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ANTIMICROBIAL ACTIVITY OF ETHANOL EXTRACT OF STRYCHNOS SPINOSALEAVES.

[*1]Suleiman, A. A, [1]Shuaibu, S. B, [1]Abdulmalik, A., [1]Ahmad, B. [1]Muhammad, A. A., [1]Garba, I., [2]Jibril, M., [2]Abdu, K. and [3]Audi, H. A.

[1]Chemistry Department Rabiu Musa Kwankwaso College of Advanced and Remedial Studies, Tudun wada, Kano.

[2]Department of Pure and Industrial Chemistry, Faculty of Physical Science, Bayero University, Kano-Nigeria.

[3] Department of Chemistry, Federal College of Education (Technical) Bichi, Kano-Nigeria.

[1]*Corresponding author: Abubakar Suleiman Abdullahi. abubakarsuleiman003@gmail.com, +2348065827693

ABSTRACT

The present study determined the antimicrobial activity of the leaf extract of strychnosspinosa. Four extracts were extracted from the leaf of the plant: ethanol, ethylacetate, acetone and chloroform. Phytochemical screening and agar well diffusion methods were employed,Phenols was the phytochemical determined due to its vasatile biological activities. Pseudomonas aeriuginosa, Escherichia coli, Salmonella typhi and Staphylococcus aureus were the test microorganisms used. Ampicilline and tetracycline were the controls. Ethanolic extract indicated promising antimicrobial activity at 10 mg/cm^3 and 100 mg/cm^3 against the tested microorganisms near the controls. These may likely be as a result of the high concentration of phenols in the ethanolic extract. Strychnosspinosa leaf ethanol extract could be a good option for antimicrobial drug development.

Key words: StrychnosSpinosa, Phytochemicals, Phenols, Ethanol Extracts, Antimicrobial and Controls

INTRODUCTION

Strychnosspinosa grow in open regions not in rain forest. It is a tree that grows up to 45m in height or as a climbing shrub, heavily branched (Isa *et. al*., 2014).The canopy in flattish and irregular, the leaves are dark green and glossy, the fruit known as monkey orange tends to appear after good rain, they are smooth hard fruit, large and green to yellow, 8-15cm in diameter. They take a long time to ripe, inside are tightly packed hard brown seeds surrounded by juicy, fleshy edible covering which is sweet sour in taste (Isa *et al*, 2014). Due to their notable pharmacological effects, some species of the genus Strychnos are widely used in traditional and modern medicine. Medicinal applicationsfrom this

plant species include antiinflammatory (Rajesh *et al* 2009), antimicrobial (Ugoh and Rejide, 2013), antiplasmodial (Genevieve, 2009, Isa *et al* 2014), antioxidant (Isa al 2014), antidiarrheal (Hoet, 2007) and antitrypanasomal (Hoet,2007) activities.

Some of the active substances from*Strychnosspinosa* are strychnine (Orhirietal 1983), a phenolics,6'-O-β-D-apiofuranosylcalleryanin (Itoh*et al* 2006),iridoid glycoside,diaboline alkaloid,methylmannopyrannosa(carsaro et al 1995), strychnoside (Piyanuch et al 2004). Secondary metabolites from plant have important biological and pharmacological activities such as antioxidant,antiallergic, antibiotic,hypoglycemic and anticarcinogenic (Chen C., et al 1992 and Katalimic Katalimic etal, 2004,). However, the concentrations and type of such phyto-medicinal compounds may depend on the extracting solvent and the method of extraction. Hence this study is aimed at comparing the optimal capacity of several solvents for the extraction of*Strychnosspinosa*with view to recommending the better option. The findings are contributions to the existing data on phyto-medicinal potency of the plant leaves.

MATERIALS AND METHODS

Solvents and Reagents

Folin-ciocalteau reagent, acetone, methanol, chloroform, ethylacetate, sodium bicarbonate, 2,2-diphenyl-1-picryhydrazyl (DPPH), Gallic acid and ascorbic acid. These chemicals andall other reagents and solvents used were of analytical grade.

Plant Materials

The leaves of Strychnosspinosa were collected from KatsiraVillage, in Gwarzo Local Government Area ofKano State, Nigeria. The voucher specimen of Strychnosspinosa was confirmed and deposited in Habarium of the Department of Plant Biology, Faculty of Life Science, Bayero University,Kano. The collected plant material was air dried, ground into fine powder and stored in a clean, separate screw cap bottle until needed.

Preparation of Plant Extract and Fractions

The fine powdered leaf sample (500g) of Strychnosspinosa was percolated using ethanol (2000 mL). The bottle was shaken at regular intervals for one week before it was decanted, filtered and concentrated using rotary evaporator at 40^{o}Cto obtain the crude ethanol extract (36.99 g). The extract was stored safely in a freezer below 0^{o}ctemperatures until use. 10g portion of the crude ethanol extract was

weighed out stored in a separate sealed container and labeled as F_1.The other crude ethanol extract (26.99g) was macerated using polarity order of solvent system; chloroform, ethylacetate and acetone. The fractions obtained were labeled as F_2, F_3 and F_4 respectively. The fractions (F_1 to F_4) were stored in air tight and sealed amber bottles and place in refrigerator at 0^{o}c temperature before analysis.

Determination of Total Phenolic Content (TPC)

The total phenolic content in the leaf extract of Strychnosspinosa was determined using Folin-ciocalteu reagent based on the procedures described by Singleton et al (1999) with some modifications. About 0.5 cm³ solution of the fraction (1 mg/cm³) was mixed with 1.5 cm³ (1:10 v/v diluted with distilled water) folin-ciocalteaus phenol reagent and allowed to stand at room temperature for 5 minutes, then 2 cm³ of sodium bicarbonate (Na_2C0_3, 7.5% w/v) was added, the mixtures were allowed to stand for 90 minutes and kept in the dark with shaking after every 10 minutes. The absorbance of blue colour that developed was measured at 517 nm using spectrophotometer (Varian cary 50 spectrophotometer). The experiments were carried out in triplicates. The total phenolic content was expressed as milligrams of Gallic acid equivalents (mg of GAE/g sample) based on the calibration curve using various concentrations of standardgallic acid solution(Milan, 2010).

Determinationof DPPH Activity

Quantitative measurements of free radical scavenging assay were carried out according to the method described by Sasidharan et al (2007) with some modifications. The quantitative measurement of the free radical scavenging properties was carried out in a test tube. The reaction mixtures contain 2.5cm³of the sample at concentrations ranging from 0.2 to 1mg/ml and 2.5cm³ of 0.04% (w/v) solution of DPPH in 80% methanol. Ascorbic acid was used as positive control. The DPPH solution in the absence of the plant fraction was used as blank. Discolorations were measured at 517nm by using spectrophometer (Variancary50,) after incubation for 30 minute in the dark. The experiment was performed in triplicates on each of the four fractions. The percentage of the DPPH free radical was calculated using the equation

%DPPH Scavenging effect = $(A-A_1)/ A X 100$

Where A = absorbance of the control and A_1 = absorbance of the DPPH in the presence

of the fraction (sample) of Strychnosspinosa (Oktayet al. 2003). The actual decrease in absorption induced by the test fraction were compared with the absorption induce by the positive controls (ascorbic acid). The IC_{50} (concentration providing 50% inhibition) values were determined using the dose of inhibition curve in linear range by plotting the fractions concentration versus the corresponding scavenging effect.

Determination of antimicrobial activity

Agar plates were inoculated with a standardized inoculums of the test micro organism. Then filter paper disc (about 6 mm in diameter) containing the test compound at 10 mg/ml and 100 mg/ml concentrations were placed on the agar surface. The petri dishes were inoculated under suitable conditions. Generally, antimicrobial agent diffuses into the agar and inhibits germination and growth of test microorganism and then the diameter of inhibition growth zone were measured in millimeters. Ampicilline and tetracycline were used as positive controls.

RESULTS AND DISCUSSION

The weight, percentage recovered and characteristics of the different fractions obtained from maceration process of the ethanol extract are shown in table 1.

Table 1: Yield of fraction after maceration of the crude extract (36.99g) from 500g powdered leaf sample.

Fraction	Weight	Characteristics
Ethanol,($F_{1)}$	10 ± 0.021*	Sticky dark green
Chloroform, ($F_{2\_}$	1.15± 0.035	Sticky green
Ethylacetate, (F_3)	0.92±0.023	Sticky light green
Acetone, (F_4)	0.62±0.047	Sticky light green
Residue	19.48±0.14	Dark green dust

*Each value is the average of three measurements ± standard deviation.

The total phenolic content in the examined fractions ranged from 32.00 to 63.14 mg GAE/g (table 2).The highest phenols concentration was found in the ethanol fraction (F_1) while the chloroform fraction was found to have the lowest phenol compounds concentration, these show that, the phenol content in the plants extract was higher in the more polar solvent. High solubility of phenols in polar solvent correlates with their high concentration in the ethanol fraction since phenol is polar compound. Phenols are very important constituents because of their scavenging ability on free radicals due to their hydroxyl groups.

Table 2: Total phenolic compounds contents in plant extract expressed in term of Gallic acid equivalent (mg of GAE/g of fraction)

FRACTION	mgof GAE/g of fraction
Ethanol, F_1	63.14 ± 0.031*
Chloroform, F_2	32.00 ± 0.251
Ethylacelate, F_3	38.60 ± 0.125
Acetone,F_4	42.00 ± 0.225

*Each value is the average of three analyses ± standard deviation.

The antioxidant activity (DPPH Value) of the fractions of Strychnosspinosa leaf sample as presented in table 3, it was determined using a methanol solution of DPPH(stable free radical). Unlike in vitro generated free radicals such asthe hydroxyl radical and superoxide anion, DPPH has the advantage of being unaffected by certain side reactions such as metal ion chelation and enzyme inhibition (Milan, 2011). A freshly prepared DPPH solution exhibit a deep purple colourwith an absorption maximum at 517 nm. This purple colour generally fades when antioxidant molecules quench the DPPH Free radicals and convert them into a colourless/bleached product resulting in a decrease in absorbance at 517 nm band (Milan, 2011).

The antioxidant activity of four different fractions from Strychnosspinosa is expressed in term of percentage of inhibition (%) and IC_{50} value (µg/ml) ,(table 3). The value of a standard compound (ascorbic acid) was obtained and compared to the value of the antioxidant activity of the fractions.

The examination of antioxidant activities of fractions from Strychnosspinosa showed different values. The values ranges from 57% to 90%.The highest capacity to scavenge DPPH radicals was found for ethanolic fraction which neutralized 90% of the free radical at the concentration of 680 µg/ml. A moderate activity was found for acetone and ethylacetate fractions. The lower capacity to inhibit DPPH radicals was found on chloroform fractions. In comparison to the IC_{50} value of the standard (Ascorbic acid), ethanol fraction from Strychnosspinosa manifested the strongest capacity for neutralization of DPPH radicals.

Table 3: Antioxidant (DPPH Scavenging) activity of investigated plant fractions presented as percentage of DPPH radical inhibition and IC_{50} value (µg/ml)

Fraction	%DPPH radical inhibition	IC_{50} Value
Ethanol , F1	90	680
Chloroform, F2	78	700
Ethylacelate, F3	76	805
Acetone, F4	57	820
Ascorbic acid	98	310

It could be inferred from the result that, increasing the concentration of the phenols or reducing the level of the free radical in 2,2-diphenyl-1-picrylhydrazylin the reaction mixture indicates the potency to scavenge free radical by the fraction of StrychnosSpinosa;hence an increase in the antioxidant properties

Table 4: Result of antimicrobial activity of the various extracts of StrychnosSpinosa leaves.

		ZONE OF INHIBITION (mm)			
Controls/Extracts	**Concentration (mg/ml)**	**S. Auriaus**	**E. Coli**	**P. Aeruginosa**	**S. Typhi**
Tetracycline	10	23	32	30	25
	100	38	35	35	34
Ampiciline	10	28	20	35	34
	100	39	35	38	39
Ethanol (F_1)	10	20	25	20	12
	100	25	33	25	23
Chloroform (F_2)	10	0	0	0	0
	100	0	0	0	0
Ethylacetate (F_3)	10	0	0	0	0
	100	0	0	0	0
Acetone (F_4)	10	0	0	0	0
	100	0	0	0	0

From the table above it can be deduced that, ethanol fraction showed significant inhibition of the growth of the tested pathogens near and above the positive controls at 10mg/ml and 100mg/ml. These extracts showed significant inhibition of the growth of staphylococcus aureus, Escherichia coli, pseudomonasaeruginosa and salmonella typhi. These may likely be as a result of more concentration of phenols good antioxidant activity as the most polar solvent/compound.

CONCLUSION

The results obtained indicates great value of the Strychnosspinosa species for use in pharmacy and phytotheraphy. Based on this information, it could be concluded that this plant is a natural source of antimicrobial and antioxidant substances.

The study has shown that, highest concentration of phenolic compounds in the ethanol extract which correlate with its high free radicals and antimicrobial activities. The high content of phenolic compound which give rise to high antimicrobial activity indicated phenolic compounds contribute to the strong antimicrobial activity.

RECOMMENDATION

Further studies on this plant should be directed to carry out more phytochemical screening on the *S. spinosa* leaf sample to investigate more on its medicinal active component in order to prepare a natural pharmaceutical product of high value. Also antimicrobial test should be conducted on the solvents used (despite that, they were completely evaporated after the maceration process) to assess the actual potentiality of the plant materials.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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