



Original Research Article

***Strychnos innocua* (Delile): Phytochemical and Antimicrobial Evaluations of its root bark extracts**

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ABSTRACT

The antifungal and antibacterial effects of medicinal plants have long been known. The root bark decoction of *Strychnos innocua* (a *Loganiaceae* plant) has been used to treat skin infections, candidiasis, and other disorders. Hence, the aim of this study is to determine the phytochemical, antibacterial, and antifungal properties of root bark extracts of *S. innocua*. This is the first time a study of this nature is been conducted using the root bark of the plant. Maceration with n-hexane, ethyl acetate, and methanol as solvents was used in the extraction process, while the phytochemical analysis of the extracts followed a standard procedure. This confirmed the presence of flavonoids, coumarines, triterpenes, steroids, saponins, tannins, phenols, anthraquinones, quinones, carbohydrates, phlobatannins, glycosides, cardiac and alkaloids. The extracts exhibited antimicrobial activities against MRSA, *B. subtilis*, *P. aeruginosa*, *S. aureus*, *C. krusei*, *A. fumigatus*, *C. albicans* while no activity against *E. coli*, *S. pyogenes*, *S. typhii*, *A. niger* and *K. Pneumoniae* were observed. MIC and MBC/MFC were also determined. In conclusion, the root bark of *S. innocua* is thought to be rich in phytochemicals and has antifungal and antibacterial effects against some of the tested pathogens.

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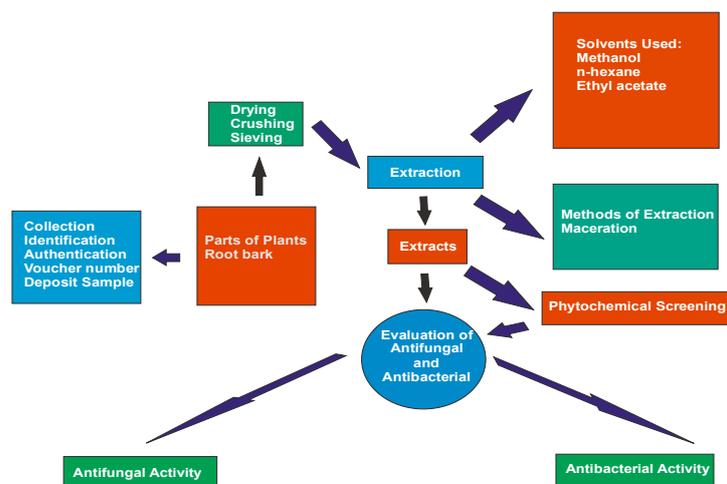
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GRAPHICAL ABSTRACT



Introduction

Phytochemicals from plants have been shown to have medicinal properties, including antioxidant, insecticidal, antifungal, antibacterial, anticonstipative, antiplasmodial, and spasmolytic properties [1]. Antimicrobials activities of a plant origin have been found to have great therapeutic potentials. The majority of studies relating to antibacterial and antifungal of medicinal plant extracts exhibited promising antibacterial and antifungal activity [2].

Herbs have long been used in pharmacology to cure diseases and are known as folk healing. This has become a part of people's traditions all around the world [3]. Herbal plants are used by over 80% of the world's population for primary health care. This is because medicinal herbs are more compatible with the human body, and have less adverse effects [4].

The complex chemical structure of secondary metabolites such as flavonoids, alkaloids, triterpenes, and other secondary metabolites is associated with medicinal plants' therapeutic qualities [5].

Flavonoids are a type of polyphenol found widely in plant flora and are used in many herbal medicines as active ingredients [6] Alkaloids are one of the most active secondary

metabolites in plants, anticancer, analgesic, and antispasmodic effects were demonstrated in some of them. [7]. Terpenes are cytotoxic to a broad variety of bacteria, fungi, insects and vertebrates, and have long been employed in herbal therapy to treat infections [8].

Strychnos innocua belong to *Loganiaceae* family and is a deciduous shrub or straight stemmed tree with much branches usually growing from 2 – 12 metres tall. It is a perennial plant and grows best in areas where annual daytime temperatures are within the ranges 19 - 31°C, but can tolerate up to 36°C [9]. The plant is harvested in South Sudan, Uganda, Nigeria, Tanzania, Malawi, Zambia, Cameroon, Congo, D. R Congo (Zaire) and Gabon [10, 11]. In Nigeria, the plant is also harvested from the wild around Samaru, Zaria, Kaduna State.

The decoction of root bark of *S. innocua* is use locally in northern Nigeria for the treatment of, skin infections, candidiasis and other diseases. This work is aimed to investigate the antifungal, antibacterial activities and phytochemical constituents of *S. innocua* (Delile) root bark extracts.



Fig. 1: *Strychnos innocua*, displaying the leaves

2.0 Methods

2.1 Collection of Plant Sample

The plant's parts were harvested from the forest of Soba district in Kaduna State, Nigeria. Mallam Namadi Sunusi of the Biological Sciences Department at ABU Zaria in Nigeria identified it. Voucher number (V/N - 01884) was allocated to the specimen and then deposited at herbarium for main purpose of reference. The plant's root bark was then shade-dried and finely ground to powder as described by [12].

2.2 Extraction of the Root Bark

The pulverize plant powder was macerated successively using n-hexane, ethyl acetate and methanol solvents in order of increasing polarity of 0.009, 0.228 and 0.762 respectively. The cool maceration involved transferring 2000 g of the pulverize powder into an aspirator and then firstly soaked with n-hexane. It was allowed to remain for period of three (3) days under room temperature and with frequent agitation. Other solvents (methanol and ethyl acetate) were used sequentially after exhaustive extraction in n-hexane as described by [13].

2.3 Phytochemical Investigation

Chemical test was carried out on the extracts of *S. innocua* in order to investigate their phytochemical constituents using standard methods as described by [14-19].

2.3.1 Alkaloids Tests

To n-hexane extract (0.5 g) was transferred into test tube, 1% aqueous HCl (5 mL) was added and stir on hot bath till warm. The solution was

filtered whilst warm while the filtrate was portioned into three (3) test tubes and labelled A, B and C. For the other extracts of methanol and ethyl acetate, the same technique/method was used.

Dragendorff test: To the portion A in test tube, Dragendorff reagent, freshly produced, was added in a few drops. Occurrence of orange-red precipitate confirmed that alkaloid is presence.

Mayer test: For portion B, Mayer's reagent (1 mL) was added to it in test tube. Occurrence of creamy colour precipitate confirmed that alkaloid is presence.

Wagner test: The portion C inside test tube, Wagner reagent (few drops) was added. Appearance of brownish colour precipitate confirmed that alkaloid is presence.

2.3.2 Anthraquinones Test

Bontrager test: In test tube, n-hexane extract (0.2 g) and chloroform (5 mL) were mixed and agitated for roughly 5 minutes. The solution was filtered while 10% ammonium solution (5 mL) and filtrate were mixed and shaken. Appearance of pink colouration in the upper layer (aqueous) confirmed free anthraquinones is presence. For the other extracts of methanol and ethyl acetate, the same technique/method was used. *Benzene test:* To each of the aqueous extracts in test tube 3 mL were boiled with aqueous H₂SO₄ (3 mL) and filter while hot. Benzene (3 mL) and filtrate were mixed and shaken, then 10% ammonia (3 mL) was added after decanting benzene layer. Occurrence of red colour in the ammonical phase (lower) indicated anthraquinone derivative is presence. For the other extracts of methanol and ethyl acetate, the same technique/method was used.

2.3.3 Steroids Test

Liebermann-Burchard test: To n-hexane extract (0.3 g) in test tube was mix with acetic anhydride (2 mL). The mixture was boiled and cool, follow by careful addition of H₂SO₄. Brown ring is formed in-between junction of upper and

lower layers, then upper layer turned blue showing steroids is presence. For the other extracts of methanol and ethyl acetate, the same technique/method was used.

Salkowski test: To n-hexane extracts (2 mL), chloroform (2 mL) and H₂SO₄ (2 mL) acid were mixed and then shaken well. Appearance of greenish fluorescence at chloroform layer indicated steroids is presence steroids. For the other extracts of methanol and ethyl acetate, the same technique/method was used.

2.3.4 Terpenoid test

Liebermann-Buchard reaction: To n-hexane extract (0.3 g) in test tube mix with chloroform and dissolved. Acetic acid anhydride (few drops) was added and then allowed to cool to room temperature. Subsequently, to form upper and lower layers, H₂SO₄ (in few drops) were carefully added to the solution. Appearance of brownish red colour indicated terpenoids is presence. For the other extracts of methanol and ethyl acetate, the same technique/method was used.

Salkowski test: For n-hex, Eth, and Meth extracts (0.2 g) each in separate test tubes and mixed with chloroform (2 mL). Then H₂SO₄ (5 mL) was added and shaken well for formation of layers. Appearance of brownish red colouration at the junction indicated terpenoids is presence.

2.3.5 Glycosides test

Legal test: For n-hex, Eth, and Meth extracts (0.3 g) were made alkaline with drops of 10% sodium hydroxide solution, then addition of freshly prepared sodium nitropruside solution. Appearance of blue colouration confirmed glycosides presence.

Keller-kiliani test: For n-hex, Eth, and Meth extracts (0.4 g) in test tube and two millilitres of GAA (glacial acetic acid having FeCl₃ solution in a drop) were mixed. The solution was placed in test tube and conc. H₂SO₄ (1 mL) was then carefully added to the test tube for formation of lower layer. Occurrence of purple-brown

between interphase confirmed deoxy sugars is presence, characteristic of cardinolides while the appearance of a pale green colouration at the upper acetic acid confirmed cardiac glycoside is presence.

Salkowski's test: The n-hexane extract (0.4 g) was transfer into test tube and chloroform (2 mL) was added to dissolve. Then H₂SO₄ (2 mL) was carefully added and the mixture shaken gently. Appearance of colouration (reddish brown) indicated steroidal ring (that is, aglycone portion of cardiac glycoside) is presence. For the other extracts of methanol and ethyl acetate, the same technique/method was used.

Liebermann's test: To each of the extracts (0.4 g) was dissolved with 2 mL of chloroform in test tube, acetic acid (2 mL) was added, then the solution was cool well in ice. H₂SO₄ (few drops) was carefully added. Change in colouration to blue from violet indicated steroidal nucleus which is aglycone portion of glycoside) is presence.

2.3.6 Test for saponin

To each of the extracts (0.4 g) in separate in test tubes and distilled water (5 mL) added to dissolved. The mixture was then boiled and the solution was filtered. The filtrate was divided into two portions in test tube and label A and B

Frothing test: To the portion A in test tube, distilled water (3 mL) was added to the portion and shaken vigorously for 3 minutes. Formation of frothing that continues on/after warming confirmed saponins. This was repeated for portion of methanol and ethyl acetate.

Fehling test: To the portion B in test tube, then addition of Fehling A and Fehling B (2.5 mL) mix in equal proportion, to the portion. Occurrence of brick-red ppt confirmed saponins is presence.

2.3.7 Tannins test

Ferric chloride test: To test tube containing 0.5 g of n-hex, Eth, and Meth extracts, distilled water was added to dissolved, then boiled and the mixture filtered. FeCl₃ added in few drops to the

filtrate. Appearance of green solution confirmed tannins is presence.

2.3.8 Flavonoids tests

Sodium hydroxide test: To each of the extracts (0.5 g) are separately dissolve in different test tube with distilled water and filter, follow by addition of 10% aqueous NaOH (2 mL) solution to the filtrate for formation of yellow colouration. A change from yellow colour to colourless after dilute HCl is added confirmed flavonoids is presence.

Shinoda's test: For n-hex, Eth, and Meth extracts (0.5 g) were transferred into different in test tube, they were separately dissolved in 95% ethanol, follow by warm and filter. To the filtrate, 3 pieces of magnesium chips was added and addition of few drops of concentrated hydrochloric acid was follow. Occurrence of purple colouration confirmed flavonoid is presence.

Ferric chloride test: In three test tubes having n-hex, Eth, and Meth extracts (0.5 g) and distilled water were mixed and boiled, then filtered. Two mL of filtrate, 10% ferric chloride solution (few drops) was mixed. Appearance of green-blue confirmed flavonoid is presence.

Test for lead acetate: For n-hex, Eth, and Meth extracts (1 mL) were measure into different test tube, follow by addition of 10% lead (II) acetate solution (1 mL). Occurrence of yellow ppt confirmed the presences of flavonoids.

2.3.9 Phenols Test

Ferric chloride test: For n-hex, Eth, and Meth extracts (0.2 g) were treated by 5% ferric chloride solution (5 mL) in test tube. Appearance of deep blue colour confirmed phenol is presence.

2.3.10 Quinone test

For n-hex, Eth, and Meth extracts (0.2 g) were treated by conc. H₂SO₄ (5 mL) or aqueous NaOH (sodium hydroxide solution) in test tube. Appearance of orange colour indicated quinone is presence.

2.3.11 Anthocyanin test

Sodium hydroxide test: For n-hex, Eth, and Meth extracts (0.2 g) in test tube, 2N NaOH (1 mL) was added, then heated at 100 °C for 5 minutes. Appearance of bluish green colouration indicated anthocyanin in presence.

2.3.12 Phlobatannins test

To each of the extracts (0.3 g) were boiled with distilled water (5 mL) in test tube then filtered, while 1% HCl (2 mL) was mixed with filtrate and boiled. Appearance of red ppt confirmed phlobatannin.

2.3.13 Test for coumarin

For n-hex, Eth, and Meth extracts (2 mL) in test tube, alcoholic NaOH (in few drops) was added. Formation of yellow colouration indicated coumarin is presence.

2.4 Fungal Activity

The selected fungal pathogens (*Candida albicans*, *Aspergillus fumigatus*, *Aspergillus nigre* and *Candida krusei*) were obtained from Medical Microbiology Department, Teaching Hospital of Ahmadu Bello University (ABUTH) Zaria - Nigeria.

The growth media used for fungi was sabouraud dextrose agar (SDA). Procedure of agar well diffusion was used for examining the stock concentration (40 mg/mL) of the extracts of root bark of *S. innocua*. Freshly prepared SDA was seeded with standard inoculum (0.1 mL) of fungi. Inocula were then spread on the media using sterile swab stick. Punching wells at the middle of all inoculated medium was achieved using sterile cork borer (6 mm in diameter). Thereafter, 0.1 mL of the appropriate solution of extracts concentration was transfer into the well of inoculated media. Extra plates containing SDA were also streaked with fungi inocula, and placed into a punched well. The plates of SDA were incubated for 7 days at 30°C. Inhibition Zone of the growth were then observed from media plates and calculated using a transparent ruler in millimetres (mm) and the values

recorded. MIC and MBC determination was achieved using broth dilution [20 - 21].

2.5 Antibacterial Activity

The selected bacterial pathogens are MRSA, *S. pyogenes*, *S. aureus*, *B. subtilis*, *E. coli*, *K. Pneumoniae*, *S. typhii*, and *P. aeruginosa*. These pathogenic microbes were obtained from Medical Microbiology Department, Teaching Hospital of Ahmadu Bello University (ABUTH) Zaria – Nigeria.

The growth media adopted for the bacteria was MHA. Agar well diffusion was used for examining the stock concentration (40 mg/mL) of the extracts of the root bark of *S. innocua*. Freshly prepared MHA was been seeded along standard inoculum (0.1 mL) of the bacterial. Inocula were then spread on media using sterile swab punching wells at the middle of all inoculated medium was achieved using sterile cork borer (6 mm diameter). Thereafter, 0.1 mL of the appropriate extracts solution concentration was added unto the well. Extra MHA plates streaked with bacteria inocula and punched, standard drugs (ciprofloxacin and sparfloxacin, 10 µg/mL) were placed into a punched well. The MHA plates were incubated for 24 hours at 37°C. Zone of inhibition of the growth were then observed from media plates and calculated using a transparent ruler in

millimetres (mm) and the value noted. MIC and MBC determination was achieved using broth dilution [20 - 21].

3.0 Results and Discussion

The Table 1 presents phytochemical constituents of extracts from the root bark of *S. innocua*.

The Table 2 shows the zone of inhibition (mm) of extracts from the *S. innocua* root bark. Zone of inhibition (mm) of Fulcin and Fluconazole is also shown in the table.

The table 3 shows the minimum concentration of n-hex extract, Eth extract and meth extracts that will inhibit growth of tested fungal.

The Table 4 shows the minimum concentration of extracts that will completely kill the tested fungi. The Table 5 shows the inhibition zones (mm) of n-hex, Eth, and meth extracts from *S. innocua* root bark. The table also includes Ciprofloxacin, Sparfloxacin, and the zone of inhibition (mm) of Ciprofloxacin. The minimal concentrations of n-hex, eth and meth extracts that will inhibit development of tested bacteria are shown in the Table 6. The Table 7 displays the n-hex extract, eth extract, and meth extracts concentrations that can totally kill the tested microorganism.

Table 1: Phytochemical Investigation

S/N	Phytochemical	Hex.	Eth.	Meth.
1	Alkaloid	-	-	+
2	Anthraquinones	+	+	-
3	Steroids	+	+	+
4	Triterpenes	+	+	+
5	Cardiac Glycoside	-	+	+
6	Saponins	-	-	+
7	Tannins	-	+	+
8	Flavonoids	-	-	+
9	Phenols	-	-	+
10	Quinones	+	+	+
11	Anthocyanins	-	-	-
12	Phlobatannins	-	-	+
13	Coumarins	+	+	+

Meth. = Methanol, Eth. = Ethyl acetate, Hex. = Hexane, + = Presence; - = Absence

Table 2: Antifungal Results

S/N	Test Organisms	The Inhibition Zone (mm)			Ful.	Flu.
		Hex.	Eth.	Meth.	10 µg/mL	10 µg/mL
1	<i>C. albican</i>	22	25	28	32	0
2	<i>C. krusei</i>	0	28	34	31	0
3	<i>A. fumigatus</i>	0	24	22	34	35
4	<i>A. nigre</i>	0	0	0	32	32

Meth. = Methanol, Eth. = Ethyl acetate, Hex. = Hexane, Ful. = Fulcin, Flu. = Fluconazole

Table 3: MIC Assay for Antifungal

S/N	Test Organisms	Extracts (mg/mL)		
		Hex.	Eth.	Meth.
1	<i>C. albican</i>	10.0	10.0	5.0
2	<i>C. krusei</i>	-	5.0	10.0
3	<i>A. fumigatus</i>	-	10	10.0
4	<i>A. nigre</i>	-	-	-

Meth. = Methanol, Eth. = Ethyl acetate, Hex. = Hexane

Table 4: MFC

S/N	Test Organisms	Extracts (mg/mL)		
		Hex.	Eth.	Meth.
1	<i>C. albican</i>	40.0	20.0	10.0
2	<i>C. krusei</i>	-	10.0	20.0
3	<i>A. fumigatus</i>	-	20.0	40.0
4	<i>A. nigre</i>	-	-	-

Meth. = Methanol, Eth. = Ethyl acetate, Hex. = Hexane

Table 5: Antibacterial Results

S/N	Test Organisms	The Inhibition Zone (mm)			Cipr.	Sparf.
		Hex.	Eth.	Meth.	10 µg/mL	10 µg/mL
1	<i>MRSA</i>	0	25	28	0	34
2	<i>S. aureus</i>	20	27	25	32	35
3	<i>S. pyogene</i>	0	0	0	35	0
4	<i>B. subtilis</i>	21	24	27	0	32
5	<i>E. coli</i>	0	0	0	38	30
6	<i>K. Pneumoniae</i>	0	0	0	0	32
7	<i>S. typhii</i>	0	0	0	42	0
8	<i>P. aeruginosa</i>	0	26	25	30	31

Meth. = Methanol, Eth. = Ethyl acetate, Hex. = Hexane, Cipr. = Ciprofloxacin, Sparf. = Sparfloxacin

Table 6: MIC

S/N	Test Organisms	Extracts (mg/mL)		
		Hex.	Eth.	Meth.
1	<i>MRSA</i>	-	10.0	5.0
2	<i>S. aureus</i>	10.0	5.0	10.0
3	<i>S. pyogene</i>	-	-	-
4	<i>B. subtilis</i>	10.0	10.0	5.0
5	<i>K. Pneumoniae</i>	-	-	-
6	<i>E. coli</i>	-	-	-
7	<i>S. typhii</i>	-	-	-
8	<i>P. aeruginosa</i>	-	10.0	10.0

Meth. = Methanol, Eth. = Ethyl acetate, Hex. = Hexane

Table 7: MBC

S/N	Test Organisms	Extracts (mg/mL)		
		Hex.	Eth.	Meth.
1	<i>MRSA</i>	-	20.0	10.0
2	<i>S. aureus</i>	40.0	20.0	20.0
3	<i>S. pyogene</i>	-	-	-
4	<i>B. subtilis</i>	40.0	20.0	20.0
5	<i>E. coli</i>	-	-	-
6	<i>K. Pneumoniae</i>	-	-	-
7	<i>S. typhii</i>	-	-	-
8	<i>P. aeruginosa</i>	-	20.0	20.0

Meth. = Methanol, Eth. = Ethyl acetate, Hex. = Hexane

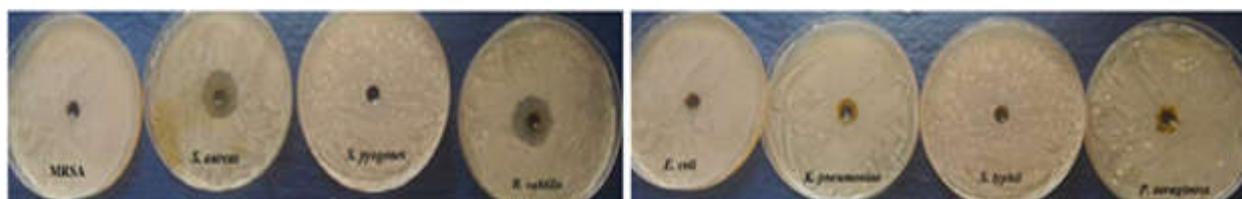


Fig. 2 a: Antimicrobial Activities of Hexane Extract



Fig. 2 b: Antimicrobial Activities of Hexane Extract

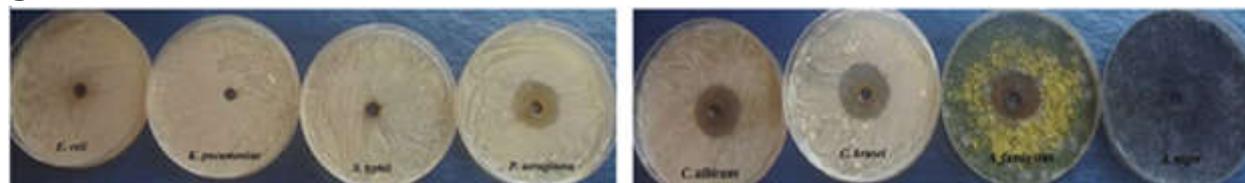


Fig 3 a: Antimicrobial Activities of Ethyl acetate Extract



Fig. 3 b: Antimicrobial Activities of Ethyl acetate Extract



Fig. 4 a: Antimicrobial Activities of methanol Extract



Fig. 4 b: Antimicrobial Activities of methanol Extract

Medicinal plants, seen as complementary therapeutics due to the presence of phytochemicals as active ingredients have showed improve management of ill-health conditions [22]. In Table 1 of this study, phytochemical such as steroids, terpenoids, quinone and coumarins are presence in all the extracts. Steroids are important in drugs discovery and are used for treatment of a number of medical ailments such as inflammation, allergic reaction, heart diseases, cancer [23]. Terpenoids have found fortuitous uses in medicine [24] and have used as antidysentery, antidiarrhea, antidiabetic, antihypertension, analgesic, anti-inflammatory, antibacterial, antibiotic, antiseptic, antioxidant [25]. Courmarines have also been reported to have antibacterial activities [26].

Eth and meth extracts revealed that tannins and cardiac glycoside are presence. n-hex and eth revealed that anthraquinone is presence. In addition, alkaloid, saponins and flavonoids and phlobatanins are presence only in the methanol extract while anthocyanins is absent in all the extracts (Table 1). This distinction in phytochemical content of the extracts is responsible for disparity seen in antifungal and antibacterial properties of extracts as shown in

tables 2 to table 7 and Fig. 2 to Fig. 4. Biological activities of saponins include effect on cell membrane, effect on cold blooded animal, hypoglycemic, effect on cholesterol metabolism, effect on animal reproduction, virucidal, anti-inflammatory, hypolipidaemic, antifungal, antimicrobial, antihemithic, antimalarial among others [27]. Alkaloids are known to have cytotoxicity activity, mutagenic and carcinogenic activity, phtoalexins, antibacterial activity, antifungal activity, antiviral activity [28].

The result of the phytochemical study also indicated that the methanol might be most efficient solvent for secondary metabolites extraction from the root bark of *S. innocua*, followed by ethyl acetate, then n-hexane. The multitarget activities of many secondary metabolites can explain the medical application of extracts from medicinal plants [29].

It was found that all the extracts showed the inhibition zone (20-28 mm) against *B. subtilis* and *S. aureus* while meth and eth extracts shows inhibition zone (25-28 mm) against *MRSA* and *P. aeruginosa* (Table 5). These bacteria have been linked to skin infections, food poisoning, pneumonia, and staph infections, among other things. However, the extracts appear to have strong antibacterial activity against *S. aureus*,

MRSA, *B. subtilis* and *P. aeruginosa*. *S. pyogene*, *E. coli*, *K. pneumoniae*, and *S. typhii* all showed no action. The extracts antibacterial activity examined is equivalent to that of conventional medicines (Ciprofloxacin, Sparfloxacin). The extract concentrations that inhibit the growth of these bacteria (MIC) vary from 5 to 10 mg/mL (Table 6), whereas the MBC concentration varies from 10 to 40 mg/mL (Table 7). The presence of several phytochemicals such as flavonoids, tannins, alkaloids, steroids, and saponins results in antibacterial action [30].

The extracts were tested for antifungal activity against a variety of fungal species, including *Candida albicans*, *Candida krusei*, *Aspergillus fumigatus*, and *Aspergillus nigre*. These plants' extracts had a wide range of actions. *C. albican*, (an opportunistic) pathogenic yeast that causes the human illness candidiasis [31], was active against all of the extracts. However, extracts of ethyl acetate and methanol exhibited action against *C. krusei* (the cause of Candidaemia) and *A. fumigatus* (causative pathogen of chronic pulmonary infections, allergic broncho-pulmonary aspergillosis). The range of activity is 22 -34 mm. No activity was observed against *A. nigre* (Table 2). The antifungal activities of extracts might be due to the combine therapeutic actions of steroids, terpenoids, quinone, coumarins, flavonoids, saponins, phlobatanins, anthraquinones, and these contributed actions against fungal strains [24]. The concentration ranges of MIC and MFC are 5 - 10 mg/mL and 10 - 40 mg/mL respectively.

4.0 Conclusions

The present study revealed the antibacterial and antifungal activities of n-hex, eth and meth extract of *S. innocua* root bark. It also revealed the available of various phytochemical constituents that might account for extracts antimicrobial activities from the plant. Pharmacognosists will find the information presented here useful in the development of antifungal and antibacterial medications.

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Authors' contributions

M.S.S. devised the phytochemical, antibacterial, and antifungal screening process, A.J.U. carried out the experiments and wrote the paper, O.R.I. aided in the experiment supervision, and H.I. evaluated/review the text.

Competing interests

There are no conflicting interests stated by the authors.

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