate was used for the following tests:

a) Two drops of Meyers reagent was added to 1 cm^3 of the extracts. A creamy precipitate observed in each extract tested, indicated the presence of alkaloids in all the extracts.
b) Two drops of Wagner's reagent was added to 1 cm^3 of the extracts. A reddish brown precipitate observed in each extract tested, indicated the presence of alkaloids in all the extracts.

1.4.2. Tannins

a) One (1) cm^3 of freshly prepared 10% KOH was added to 1 cm3 of the extracts. A dirty white precipitate observed in each extract tested indicated the presence of tannins in all the extracts.
b) Two drops of 5% $FeCl_3$ was added to 1 cm^3 of the extracts. A greenish precipitate observed in each extract tested indicated the presence of tannins in all the extracts.

1.4.3. Glycosides

a) Ten (10) cm^3 of 50% H_20_4 was added to 1 cm^3 of the extract in a test tube. The mixture was heated in cooling water for 15 minutes. 10 cm^3 of Fehling's solution was added and mixture was boiled a brick-red precipitate observed in each extract tested indicated the presence of glycosides in all the extracts.

1.4.4. Saponins

a) Frothing test: 2 cm^3 of the extract in a test tube was vigorously shaking for 2 minutes. Frothing observed in each extract tested indicated the presence of saponins in all the extracts.
b) Emulsion test 5 drops of Olive oil was added to 3 cm^3 of

the extract in a test tube and the mixture was vigously shaking. A stable emulsion formed in each extract test indicated the presence of saponins.

1.4.5. Steroids

Salkowski test: To 1 cm^3 of the extract, concentrated H_2SO_4 (5 drops) was added. No red colouration was observed in each extract tested. This result showed the absence of steroids in all the extract.

1.4.6. Flavonoids

One cm3 of 10% NaOH was added to 3 cm^3 of the extract. A yellow colouration was observed in each extract tested indicated the presence of flavonoids in all the extracts.

1.5. Antibacterial Screening

The method used was the Punch Hole Method in which a uniform and equidistant hole of 5mm in diameter was punched into the inoculated nutrient agar using a sterile cock borer.

1.5.1. Preparation of the medium

Nutrient Agar or Potato Dextrose Agar (PDA) was prepared by dissolving 2.8g of nutrient agar and 3.5g of PDA in 100ml of distilled water. The solution was sterilized in an autoclave at 121°C at I.I N pressure for 15 minutes, cooled and allowed to solidify in the sterile Petri-dishes. The agar depth of the media was 4 mm.

1.5.2. Preparation of cultures and inoculation

Pure culture of bacteria *(Scrfimvnellae lyphic; Klebsiella pheumoniae; Proteus spp; Eschenchie coil; Mucor mucedo)* and fungi *(Aspergillus fumigalus; Rhyzopus stolonifer; Mucor mucedo)* obtained from the Department of medical microbiology of the University of llorin Teaching Hospital, Ilorin, Nigeria, were separately used to inoculate the Petri-dishes. These were done by pouring 20 mL of each molten agar into culture plates and allowed to solidify. Inoculated open-wells ' of 10mm diameter were out using sterile cock-borer and then filled with 1 ml of different concentration of the extract. The inoculated plates were incubated at 37°C for bacteria for 24 hours and at room temperature for fungi for 72 hours [20, 21]

1.5.3. Assay of Extracts

The extracts were serially diluted to obtain 5%, 10%, 20% and 30% solutions of the extracts in the sterile test-tubes selected pathogens (bacteria and fungi tested organisms) were seeded on their respective media and incubated at 37°C for 24 hours (bacteria) and at room temperature for 72 hours Fungi. The clear zones of inhibition were checked in the plates . Presence of zones of inhibition indicated activity. The zones of inhibition were measured from the edges of the wells as shown in Tables 1, 2 and 3.

2. RESULTS AND DISCUSSION

The results of phytochemical analysis is shown in Table 1. The alkaloids, flavonoids, glycosides and tannins were present but the steroids and saponins were not found in both samples.

Table 1: Phytochemical Analysis of the Extract of the Fresh Leaves of *G. max* and *P. vulgaris.*

Plant (*G. max*)	**Alkanoids**	**Flavonoids**	**Steroids**	**Glycosides**	**Saponins**	**Tannins**
Petroleum ether	+	+	-	+	-	+
Ethanol	+	+	-	+	-	+
Water	+	+	-	+	-	+
Plant (*P. vulgaris*)						
Petroleum ether	+	+	-	+	-	+
Ethanol	+	+	-	+	-	+
Water	+	+	-	+	-	+

Key: + Present
- Absent

Table 2: The antimicrobial activity of the extracts of fresh leaves of glycin max and phaseolus vulgaris

Test Organisms	**Petroleum Ether**	**Ethanol**	**Water**
Salmonella Typhi	+	+	+
Klebsiella Pneumoniae	+	+	+
Proteus Spp	-	-	-
Escherichia Coli	+	+	+
Staphylococcus aureus	-	-	-
Aspergillus fumigathus	+	+	+
Rhizopus stolonifer	+	+	+
Mucor mucedo	+	+	+

All the extracts have antimicrobial activity with most of the test organisms as shown in Table 2. Antimicrobial actitivity of plant extract has been reported in literature to be due to the presence of tannins and flavonoids[17,19, 20]. Here, all the solvents were effective and contained these compounds which make them inhibit the growth of the bacteria *(Salmondlae typhi;Klebsiella pneumoniae; and Escherichia coli)*and fungi *(Aspergillus fuinigatus; Rhizopus stolonifer and Mucor mucedo).* Flavonoids play protective role and contribute to colour in plants and are recognized as having antimicrobial, anti-allergic, anti-inflammatory, hepato protective and anti-thrombic effects [4, 18]. Tannins have also been reported to form irreversible complexes with praline-rich protein thereby resulting in inhibition of cell wall synthesis [21, 22]. The results of the inhibition of bacterial and fungi growths in Table 3 shows that the extracts are close dependent since no activity was observed at very low concentrations.

Table 3: Inhibition of microbial growth of the leaves extract of glycin max (gm) and phaseolus vulgaris (pv)

Test Organism		Zone of Inhibition (mm)					
(Bacteria)	Dilution	Petroleum-Ether EEthEthEther		Ethanol		Water	
Salmonelae tychi	(%)	Gm	Pv	Gm	Pv	Gm	Pv
	30	1.0	2.0	1.2	2.5	2.5	1.5
	20	1.0	1.0	1.0	1.0	2.0	2.0
	10	-	-	-	-	1.0	-
	05	-	-	-	-	-	-
Klebsiella pneumonia	30	2.0	1.0	2.0	1.0	2.5	2.0
	20	2.0	1.0	1.5	-	2.0	1.5 .
	10	1.0	-	-	-	-	-
	05	-	-	-	-	-	-
Proteus spp.	30	-	-	-	-	-	-
	20	;	-	-	-	-	-
	10	-	-	-	-	-	-
	05	-	-	-	-	-	-
Escherichia coli	30	2.1	2.0	2.0	2.0	2.5	2.0
	20	1.0	0.5	1.0	1.0	1.5	1.0
	10	-	-	-	-	1.0	-
	05	-	-	-	-	-	-
Staphylococcus Asureus	30	-	-	-	-	-	-
	20	-	-	-	-	-	-
	10	-	-	—	-	-	-
	05	-	-		-	-	-
Aspergillus fumiga tus	30	2.0	2.0	3.0	2.0	3.0	2.0
	20	-	1.0	-	1.0	-	2.0
	10	-	-	-	-	-	-
	05	-	-	-	-	-	-
Rhizopus Stolonifer	30	2.0	2.0	2.0	3.0	2.0	2.0
	20	1.0	2.0	2.0	2.0	2.0	2.0
	10	-	1.0	-	-	-	-
	05	-	-	-	-	-	-
Mucor mucedo	30	2.0	2.0	4.0	2.0	4.0	2.5
	20	1.0	1.0	2.0	2.0	1.5	2.0
	10	1.0	1.0	-	1.0	-	-
	05	-	-	-	-	-	-

Key: + Present
- Absent

However, the mucor mucedo was the most sensitive to the inhibiting actions of both ethanol and aqueous extracts of Glycin. The extracts did not exert any inhibitory effects on the Proteus spp. and Staphylococcus aureus, showing the great

antibiotic potential against specific organism [18]. Minimum inhibitory concentration was 20% for all extracts.

CONCLUSION

The leaves extracts of *Glycin max* and *Phaseolus vulgaris* using three different solvents (petroleum ether, ethanol and water) were found to contain alkaloids, flavonoids, glycosides and and tannins. The extracts showed inhibitory effects on the growth of test bacteria (*Salmomonela tychi, Klebsiella pneumonia*; *Escherichia coli*) and fungi (*Aspergillus fumigatus; Rhizopous stolonifer; Mucor mucedo).* Saponins and steroids were absent. The inhibitory potential of the extracts of the leaves of 'Soyabean' (Glycin max) and 'black-eyed bean' (Phaseolus vulgaris) promises potential application in the treatment of ailments.

REFERENCES

1. J. Hutching, J..M Dalziel,. Floral of West Tropical Africa: The University of Chicago Press; Vol. 1. Part 2, 2000, pp 111
2. W. Liu, J. Liu, D. Yin, X. Zhao. Influence of Ecological Factors on the Production of Active Substances in the Anti-Cancer Plant Sinopodophyllum hexandrum (Royle). PLoS ONE. 2015;10(4):e0122981.
3. S.B Mishra, CV Rao, SK Ojha, MV Vijayakumar.. An analytical review of plants for antidiabetic activity with their phytoconstituent & mechanism of action A. Int. J. Pharm. Sci. Res. 2010, 1(1), 9-44.
4. N.G Konkon, A.L Adjoungoua, P. Manda, D.Simaga,K.E N'Guessan, B.D. Kone. Toxicological and phytochemical screening study of *Mitragyna Inermis* (willd.) O ktze (Rubiaceae), anti diabetic plant J. Med. Plants Res. 2010, 2(10), 279-284.
5. C.F Massaro, D. Shelley, T.A Heard, P Brooks In vitro antibacterial phenolic extracts from "sugarbag" pot-honeys of Australian stingless bees (Tetragonula carbonaria). J of Agric and food chemi. 2014;62(50):12209-12217.
6. N.M Lerato, T. Samkeliso, P. Michael. Preliminary Phytochemical Screening of Crude Extracts from the Leaves, Stems, and Roots of Tulbaghia violacea Int. J. Pharm. & Phyto. Res. 2017, 9(10), 1300-1308
7. R.C Ambady, Early diagnosis and prevention of diabetes in developing countries. S. Rev. End. Met. Dis. 2008, 9(3), 193-201.
8. Lim, T. Hovenia dulcis. Edible medicinal and non-medicinal plants: Springer, 2013, 568-77.
9. L. Hangen, M. Bennink. Consumption of Black Beans and Navy Beans (P. vulgaris) Reduced azoxymethane-induced colon cancer in rats. Nutr. Cancer. 2002, 44, 60-65.
10. A.L Ocho-Anin, K.D Atchibri, T.H Brou, Y.J Kouakou, K.D. Gnakri. Screening for antidiabetic activity and phytochemical constituents of common bean (Phaseolus vulgaris L.) seeds J. Med. Plants Res. 2010, 4(17), 1757-1761.
11. A. Helmstädter. Beans and Diabetes: Phaseolus vulgaris Preparations as Antihyperglycemic Agents. J. Med. Food, 2010, 13(2), 251-254.
12. M. Yadav, S. Chatterji, S.K Gupta, G. Watal. Int. Preliminary phytochemical screening of six medicinal plants used in traditional medicine.J Pharm. Sci. 2014, 6(5):539-42.
13. D.R Sheel, K. Nisha, P.J Kumar Preliminary. Phytochemical Screening of Methanolic Extract of Clerodendron infortunatum. J Appl. Chem. 2014, 7(1), 10-3.
14. J.B Harbone. Phytochemical Methods, 3rd Ed. Chapman and Hall: London, 1988, pp. 135-203
15. E.A Sofowora. Medicinal plants and traditional medicine in Africa. Spectrum Books Ltd: Ibadan, 1984, pp. 97-145.
16. G.E Trease, W.C Evans. Pharmacog. Ed. London: 1996, pp. 282-396.
17. A.O Adetuyi, A.V Popoola. Extraction and Dye ability Potential Studies of the Colourant in Zanthoxylum Zanthoxyloides Plant on Cotton Fabric J. Sci. Eng. Technol., 2001, 8(2), 3291-3299.
18. K.N Venugopala, V Rashmi, B Odhav Review on natural coumarin lead compounds for their pharmacological activity.BioMed Res. Int. 2013, 2013.
19. A. Wadood, M. Ghufran, S.B Jamal, M. Naeem, A. Khan, R. Ghaffar. Phytochemical analysis of medicinal plants occurring in local area of Mardan. Anal Biochem. 2013, 2 (4), 1-4.
20. C.F Massaro, D. Shelley, T.A Heard, P. Brooks. In vitro antibacterial phenolic extracts from "sugarbag" pot-honeys of Australian stingless bees (Tetragonula carbonaria). 2014, 62 (50), 12209-12217.
21. S.L Lyantagaye. Methyl-α-D-glucopyranoside from Tulbaghia violacea extract induces apoptosis in vitro in cancer cells. J Pharm. 2013, 8 (2), 93-101.
22. J. Mutembei, P. Kareru, F. Njonge, G. Peter, H. Kutima, J. Karanja, D.J Kimani. In-vivo anthelmintic evaluation of a processed herbal drug from Entada leptostachya (Harms) and Prosopis juliflora (Sw.) (DC) against gastrointestinal nematodes in sheep. Poly. & Text. Engr. 2015, 2 (1), 6-10.

Citation: Mustapha Aliru Olajide (2020). Phytochemical screening and medicinal attributes of crude extracts of soyabean (*glycin max*) and black-eyed bean (*phaseolus vulgaris*) leaves. J. of Advanced Botany and Zoology, V7I4.04. DOI: 10.5281/zenodo.3840043.