Unveiling the Antimicrobial Constituents of *Ipomoea Cairica*: A Phytochemical Study of Methanolic Extracts

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Abstract

The emergence of new infectious diseases, the resurgence of several infections and the occurrence of antimicrobial resistance (AMR) has put the people in Saharan and sub-Saharan Africa to an assiduous risk. This has created the necessity for studies directed towards the development of new therapeutic alternatives for antimicrobial agents. In this study the air dried and pulverized aerial parts of *Ipomoea cairica* was soaked in a mixture of dichloromethane and methanol (1:1, v/v). The study investigates the antimicrobial properties of methanolic extracts of *I. cairica* and evaluates their phytochemical composition. The crude extract from the aerial parts of this plant were evaluated for antimicrobial activities against four different bacterial strains; *Escherichia coli, Salmonella typhi, Pseudomonas* and *Staphylococcuss aureus,* and three fungal strains; *Aspergillus niger, Penicillum chrysogenum* and *Candida albicans* using well agar diffusion assay and their minimal inhibitory concentration (MIC) values determined using a 2-fold dilution technique. The crude extract exhibited good antimicrobial activities with zones of inhibitions; 19 ± 0.25, 25 ± 0.10, 23 ± 0.12 and 13 ± 0.05 mm for *E. coli, S. typhi, P. aeruginosa* and *S. aureus* respectively, and the zones of inhibitions for fungal strains were as follows; 15 ± 0.5, 23 ± 0.00 and 19 ± 0.41 mm for *A. niger, P. chrysogenum* and *C. albicans* respectively. The findings reveal that *I. cairica* is a promising source of natural antimicrobial agents and form a foundation for future studies targeted at isolation and characterization of the active constituents responsible for its bioactivity. Phytochemical analysis revealed the presence of key secondary metabolites, including phenolics, flavonoids, terpenoids, and alkaloids, which may contribute to the observed biological effects.

Keywords: *Ipomoea cairica*; Methanolic extract; Antimicrobial activity; Phytochemicals; Secondary metabolites; Natural products.

Introduction

Microbial infections are ailments or sicknesses caused in animals and humans by the introduction of the following microbes such as bacteria, viruses, fungi and protozoa (Baumgardner, 2012). An infected person can transmit microbes to another person through the following ways; **i**) Sneezing or coughing which can transmit viruses that cause colds or flue or the bacteria that causes tuberculosis, **ii**) Shaking hands or touching surfaces which are contaminated such as door shutters, computer key boards to mention but a few,

iii) Sexual intercourse can transmit microbes such as herpes simplex virus which causes genital herpes, gonorrhea bacteria from one person to another (Chakraborti *et al*., 2019).

The global rise of antimicrobial resistance (AMR) has intensified the search for novel, effective, and safer therapeutic agents from natural sources. Pathogenic microorganisms are rapidly evolving resistance to existing antibiotics, creating an urgent need for alternative compounds with broad-spectrum antimicrobial potential. Medicinal plants remain one of the most promising reservoirs of new bioactive molecules, owing to their chemical diversity and long-standing ethnomedicinal relevance. *Ipomoea cairica* (Convolvulaceae), a fast-growing climbing herb distributed widely across tropical and subtropical regions, has garnered scientific interest for its reported pharmacological properties.

Global estimates indicate that over 75% of world population cannot afford the products of Western pharmaceutical industry and have to rely upon the use of herbal medicines for treatment of various diseases (Kour *et al., 2021)*.

The potential of plants as sources for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller. Although, for example, the National Cancer Institute (NCI) of the United States screened some 35, 000 plant species for antitumour activity from 1957 to 1981, these plants will still have to be considered as 'uninvestigated' with respect to any other pharmacological activity (Newman & Cragg, 2020). Plants contain hundreds or thousands of metabolites. Thus, any phytochemical investigation of a given plant will reveal only a very narrow spectrum of its constituents. This is due to the fact that, the process that leads from the plant to a pharmacologically active, pure constituent is very long and tedious, and requires a multidisciplinary collaboration of botanists, pharmacognosists, chemists, Pharmacologists and toxicologists (Ramawat *et al*., 2009). This approach involves the following steps; **i**) Collection, proper botanical identification, authentication and drying of the plant materials, **ii**) Preparation of appropriate extracts and preliminary chromatographic analysis by CC, TLC and HPLC where each fraction obtained has to be submitted for bioassay in order to follow the activity (activity guided fractionation), **iii**) Biological and pharmacological screening of crude extracts, **iv**) Bio-assay of pure constituent(s).

Most of the bioassays require specialized facilities for cell culture and the technical know-how of a biochemist, biologist or pharmacologist, **v**) Structure elucidation and structure modification (Aribi *et al*., 2022; Mroczek *et al*., 2020). Traditionally, various parts of the plant have been used for treating inflammation, infections, fever, and skin ailments, suggesting the presence of potent bioactive constituents. Among extraction solvents, methanol is particularly effective at solubilizing a broad range of polar and moderately non-polar phytochemicals, making methanolic extracts a valuable starting point for identifying potential antimicrobial compounds. Despite scattered reports of biological activities within the genus *Ipomoea*, systematic evaluation of the antimicrobial properties of methanol extracts from *I. cairica* remains limited. By exploring this plant as a potential source of novel antimicrobial agents, through a comprehensive assessment of their phytochemical profile and biological activity against selected pathogenic microorganisms, the work contributes to ongoing efforts to identify plant-based alternatives that may support future drug discovery and help address the escalating challenge of AMR. These findings therefore, would justify the traditional medicinal use of the plant by people in Uganda and globally.

Botanical information of the plant under study

The *I. cairica* belongs to Convolvulaceae family. It is a climbing herb which is found abundantly in tropical and sub-tropical regions. It has many common names such as railroad creeper, Cairo morning glory, five fingered- morning glory, messina creeper, mile-a-minute vine among others (Sengupta & Dash, 2020). It is a rampant long-lived (perennial) climber reaching up to 5 m or more in height, or creeping along the ground and fences. It has very distinctive leaves with 5-7 finger-like lobes, large purple, purplish-pink or whitish tubular flowers (4-6 cm long and 5-8 cm across) with a darker centre. (Srivastava & Shukla, 2015, ançerli *et al*., 2018).



Fig. 1: Shows *Ipomoea cairica* (leaves, flowers and stem) plant from its natural habitat

The *I. cairica* has the following taxonomy as shown in the **Table 1**.

Domain	**Eukaryota**
Kingdom	Plantae
Phylum	Spermatophyta
Sub phylum	Angiospermae
Class	Dicotyledonae
Oder	Solanales
Family	*Convolvulaceae*
Genus	*Ipomoea*
Species	*Ipomoea cairica*

Table .1: Taxonomy of *Ipomoea cairica*

The generic name is derived from the Greek words meaning "resembling" which refers to their twining habit. The genus *Ipomoea* has over 400 species all over the world from *Ipomoea palma*te forks or *Ipomoea cairica* L. which grows abundantly in Egypt (Kishore et al., 2014). The different species of *ipomoea* other than the *Ipomoea cairica* are listed below *I. batatilla*, *I. crasscaulis, I. fistulosa, I. albiflora, I. fruticosa I. gossypioides, I. nicaraguensis, I. transvaalensis and I. texana.*

Materials And Method

General:

The solvents (methanol, Dichloromethane) used in the extraction were analytical grade reagents, while n-Hexane and Ethyl acetate used as mobile phase 98:2) in TLC tanks were double glass distilled. The Analytical TLC was done on silica gel 60 (F_{254} Merck) pre-coated Aluminium plates. The visualization of the spots on the TLC was carried out using the UV light (254 or 366 nm), iodine vapour and/or Draggendroff's reagent sprayed to view unclear or faint spots clearly.

Collection of Plant materials

The plant materials (aerial parts) were collected from Kyambogo University ward, Nakawa division, Kampala district – Central Uganda in April 2020 and dried under shade. The sample specimen was taken to Makerere University for proper identification and authentication in the Herbarium. The identification was done at the department of Botany, Makerere University where a voucher specimen (IG005) was deposited. The plant materials were thoroughly cleaned under water as described by Ogwal-Okeng *et al.*(2003) and Lamorde *et al.* (2010).

Extraction

Extraction was done in the laboratory, at the Department of Chemistry, Kyambogo University. The air-dried material (aerial parts), were crushed into powdered form by using a blender and 700 grams were obtained. The pulverized materials (powdered form) was kept in an airtight glass as advised by Sofowora, (1993). 620g of the grounded sample of the plant was soaked in a mixture of dichloromethane (DCM) and Methanol (MeOH) in the ratio of 1:1. The initial extraction required 1.3 litres of each solvent, and it was carried out after a 24-hour soaking period. The crude extract was filtered twice using a filter funnel packed with cotton wool as recommended by Yenesew *et al*. (2012). Second and third extractions were done to ensure exhaustive removal of the bioactive compounds present as described by Azmir *et al*. (2013). 52.6082 g of the sample were extracted during the concentration of the crude extract, which was carried out under low pressure with the help of a rotary evaporator to get rid of the solvent.

Qualitative Phytochemical test

Qualitative determinations of the phytochemical constituents was carried out on the methanol extracts of *I. cairica* as described by Prashant *et al*. (2011). The different extracts of the aerial parts of *I. cairica* were tested for various components by their specific tests such as; Mayer's test, Dragendroff's test, for alkaloids; Ferric chloride test, Lead acetate test for phenolic compounds, Acetic anhydride and concentrated sulphuric acid test for terpenoids; Benedict's test, Fehling's solution test for reducing sugars and Ninhydrine test for amino acids.

Test for Phenolic Compounds

a) The extract (50 mg) was dissolved in 5 ml of distilled water. A few drops of neutral 5% ferric chloride solution was added. A dark green colour indicated the presence of phenolic compounds.

b) Lead Acetate Test: The crude extract was dissolved in distilled water and 3 ml of 10% lead acetate solution was added. A bulky white precipitate indicated the presence of phenolic compounds.

Test for flavonoids (Alkaline reagent test)

An aqueous solution of the extract was treated with 10% ammonium hydroxide solution. Yellow fluorescence was seen as a sign of flavonoids. (Saeed *et al*., 2012).

Test for Alkaloids

The crude extract was dissolved in dilute hydrochloric acid and filtered. The filtrate was further tested with following reagents for the presence of alkaloids.

a) Dragendroff's test

The filtrate was treated with potassium bismuth iodide solution followed by heating. The development of an orange-red precipitate served as evidence that alkaloids were present.

b) Mayer's test

The extract was treated with potassium mercuric iodide solution. Formation of a whitish yellow or cream coloured precipitate indicated the presence of alkaloids.

Test for quinones

The extract (0.5g) was mixed with 10 ml of toluene before filtering. The filtrate was then mixed with 5ml of 10% ammonia. After shaking the mixture, pink, crimson, or violet colorations appeared, indicating the presence of quinones.

Test for Saponins

The solid extract (1.0 g) was boiled with 5 ml of distilled water and filtered off. To the filtrate, about 3 ml of distilled water was added and shaken vigorously for about 5 minutes. Frothing which persists on warming showed the presence of saponins.

Test for terpenoids

A little of the crude extract was dissolved in ethanol. To it, 1 ml of acetic anhydride was added followed by the addition of concentrated sulphuric acid (conc H_2SO_4). A change in colour from pink to violet was an indication of the presence of terpenoids (Sofowora, 1993).

Test for Amino acids

Ninhydrin Test: Extract solution was treated with Ninhydrin (Tri-ketohydrindene hy-drate) at the pH range of 4 - 8. Development of purple colour indicated the positive response for amino acids.

Test for reducing sugars

a) Fehling's test: About 0.5 g of the extract was dissolved in distilled water and filtered. The filtrate was heated with 5 ml of equal volumes of Fehling's solution A and B. Formation of a red precipitate of cuprous oxide was an indication of the presence of reducing sugars.

b) Benedict's Test: To 5 ml of the extract solution, 5 ml of Benedict's solution was added in a test tube and boiled for few minutes. Development of brick red precipitate confirmed the presence of reducing sugars.

Antimicrobial Activity Tests

Using the agar well diffusion method, the antibacterial activity of crude extract was evaluated against a few selected species of gram positive and gram-negative bacteria and fungi.

a). Anti- bacterial Activity Test

The antibacterial assay of the crude extract was carried in the biology laboratory, department of Biology, Kyambogo University. The bacterial cultures used in the study were obtained from the Department of Biology and Natural Sciences, Kyambogo University. The antibacterial activity was determined after incubation, by measuring the inhibition zone on the selected common human pathogenic bacterial strains. The antibacterial assay involves growing of the organisms (culturing), preparation of the extract to be used and carrying out the test on the selected micro-organisms (bacterial stains)

Culturing; the stock organisms were cultured in broth bacteriological water. Sub-culturing was done in Nutrient Agar by streak plate technique and incubated at 37 ^{0}C for 24 hours.

Preparation of the extract; the crude extract (1g) was weighed and dissolved in 2mls of Dimethyl sulphoxide (DMSO).

Test method; Agar well Diffusion method was used. 15 mL of the Muller Hinton Agar (MHA) was dispensed on a clean petri-dish and left to cool before the test organisms were inoculated. Four wells of about 5 mm were made using sterile cork borer. Approximately 1 ml of each; crude extract was dispensed in wells, tetracycline and DMSO as positive and negative control for antimicrobial activity respectively. After 24 hours of incubation at 37 ^{0}C, the sensitivity was determined by measuring the zone of inhibition. (Khatiwora et al., 2012).

b). Antifungal activity Tests

In this study, the antifungal activity was studied against the microorganism viz. *Aspergillus niger, Penicillum chrysogenum* and *Candida albicans.* The cultures were obtained from the standard cultures maintained in the Microbiology Department of Kyambogo University. These cultures were maintained on Sabouraud Dextrose Agar (SDA) at first being incubated at 25 ^{0}C for about 72-96 hours and then stored at 4 ^{0}C as stock cultures for further antifungal activity. Fresh cultures were obtained by transferring a loop full of cultures into sabouraud dextrose broth and then incubated at 25 ^{0}C for 72 hrs.

To test the antifungal activity, the well diffusion method was used. Here culture media was prepared in sabouraud dextrose agar (SDA) and incubated for a period is 72 hours at 25 ^{0}C. The rest of the method/procedure is as described for antibacterial activity. The concentration used for antifungal activity is 200 mg/ml (Agarry & Osho, 2005).

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration is a test aimed at determining the lowest concentration of the extract (drug compound) that will inhibit the growth and activity of the microorganisms. This was carried out within a 24hr period. The concentration for MIC was done by serial dilution of the extract by using a 2-fold method; 1/2, 1/4, 1/8, 1//16, 1/32, 1/ 64, 1/128 and 1/256 by following a procedure previously described by Rabe et al. (2002), with some modifications. An inoculum of 100µl (0.5 McFarland standard) of overnight microbial cultures of each bacterium; *E. coli*, *S. typhi*, *S. aureus*, and *P. aeruginosa* were added in each of the vails. Triplicate of each vails was made and the procedure repeated for each of the test

organisms. The value of each dilution in $\mu g/ml$ was obtained, a volume of each was taken and made up to 20 ml with a corresponding volume of nutrient agar, which together was poured aseptically into petri dish and allowed to set after swirling. The agar plates were then taken to the oven for dryness at 60 ℃ for 20 minutes. Then the microorganisms present in the broth media were then sub cultured to reduce their viable count to about 1 in 10^6. To each vial was then added 10 μl of each organism. Then the vials were incubated for 24 hours. The MIC was taken based on the inhibition of organisms added (Rabe et al., 2002).

Results and Discussion

Introduction

The 620 g of pulverized aerial parts of *Ipomoea cairica* yielded 52.6082 g of the crude extract. Therefore, the Percentage yield of the extract = 52.6082/620 x 100% = 8.4852 % per the total extractions. The extract was analyzed by TLC which showed the presence of several compounds as judged from the many spots on the TLC plate visualized under UV light (254 and 366 nm). Furthermore, the extract showed a strong UV absorption (298-380 nm), a typical of the occurrence of aromatic compounds.

Results of Qualitative Phytochemical Test

The **table. 2:** shows the results of the phytochemical tests of the various bioactive compounds of *Ipomoea cairica* methanol/DCM extract.

Table.2: Phytochemical Tests of the Bioactive Compounds

Plant Constituents	Test/Reagents	Present / Absent
Alkaloids	Dragendorff's	++
	Mayer's	+
Sterols	Sulphuric acid	+
Flavanoids	NaOH Solution	+
	H_2SO_4	+
Reducing Sugars	Fehling solution	-
	Benedicts Solution	-
Tannins	Dichromate	+
	Lead Acetate	+
	$FeCl_3$	+
Saponins	Distilled water	+
Terpenoids	Acetic acid + H_2SO_4	++
Phenols	FCl_3	+
	Lead Acetate	+
Amino Acids	Ninhydrin	-

The phytochemical compounds observed in the extracts of the plants are known to play important roles in bioactivity of medicinal plants and these secondary metabolites exert antimicrobial activity through different mechanisms. The medicinal values of medicinal plants lie in these phytochemical compounds, and as such, produce definite physiological actions on the human body. Tannins, which are part of the phytochemical constituents, have been found to form irreversible complexes with proline rich protein

resulting in the inhibition of cell protein synthesis. Parekh and Chanda (2007) reported that tannins are known to react with proteins to provide typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery. Hence supportive of the use of *I. cairica* as herbal medicine. Li and Wang (2003) reviewed the biological activities of tannins and observed that tannins have anticancer activity and can be used in cancer prevention, thus suggesting that *I. cairica* has potential as a source of important bio-active molecules for the treatment and prevention of cancer. Another secondary metabolite compound observed was alkaloid. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines. Alkaloids which are one of the largest groups of phytochemicals in *I. cairica* have amazing effects on humans and this has led to the development of powerful antinociceptive agents. It is documented that the presence of Saponins can control human cardiovascular disease and reduce cholesterol, also tannins may provide protection against microbiological degradation of dietary proteins in the semen (Just *et al*.,1998) revealed the inhibitory effect of saponins on inflamed cells. Saponin was found to be present in MeOH/DCM extract of the aerial parts of *I. cairica* and has supported the usefulness of this plant in managing inflammation (Hammuel *et al*., 2011; Ralte, 2014). Steroidal compounds present in the extracts are of importance and interest due to their relationship with various anabolic hormones including sex hormones (Quinlan *et al*., 2000), worked on steroidal extracts from some medicinal plants which exhibited antibacterial activities on some bacterial isolates. Neumann *et al*. (2004), also confirmed the antiviral property of steroids. Flavonoids, another constituent of *I. cairica* leaves and flower extracts exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, analgesic, antiallergic, cytostatic and antioxidant properties. One of the ability of flavonoids is their ability to scavenge for hydroxyl radicals, and superoxide anion radicals and thus health promoting in action.

Results of the antimicrobial test of the crude extract

Table 3, shows the results of the bioassay of the methanol /DCM crude extract of *I. cairica* on four different strains of bacteria. 1 mL of the crude extract was dispensed in wells for antimicrobial activity, followed by a positive control using tetracycline and a negative control using DMSO. The average diameter of zones of inhibition was measured in millimeter (mm).

Table. 3: Antibacterial activity of the crude extract on selected strains of bacteria

Test organisms	Diameter of zone of inhibition (mm)		
	Crude extract	Positive control (Tetracycline)	Negative control (DMSO)
S. typhi	19 ± 0.25	31 ± 0.10	0.00
E. Coli	25 ± 0.10	44 ± 0.32	0.00
P. aeruginosa	23 ± 0.12	35 ± 0.45	0.00
S. aureus	13 ± 0.05	41 ± 0.00	0.00

All the bacterial strains were found to be susceptible to the methanol/DCM extract as the zone of inhibition diameters (19 mm, 25 mm, 23 mm 13 mm for *S. typhi*, *E.coli* , *P.aeruginosa* and *S. aureus* respectively, **Table 3**) were within the range for standard antibiotics such as ampicillin (inhibition diameter 16-22 mm), doxycycline (inhibition diameter 18-24 mm) and tetracycline (inhibition diameter 18-45 mm), as reported by the Clinical and Laboratory Standards Institute (Wayne, 2010). Similarly, **Table 4** below shows the results of the bioassay of the methanol crude extract of *I. cairica* on three different strains of fungi. 1 mL of the extract of concentration 200 $\mu g/ml$ was dispensed in wells for antifungal activity, followed by a positive control using ketoconazole and a negative control using DMSO. The average diameter of zones of inhibitions was measured too, in the same unit.

Table .4: Antifungal activity of the crude extract

Fungal strains	Diameter of zone of inhibition (mm)		
	DCM:MeOH extract	Ketoconazole/positive control	DMSO Negative control
A.nigar	15 ± 0.50	22 ± 0.10	0.00
C. albicans	18 ± 0.00	20 ± 0.00	0.00
P. chrysogenum	20 ± 0.41	29 ± 0.50	0.00

The extract showed a strong UV absorption (298-380 nm), a typical of the occurrence of aromatic compounds. The results are in agreement with Convolvulaceae chemistry that the metabolites are well known for their biological and pharmacological potentials. This is due to the presence of; alkaloids, terpenoids, flavonoids, saponins, tannins and other bioactive molecules.

The antibacterial studies of the methanol/DCM extract of the aerial parts of *I.C* showed inhibition zone against bacterial strains *E. coli* (25 mm), *S. typhi* (19 mm), *P. aeruginosa* (23 mm) and *S. aureus* (13 mm) in comparison with the standard drug tetracycline with inhibition zone against *E. coli* (31 mm), *S. typhi* (44 mm), *P. aeruginosa* (35 mm) and *S aureus* (41 mm) as given in table 4.3.1.

The antifungal results of the crude extract of the aerial parts of *I.C* revealed the inhibition zone of three different strains of fungi, i.e., *A. nigar* (15mm), *C. albicans* (18 mm) and *P. chrysogenum* (20mm) in comparison with the standard drug, ketoconazole which showed the inhibition zone against; *A. nigar* (22mm), *C. albicans* (20mm) and *P. chrysogenum* (29mm) as given in **Table 4**. The bioassay of the crude extract of *I.C* provides evidence of the occurrence of the anti-pathogenic natural products against both bacteria and fungi.

Minimum inhibitory concentration (MIC)

This is the minimum concentration of an antimicrobial drug (extract) that stops the visible growth of a specific microorganism (bacteria or fungi) in a laboratory setting after an overnight (**24 h**) incubation. It reflects the **growth-inhibitory (bacteriostatic)** potential of the compound and this was determined by broth dilution.

Table. 5: Minimum Inhibitory Concentration (MIC) on bacteria

Concentration ($\mu g/ml$)	500.00	250.00	125.00	62.50	31.25	15.63	7.82	3.91
E. coli	-	-	-	+	+	+	+	+
P. aeruginosa	-	-	+	+	+	+	+	+
S. typhi	-	-	+	+	+	+	+	+
S. aureus	-	-	-	+	+	+	+	+

Where: The minus sign (-) showed no growth of organisms occurred (colourless).
The positive sign (+) indicated growth occurred (turbidity)

The methanol crude extract showed a minimum inhibitory concentration (MIC) of 125 $\mu g/ml$ on *E. coli,* 250 $\mu g/ml$ on *P. aeruginosa,* 250 $\mu g/ml$ on *S. typhi and* 125 $\mu g/ml$ on *S. aureus*. This showed that the extract was more effective against *E. coli* and *S. aureus* as compared to *P. aeruginosa* and *S. typhi*.

Conclusions And Recommendations

Conclusion:

The crude extract from the aerial parts of *I. cairica* showed good antimicrobial activities against all the selected microbial strains for both bacteria and fungi. Phytochemical analysis revealed the presence of key secondary metabolites, including phenolics, flavonoids, terpenoids, tannins, saponins and alkaloids, which may contribute to the observed biological effects.

Recommendations:

1. The aerials parts of *I. cairica* should be investigated further using the different modern separation techniques such as MPLC or HPLC to exhaustively isolate most of the phytoconstituents.
2. Additional work be done to establish the **bactericidal** activity of the agent. The MIC and MBC values would help to evaluate whether an antimicrobial compound is both bacteriostatic and bactericidal.
3. Further study is necessary to identify and isolate the possible active phytoconstituents responsible for the antimicrobial activity. The isolated phytochemicals should then be evaluated for their anti-microbial potential against some other pathogenic strains which are purposively relevant in the clinical investigations.
4. The respective chemical structures of the isolated compounds should be determined by a combination of spectroscopic & spectrometric techniques; Mass spectrometer (**MS**), Infra- red (**IR**), Ultraviolet Visible (**UV Vis**) and Nuclear magnetic resonance (**NMR**).

Disclosure statement

The authors declare no conflict of interest.

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