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Full Length Research Paper

Antibacterial activities of aqueous and methanol leaf extracts of *Solanum incanum* Linn. (Solanaceae) against multi-drug resistant bacterial isolates

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It is estimated that about 80% of the world's population use medicinal plants either in their crude unmodified form or partially in their modified semi-synthetic form for their medical care. The present study investigated the antibacterial activity of aqueous and methanol leaf extracts of *Solanum incanum* Linn. (Solanaceae) against multiple drug resistant (MDR) clinical isolates (*Streptococcus pyogenes, Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). The extraction was done by cold maceration. The antibacterial susceptibility of the bacteria was carried out using agar well diffusion method. The phytochemical screening revealed presence of cardiac glycosides, carbohydrates, reducing sugars and ketoses in both extracts. In addition, resin, flavonoid, terpenoids and steroids were found in the methanol extract while saponins and alkaloids were found in the aqueous extract. Evaluation of the antibacterial activities of *S. incanum* Linn. showed that the aqueous and methanol extracts have significant activities against *S. pyogenes, S. aureus, K. pneumoniae* and *P. aeruginosa*. Highest antibacterial activity was shown for both aqueous (MIC=2.62 mg/ml, MBC= 60 mg/ml) and methanol (MIC=7.50 mg/ml, MBC>80 mg/ml) extracts against *P. aeruginosa*, respectively. The least antibacterial activity was shown for both aqueous (MIC=0.05 mg/ml, MBC=20 mg/ml) and methanol (MIC=5.00 mg/ml, MBC=80 mg/ml) extracts against *K. pneumonia*. Thus, *S. incanum* Linn. (Solanaceae) can be said to have antibacterial activities against MDR bacterial isolates.

Key words: *Solanum incanum* Linn., phytochemical, antibacterial activities, multi-drug resistant, antibacterial.

INTRODUCTION

Plants have always been part of human cultures due to their usefulness in prevention and treatment of human and animal diseases (Anselem, 2004; Rios and Recio, 2005). According to experts, World Health Organization (WHO) estimated that 80% of the population of some Asian and African countries makes use of herbal medicine

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especially in some aspect of primary health care (Augustine et al*.,* 2017). The emergence and widespread of antibacterial resistant strain of bacteria has further compounded the global threat of infectious diseases. Medicinal plants have been reported as alternative treatment of disease in order to overcome the problem of antibacterial resistance by pathogenic micro-organisms (Emad, 2011; Ibrahim, 2014). The emergence and widespread of antibacterial resistant strain of bacteria has further compounded the global threat of infectious diseases. Alternative antimicrobial strategies are needed thus, this lead to re-evaluation of the therapeutic use of ancient remedies, such as plants (Mandal et al., 2010). The use of medicinal plants for medical treatment has become popular when people realized that the effective lifespan of antibiotics is limited and over prescription and misuse of traditional antibiotic are causing microbial resistance (Alam et al., 2009).

Solanum incanum L. (Solanceae) is a perenial wild shrub-like herb commonly called Sodom/bitter apple or bitter garden egg usually found in the middle East, East Asia and many regions in Africa (Nigeria inclusive). It is used traditionally in the treatment of sore throat, angina, stomach-ache, colic and headache (Kokwaro, 1993). The fruits of *S. incanum* has been reported to have marked antibacterial effect against several Gram positive and Gram negative bacteria such as *Streptococcus pyogenes, Staphylococcus aureus, Clostridium perfringens, Bacillus anthracis, Brucella arbutus* and *Salmonella* species (Alamri and Moustafa, 2012; Mwonjoria et al*.,* 2014). Similarly, the leaf extracts showed antibacterial activity against *Escherichia coli* (Britto and Senthinkumar, 2001), *S. pyogenes*, *S. aureus* and *P. aeuruginosa* (Taye et al*.*, 2011). The antifungal activities have also been demonstrated (Mwonjoria et al*.,* 2014; Mbaya and Muhammed, 1976). However, the activities of this plant against multi drug resistance have not been adequately investigated; hence, the present study investigated the phytochemical constituents and antibacterial activities of leaf extracts of *S. incanum* L. against MDR bacterial isolates.

MATERIALS AND METHODS

Plant collection and identification

Fresh leaves of *S. incanum* L. were collected from Biu Local Government Area, Borno State, Nigeria between January-March, 2017. Identification and authentication of the plant was done by plant taxonomist, Professor S. S. Sanusi of Department of Biological Science, Faculty of Sciences, University of Maiduguri and voucher specimen (Voucher No. 014) deposited in Pharmacology Laboratory, Department of Clinical Pharmacology and Therapeutics of the University.

Preparation of the leaf extracts

The leaves were washed in clean water, shade-dried at room temperature and pulverized using a blender. The powdered material was weighed and stored. Fifty (50) grams each of the powdered material was subjected to maceration using 500 ml of each of the solvents (99% methanol and water). The solutions were allowed to stand for 24 h with periodic shaking and then filtered. The filtrates were evaporated using a water bath at 56°C. The percent yields were determined using the formula below:

Percentage yield (%) = Final weight (g) / Initial weight (g) × 100

Phytochemical analysis of the leaf extracts

The extracts were subjected to phytochemical screening to determine the presence of the following constituents: alkaloids, carbohydrates, flavonoids, saponins, tanins, glycosides, (cardiac, steriodal), terpenes/terpenoids, fatty acids, resins using procedures described by Brian and Turner (1975), Vishnoi (1979), Markham (1982), Silva et al. (1998), Sofowora (2008), and Evans (2009) as described next.

Test for carbohydrates

General test (Molisch's test): Few drops of Molisch's reagent were added to the extract which was dissolved in distilled water. This was followed by the addition of 1 ml of concentrated tetraoxosulphate (IV) acid (H_2SO_4) by the side of the test tube, so that the acid formed a layer beneath the aqueous layer. The mixture was then allowed to stand for two minutes and then diluted with 5 ml distilled water. The formation of a dull violet colour at the interface of the layers showed a positive test (Evans, 2009).

Test for reducing sugar (Fehling's test): Approximately 0.2 g of the extract was dissolved in distilled water and filtered. The filtrate was heated with 5 ml equal volumes of Fehling's solutions A and B (which gives a deep blue coloration). Formation of a red precipitate of cuprous oxide (Cu_2O) indicated the presence of reducing sugar (Evans, 2009).

Test for combined reducing sugars: Approximately 0.2 g of the extract was hydrolysed by boiling with 5 ml diluted hydrochloric acid and the resulting solution was neutralized with sodium hydroxide solution. Few drops of Fehling's solution were added and then heated on a water bath for 2 min. Formation of cuprous oxide indicated the presence of combined reducing sugar (Evans, 2009).

Standard test for ketones (Salivanoff's test): Few crystals of resorcinol and 2 ml of hydrochloric acid were added to a small quantity of the extract and the solution boiled for 5 min. A red colouration indicated the presence of ketoses (Vishnoi, 1979).

Test for monosaccharides (Barfoed's test): Approximately 0.5 g of the extract was dissolved in water and filtered. One (1) ml of the filtrate was mixed with 1 ml of Barfoed's reagent in a test tube. This was then heated on a water bath for 2 min. A red precipitate of cuprous oxide indicated the presence of monosaccharides (Brian and Turner, 1975).

Test for soluble starch: A small quantity of the extract was boiled with 1 ml of 5% potassium hydroxide (KOH), cooled and acidified with H_2SO_4. A yellow colouration showed the presence of soluble starch (Vishnoi, 1979).

Test for anthraquinones

Test for free anthraquinones (Bontrager's test): The extract (0.5 g) was shaken with 10 ml of benzene and filtered. Then, 5 ml of

10% ammonia solution was added to the filtrate. The mixture was then shaken and appearance of a pink, red or violet colour in the ammonial (lower) phase indicated the presence of free anthraquinones (Evans, 2009).

Test for combined anthraquinones: The plant extract (0.5 g) was shaken with 10 ml of aqueous H_2SO_4 and then filtered while hot. The filtrate was the shaken with 5 ml of benzene, thereafter the benzene layer was separated and 10% ammonia solution on half of the benzene volume was added. The presence of pink, red or violet colouration in the ammonial (lower) phase indicated the presence of combined anthraquinones (Evans, 2009).

Test for cardiac glycosides

Test for steroidal nucleus (Salkowiski's test): The extract (0.5 g) was dissolved in 2 ml of chloroform. Tetraoxosulphate (VI) acid was carefully added by the side of the test tube to form a lower layer. Appearance of a reddish brown coloration at the interphase indicated the presence of a steroidal ring (that is, aglycone portion of the cardiac glycoside structure) or methylated steroids (Silva et al., 1998).

Test for steroidal nucleus (Liebermann-Burchard's test): Three millilitres (3 ml) of acetic anhydride was added to 0.5 g of the extract. After it dissolved, it was cooled in ice. Concentrated tetraoxosulphate (VI) acid was carefully added. Colour development from violet to blue or bluish-green indicated the presence of a steroidal ring (Silver et al., 1998).

Test for terpenoids: A small quantity of the extract was dissolved in ethanol. One millilitre (1 ml) of acetic anhydride was added, followed by the addition of concentrated tetraoxosulphate (VI) acid. The colour change from pink to violet indicated the presence of terpenoids (Silva et al., 1998).

Test for flavonoids

Ferric chloride test: The extract (small quantity) was boiled with distilled water and then filtered. To 2 ml of the filtrate, few drops of 10% ferric chloride were added. A green-blue or violet colouration indicated the presence of phenolic hydroxyl group (Evans, 2009).

Shinoda's test: The extract (0.5 g) was dissolved in ethanol, then warmed and filtered. Few magnesium chips were added to the filtrate followed by few drops of concentrated hydrochloric acid. A pink coloration indicated the presence of flavonoids (Markham, 1982).

Lead ethanoate test: The extract (small quantity) was dissolved in water and then filtered. To 5 ml of the filtrate, 3 ml of lead ethanoate was added. The appearance of a buff-coloured precipitate indicated the presence of flavonoids (Brian and Turner, 1975).

Sodium hydroxide test: The extract (small quantity) was dissolved in water and filtered. To the filtrate, 2 ml of 10% aqueous sodium hydroxide was added to produce a yellow coloration. A change from yellow to colourless on addition of dilute hydrochloric acid indicated the presence of flavonoids (Evans, 2009).

Test for saponin glycoside (Frothing test)

The extract (1 g) was boiled with 5 ml of distilled water and filtered. The filtrate was divided into two portions. To the first portion, about3 ml of distilled water was added and shaken for about 5 min. Frothing which persisted on warming was an evidence for the presence of saponins (Sofowora, 2008). To the second portion, 2.5 ml of a mixture of equal volume of Fehling's solution A and B was added. The appearance of brick-red precipitate was an indication for saponin glycosides (Vishnoi, 1979).

Test for phlobatannins

A small quantity of the extract was boiled with distilled water and then filtered. The filtrate was further boiled with 1% aqueous hydrochloric acid. The appearance of red precipitate indicated the presence of phlobatannins (Evans, 2009).

Test for tannins

To the extract (0.5 g), 10 ml of distilled water was added and stirred. The mixture was filtered and the filtrate was then used for the following test:

i) To 2 ml of the above filtrate, few drops of 1% ferric chloride solution was added. The occurrence of blue-black precipitate indicated the presence of tannins.
ii) A mixture of equal volume of 10% lead ethanoate was added to 2 ml of the filtrate. The formation of a white precipitate indicated the presence of tannins.
iii) The filtrate of the extract was boiled with 3 drops of 10% hydrochloric acid, and a drop of methanol. A red precipitate was an indication of the presence of tannins (Sofowora, 2008; Evans, 2009).

Test for alkaloids

The extract (0.5 g) was stirred with 5 ml of 1% aqueous hydrochloric acid on water bath and then filtered. Three millilitres (3 ml) of the filtrate was divided into two portions and used as follows:

i) To the first portion, few drops of Dragendoff's reagent were added. The occurrence of orange red precipitate indicated the presence of alkaloid.
ii) To the second portion, 1 ml of Mayer's reagent was added. The appearance of buff-coloured precipitate was an indication for the presence of alkaloids (Brian and Turner, 1975).

Test for resin

one (1) ml of the extract was dissolved in acetone and then 1 ml of distilled water is added. Turbidity indicates the presence of resin (Tripathi and Mishra, 2015).

Test for steroids

Approximately, 0.5 mls of each of the aqueous and methanol extracts of *S. incanum* L. was evaporated and dissolved in 2 ml chloroform, 2 ml of conc H2SO4 was carefully introduced by the side wall of the test-tube. Formation of red colour ring will confirm the presence of steroid (Pavitra et al., 2010).

Isolation of the multi-drug resistant bacterial isolates

A total of four isolates were used in this study: two Gram positive bacteria (*S. aureus* and *S. pyogenes*) and two Gram negative bacteria (*K. pneumoniae* and *P. aeruginosa*). The isolates were obtained from the samples analyzed in the Department of Microbiology, University of Maiduguri Teaching Hospital (UMTH). They were identified by morphological features on culture plates

followed by biochemical analysis (Bonev et al., 2008). They were subjected to antibacterial sensitivity testing using Kirby-Bauer method (Bauer et al., 1966). Briefly, each isolate was suspended in peptone water to match McFarland turbidity standard. The Mueller-Hilton Agar plates were prepared by pouring 15 ml of molten media into sterile petri plates. The plates were allowed to solidify for 5 min and 0.1% inoculum suspension was swabbed uniformly using sterile L spreader on separate plate by lawn culture technique and the inoculum was allowed to dry for 5 min. Using a sterile forceps, antimicrobial discs were evenly distributed on the inoculated plate and lightly pressed. The plates were incubated aerobically at 37°C for 24 h. After incubation, the zone of inhibition was measured using a meter ruler and the results were interpreted as sensitive and resistance (Vineetha et al., 2015). The isolates resistant to at least three drugs of different classes were identified and used for subsequent analysis.

Determination of antibacterial activity of the leaf extracts

In the determination of antibacterial activity, the bacteria were preserved on Mueller-Hilton Agar plates at 37°C using Kirby-Bauer assay method (Bauer et al., 1966). Bacterial cultures were adjusted to 0.5 McFarland and incubated at 37°C for 24 h. Assessment of antibacterial activity of leaf extract of *S. incanum* L. of different concentrations (20, 40, 80 and 160 mg/ml) was based on measurement of the diameter of the inhibition zones around the discs after 24 h. Ciprofloxacin (10 µg) was used as standard antibiotic in this study. All tests were performed in triplicate and the mean values were determined.

Determination of minimum inhibitory concentration and minimum bactericidal concentration

The modified method used by Usman and Osuji (2007) was employed to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Briefly, 20, 40, 80 and 160 mg/ml of the extracts were prepared in microtitre plate in duplicates in sterile water. Selected plant extracts were subjected to serial dilution using sterile nutrient broth medium as a diluent. After 24 h of incubation at 37°C, the microtitre plate was observed for the presence of turbidity. The least concentration where no turbidity was observed was recorded as the MIC. For MBC, a portion of the liquid from the plates that exhibited no growth were inoculated and incubated at 37°C for 24 h. The lowest concentration that revealed no visible growth after sub-culture was taken as the MBC (Usman and Osuji, 2007).

Data analysis

The data were expressed as mean ± standard deviation and the analysis was done by One Way Analysis of Variance (ANOVA) using Statistical Package for Social Sciences version 21 (SPSS, 2006) followed by Student Newman-Keul post- hoc test. $P<0.05$ was considered significant.

RESULTS

Yield of the extracts

The yield of the aqueous and methanol leaf extracts of *S. incanum* L. appeared dark brown to greenish colour, pasty and sticky. The assessment of the percentage yield of the extracts indicated that aqueous extract yielded 34.51 g (69.0% w/w) while methanol extracts yielded 30.64 g (61.3% w/w) [p=0.999].

Phytochemical constituents of the extracts

The result of the phytochemical screening of the aqueous and methanol leaf extracts of *S. incanum* L. leaf extracts is summarized in Table 1. This study reveals the presence of phytochemicals considered as active medicinal chemical constituents. Important medicinal phytochemicals such as carbohydrate, reducing sugar, cardiac glycosides, alkaloids and saponin were found in aqueous leaf extract while carbohydrate, reducing sugar, cardiac glycosides, flavonoids, terpenoids, resin, steroid and tannins were found in methanol leaf extract.

Profile of the multi-drug resistant bacterial isolates

The antibacterial susceptibility profile of the MDR bacterial isolates obtained from the samples of the patients seen at the Department of Microbiology, UMTH are shown in Table 2. The isolates were resistant to at least five (5) drugs. While all the isolates were resistant to amoxacillin, chloramphenicol and quinolones, they were all sensitive to aminoglycosides.

Antibacterial activity of *S. incanum* L. leaf extracts

The antibacterial activity of *S. incanum* L. was evaluated *in vitro* against MDR *S. pyogenes*, *S. aureus*, *P. aeuruginosa* and *K. pneumoniae*. In general, aqueous and methanol leaf extracts of *S. incanum* L. exhibited antibacterial activity (Tables 3 and 4).

The aqueous and methanol extracts demonstrated antibacterial activities against all the isolates; however, the activity was significantly lower than that of Ciprofloxacin even at highest concentration of 160 mg/ml ($P<0.05$).

Minimum inhibitory concentration and minimum bacteriocidal concentration

The MIC and MBC of the isolates were determined and presented in Table 5. The overall mean MIC for the aqueous and methanol leaf extracts were 0.8 mg/ml and 5.78 mg/ml while the overall mean MBC for the aqueous and methanol extracts was 37.5 and ≥320 mg/ml. Methanol extracts shows more activity against aqueous extracts.

DISCUSSION

Morbidity and mortality of bacterial infections are on the increase partly due to inadequacy and high cost of new generation antibacterials as well as widespread

Table 1. Phytochemical profile of aqueou and methanol leaf extracts of *S. incanum* L.

Test	Results	
	Aqueous extract	**Methanol extract**
Carbohydrates		
General test (Molisch's Test)	-	+
Test for free reducing sugar (Fehling's test)	+	+
Test for combined reducing sugar	+	+
Test for ketoses	+	+
Test for anthraquinones		
Test for free anthraquinone	-	-
Test for combined anthraquinone	-	-
Test for cardiac glycosides		
Salkowski's test	-	-
Lieberman-Burchard's test	+	+
Test for terpenoids	-	+
Test for flavonoids		
Shinoda's test	-	+
Ferric chloride	-	+
Lead acetate	-	+
Sodium hydroxide	-	+
Test for saponins		
Frothing test	+	-
Test for phlobatannins	-	-
Test for tannins		
Ferric chloride	-	+
Lead acetate	-	+
Test for alkaloids		
Dragendoff's reagent	+	-
Mayer's reagent	+	-
Resin	-	+
Steroids	-	+

+ Present, - Absent

Table 2. Profile of the multi-drug resistant bacterial isolates used in the study.

Isolate	Antibiogram	
	Sensitive	**Resistance**
S. aureus	Ery, Cpx, S, Na, Cot	Am, Cn, Pn, Ch, Ofx
S. pyogenes	S, Pef, Cot, Cpx	Cn, Am, Ch, Ery, Pn
K. pneumoniae	Ofx, Cn, S, Cpx	Na, Am, Ch, Cot, Ery
P. aeruginosa	Ofx, Pef, Cn, Cpx	Pn, Na, Am, Ery, Ch

Am: Amoxacillin; Na: Nalidixic acid; Pef: Pefloxacin; Ery: Erythromycin; Pn: Penicillin; Cn: Gentamycin; S: Streptomycin; Ofx: Ofloxacin; Cpx: Ciprofloxacin; Ch: Chloramphenicol; Cot: Cotrimoxazole.

resistance to old generation antibacterials (Williams, 2000; Fair and Tor, 2014; Li and Webster, 2018). Therefore, there is need to look for new substances from other sources with proven antimicrobial activity. Consequently, this has led to the search for effective antimicrobial agents of plant origin. The present study evaluated the antibacterial activities of aqueous and methanol leaf extracts of *S. incanum* L against MDR.

Table 3. Zones of inhibition (mm) produced by aqueous leaf extract of *S. incanum* L.

Extracts concentrations (mg/ml) and Ciprofloxacin (µg/ml)	Zones of inhibition (mm)			
	S. pyogenes	***S. aureus***	***K. pneumoniae***	***P. aeruginosa***
20	2.50±0.50*	2.00±1.00*	1.50±0.25*	2.50±1.50*
40	2.00±0.75*	2.00±0.75*	1.50±0.25*	2.00±1.00*
80	1.50±0.75*	2.50±0.25*	2.50±0.25*	2.25±0.25*
160	2.50±0.75*	2.50±0.25*	3.00±0.00*	2.50±0.25*
Ciprofloxacin (30 µg/ml)	19.0±2.00	20.0±3.00	15.0±2.00	18.0±3.00

Results expressed as Mean ± SEM, n=3 and *P< 0.05 is statistically different when compared with the standard.

Table 4. Zones of inhibition (mm) produced by methanol leaf extract of *S. incanum* L.

Extracts concentrations (mg/ml) and Ciprofloxacin (µg/ml)	Zones of inhibition (mm)			
	S. pyogenes	***S. aureus***	***K. pneumoniae***	***P. aeruginosa***
20	1.50±0.25*	1.00±0.25*	1.50±0.25*	1.00±0.00*
40	2.50±1.00*	2.50±0.50*	1.50±0.25*	2.00±0.00*
80	2.50±0.50*	2.50±0.50*	2.00±0.25*	1.50±0.25*
160	2.50±0.25*	2.50±0.75*	3.00±0.00*	2.50±0.25*
Ciprofloxacin (30 µg/ml)	19.0±3.00	20.0±3.00	15.0±2.00	18.0±3.00

Results expressed as Mean ± SEM, n=3 and *P< 0.05 is statistically different when compared with the standard.

Table 5. The minimum Inhibitory concentration and minimum bacteriocidal concentration of the aqueous and methanol extracts of *S. incanum* L.

Resistant strain	MIC(mg/ml)			MBC (mg/ml)		
	Aqueous	**Methanol**	**P value**	**Aqueous**	**Methanol**	**P value**
S. aureus	0.15	5.62	<0.05	20	80	>0.05
S. pyogenes	0.38	5.00	<0.05	50	>80	<0.05
K. pneumoniae	2.62	7.50	>0.05	60	>80	>0.05
P. aeruginosa	0.05	5.00	<0.05	20	80	<0.05

MIC: Minimum inhibitory concentration, MBC: minimum bacteriocidal concentration.

In this study, the antibiogram reflected all isolates sensitive to Ciprofloxacin but resistant to amoxacillin and chloramphenicol. Each of the isolate was resistant to at least five (5) drugs; therefore, the antibiogram of the bacterial isolates used in this study indicated that the isolates are multi-drug resistant. The resistance of strains of these bacterial (*S. aureus, S. pyogenes, K. pneumoniae, P. aeruginosa*) to numerous antibacterial drugs observed in this study is in accordance with previous studies that reported MDR among bacteria (Khan and Musharraf, 2004). Several factors may lead to increase in antimicrobial resistance which include; previous antibiotic used, inappropriate use of antimicrobial agents and inadequate adherence to infection control practice (Shu-Hui et al., 2011). Resistance of *S. aureus, S. pyogenes* and *K. pneumoniae* isolates to penicillins (amoxycillin) and chloramphenicol could be an indication of wide use of the drugs. Poor sensitivity to amoxycillin may be due to production of alpha-lactamase by resistant strains of isolates (Jhambh et al., 2012).

Secondary metabolites of plants have large economical value because of their involvement in production of colour or fragrance of flowers, taste and colour of food and resistance against diseases. The judicious use of different plant parts by folkloric medicine has played a key role in reducing human diseases (Mazid et al., 2012; Doss and Anand, 2012).

Flavonoids, resin, alkaloids, terpenoid, steroids and tannin have been reported to possess antimicrobial activities (Usman and Osuji, 2007; Doss and Anand, 2012; Sheel et al*.,* 2014; Ghalem and Ali, 2017).

The results of this study suggested that the presence of potential pharmacologically active substances such as resin, flavonoids, alkaloids, steroids and saponins are responsible for antibacterial activities. The antibacterial activities of *S. incanum* L. showed that the aqueous and

methanol leaves extract have activities against the bacterial tested (*S. pyogenes, S. aureus, K. pneumoniae* and *P. aeruginosa*) and this was in conformity with previous work done by Alamri and Moustafa (2012) in Saudi Arabia. Also, Owino et al. (2015) reported that the extract of *S. incanum* L. was found to be bacteriostatic and bacteriocidal against *S. pyogenes, S. aureus, K. pneumoniae* and *P. aeruginosa*.

Conclusion

The leave extracts of *S. incanum* L. consist of flavonoids, saponins, resins, steroids, glycosides, terpenoids, alkaloids, carbohydrates, reducing sugars and ketoses. The aqueous and methanol leaf extracts of *S. incanum* L. demonstrated antibacterial activity against MDR isolates of Gram positive and Gram negative bacteria. However, these activities at the concentration used are significantly lower than the antibacterial activity of Ciprofloxacin. The present study therefore shows that *S. incanum* L. aqueous and methanol leaf extracts have useful antibacterilal properties. Further work is needed to isolate the active principles from the plant in order to test the specific antibacterial activity of the respective phytochemical constituents.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Alam N, Shim MJ, Lee MW, Shin PG, Yoo YB, Lee TS (2009). Phylogenetic relationship in different commercial starins of *Pleurotus nebrodensis* based on ITS sequence and RAPD. Mycology 37:183-188.

Alamri SA, Moustafa F (2012). Antimicrobial properties of 3 medicinal plants from Saudi Arabia against some clinical isolates of bacteria. Saudi Medical Journal 33(3):272-277.

Anselem A (2004). Herbs for healing pax herbals.Edo state, Nigeria.

Augustine EC, Ugoha R, Azubuike MI (2017). The contributions of African traditional medicine to Nigeria's health care delivery system. IOSR Journal of Humanities and Social Science 22(5):32-43.

Bauer AW, Kirby WM, Sherris JC, Turck M (1966). Antibiotic susceptibility testing by a standard single disk method. *A*merican Journal of Clinical Pathology 45:493-496.

Bonev B, Hooper J, Parisot J (2008). Principle of assessing bacterial susceptibility to antibiotic using the agar well diffusion method. Journal of Antimicrobial Agent Chemotherapy 61(9):1295-1301.

Brian KR, Turner TD (1975). Practical Evaluation of Phytochemicals. Wright Scentechnical, Bristol, UK. pp. 57-59.

Britto SJ, Senthinkumar S (2001). Antimicrobial activities of *Solanum incanum* leaf extract. Asian Journal of Microbiology, Biotechnology and Environmental Sciences 3(1-2):65-66.

Doss A, Anand SP (2012). Preliminary phytochemical screening of asteracantha longifolia and pergularia daemia. World Applied Sciences Journal 18(2):233-235.

Emad MA (2011). Plants: An alternative source for antimicrobials. Journal of Applied Pharmaceutical Science 01(06):16-20.

Evans CW (2009). Trease and Evans Pharmacognosy. 16th edition. Saunders Elsevier: Edinburgh pp. 196-197, 225-227, 229-232, 561.

Fair RJ, Tor Y (2014). Antibiotics and bacterial resistance in the 21st century. Perspectives in Medicinal Chemistry 6:25-64.

Ghalem BR, Ali B (2017). Preliminary phytochemical screening of five commercial essential oils. World Journal of Applied Chemistry 2(4):145-151.

Ibrahim S (2014). Application of medicinal plants to overcome antibiotic resistance in some selected multi-drug resistant clinical isolates. Research and Reviews: Journal of Pharmacognosy and Phytochemistry 2(4):48-52.

Jhambh R, Dimri U, Gupta VK, Rathore R (2012). Identification and antibiogram of bacterial isolates from dairy cows with clinical mastitis. Veterinary Practitioner 13(2):358-359.

Khan AU, Musharraf A (2004). Plasmid-mediated multiple antibiotic resistance in Proteus mirabilis isolated from patients with urinary tract infection. Medical Science Monitor 10:598-602.

Kokwaro JO (1993). Medicinal plants of East Africa, 2nd Edition East African Literature Bureau, Nairobi pp. 222-223.

Li B, Webster TJ (2018). Bacteria Antibiotic Resistance: New challenges and opportunities for implant-associated orthopaedic infections. Journal of Orthopaedic Research 36(1):22-32.

Mandal SM, Chakraborty D, Dey S (2010). Phenolic acid acts as signaling molecules in plants microbes symbioses. Plant Signaling and Behavior 5:359-368.

Markham KR (1982). Techniques of Flavonoids Identification. Academic Press: New York, USA. pp. 1-113.

Mazid M, Khanb TA, Mohammada F (2012). Medicinal Plants of Rural India: A Review of Use by Indian. Folks Indo Global Journal of Pharmaceutical Sciences 2(3):286-304.

Mbaya B, Muhammed S (1976). Antibiotic action of *Solanum incanum* L. Antimicrobial Agents *and* Chemotherapy 6:920-927.

Mwonjoria JK, Ngeranwa JJ, Kariuki HN, Githinji CG, Sagini MN, Wambug SN (2014). Ethno medicinal, phytochemical and pharmacological aspects of *Solanumincanum* (Lin.). International Journal of Pharmacology and Toxicology 2(2):17-20.

Owino J, Omundi J, Njeru SN (2015). Antibacterial activity of methanol crude extracts of Solanum incanum L. Kenyan traditional medicinal plants. International Journal of Science and Research 4(2):560-563.

Pavitra PS, Janani VS, Charumathi KH, Indumathy R, Sirisha P, Rama SV (2010). Antibacterial activity of plants used in Indian herbal medicine. International Journal of Green Pharmacy 4(1):23-28.

Rios JL, Recio MC (2005). Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology 100:80-84.

Sheel R, Nisha K, Kumar J (2014). Preliminary phytochemical screening of methanolic extract of *Clerodendron infortunatum*. IOSR Journal of Applied Chemistry 7(1):10-13.

Shu-Hui TA, Chung-ming LB, Tzoa-Yin LC, Shan-Chwen CD, Feng-Yee C (2011). Emergence and spread of multi-drug resistant organisms. Journal of Microbiology, Immunology and Infection 44:157-165.

Silva LG, Lee IS, Aflinnghorn DA (1998). Special problem with extraction of plant in Natural product isolation. Human Press Inc. 999, Review drive, suite 208, Totowa, New Jersey, USA 072512. pp. 343-364.

Sofowora A (2008). Medicinal plants and traditional medicine in Africa.3rd Edition, Spectrum Books limited, Ibadan, Nigeria. pp 289.

SPSS (2006). Statistical Package for Social Science Windows version 16.0. SPSS Inc, Chicago, IL, USA.

Taye B, Giday M, Animut A, Seid A (2011). Antimicrobial activity of selected plants in traditional treatment of wounds in Ethiopia. Asian Pacific Journal of Tropical Biomedicine 1(5):370-375.

Tripathi IP and Mishra C (2015). Phytochemical screening of some medicinal plants of Chitrakoot region. Indian Journal of Applied Research 5(12):56-60.

Usman M, Osuji JC (2007). Phytochemical and *in vitro* antimicrobial assay of the leaf extracts of new bouldia leaves. African Journal of Traditional, Complementary and Alternative Medicines 4(4):1476-1480.

Vineetha N, Vignesh RA, Sridhar D (2015). Preparation, standardization of antibiotic discs and study of resistance pattern for first-line antibiotics in isolates from clinical samples. International Journal of Applied Research 1(11):624-631.

Vishnoi NR (1979). Advanced practical chemistry. Ghaziabad-India: Yikas Publication House, PVT Ltd. pp. 447-449.

Williams R (2000). Antimicrobial resistance a global threat. Essential Drug Monitor 1:28-29.

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