Original Article

Antimicrobial Activity of Garlic (*Allium sativum*) on Selected Uropathogens from Cases of Urinary Tract Infection

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Abstract

Introduction: The etiologic agents of urinary tract infection (UTI) occur among both the Gram-positive and Gram-negative bacteria including those that exhibit resistance to commonly used standard antibiotics. Both the male and female individuals can be prone to developing UTI. Garlic (*Allium sativum)* has been associated with antibacterial activity, though with scanty reports on uropathogens. This study was, therefore, carried out to determine the antibacterial efficacy of aqueous and methanol extracts of garlic (*A. sativum*) *in vitro* against six uropathogens (*Escherichia coli*, *Staphylococcus saprophyticus, Pseudomonas aeruginosa, Proteus* spp., *Klebsiella* spp., and *Serratia marcescens*) and a reference strain, *E. coli ATCC-25922*. **Materials and Methods:** The bacterial isolates were collected from the Routine Microbiology Laboratory, University College Hospital, Ibadan, and were authenticated by Gram staining and some conventional biochemical tests. The isolates were then subjected to antimicrobial susceptibility testing against both the aqueous and methanol extracts of garlic. Phytochemical screening was also carried out on the plant. **Results:** Both aqueous and methanol extracts had maximum zones of growth inhibitions of 22 mm at 200 mg/mL and 25 mm at 200 mg/mL against *E. coli,* followed by *P. aeruginosa* with zones of growth inhibitions of 21 mm and 25 mm at the same concentration. *Proteus spp.,* exhibited the lowest zones of growth inhibition of 16mm at 200mg/mL.and 10mm at 25mg/mL to methanol and aqueous extracts respectively. The minimum inhibitory concentrations MICs and MBCs of the aqueous and ethanol extracts of garlic varied for each organism. *Escherichia coli* had the MICs' of 100mg/mL and MBC of 220mg/mL while the MIC and MBC of the aqueous extract on the *Proteus spp.* are 100mg/mL and 320mg/mL respectively. The MBCs recorded were relatively higher in comparison to the MIC values. **Conclusion:** These findings showed that garlic could be of therapeutic use in the management of bacterial mediated UTI.

Keywords: Garlic (*Allium sativum*), urinary tract infection, uropathogens

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Introduction

Urinary tract infections (UTIs) are the inflammatory disorders of the urinary tract that are caused by the presence of nomadic microbes that escape the antimicrobial defense mechanisms of the urinary system. UTI is known to cause short-term morbidity in terms of fever, dysuria, and lower abdominal pain, which may result in permanent scarring of the kidney. UTI is the second most common infectious disease encountered in community clinical practice. UTIs are defined as complicated when they occur in patients with immunosuppression, including diabetes, or in the context of structural or functional abnormalities of the urinary tract.[1]

Worldwide, about 150 million people are diagnosed annually with UTI, at a total treatment cost in the billions of dollars. UTIs can be nosocomial or community acquired. Although UTI is usually treated with antibiotics, emerging antimicrobial resistance triggers our curiosity to attempt traditional medicines or herbal products as an alternative therapeutic option.[2]

Uropathogens have inherent potential to adhere to the epithelial cell of the bladder in immunocompromised status and cause UTI. In general, *Escherichia coli* is the

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most common uropathogen, responsible for approximately 80% of UTIs. Non-*E. coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas aeruginosa, Staphylococcus aureus, Serratia marcescens, Staphylococcus epidermidis, Enterobacter cloacae,* and *Enterococcus faecalis* infection are considerably more common (44% to 72%) in the subset of patients with complicated UTIs. However, little is known about associations between uropathogen species and host characteristics.[3]

Uropathogens differ in terms of the virulence factors and pathogenic mechanisms that allow them to colonize and infect the urinary tract. For example, some uropathogens, especially *Proteus* spp., produce the enzyme, urease, which hydrolyzes urea to ammonia and carbon dioxide. The release of ammonia raises the urinary pH, which favors the precipitation of urinary salts in the form of kidney or bladder stones, which frequently serve as a focus for recurrent *P. mirabilis* infection. Another mechanism for colonization of the urinary tract, particularly relevant to stones and other foreign bodies, is biofilm formation. *P. aeruginosa* is well known to be adept at biofilm formation because of genes that mediate the formation of the exopolysaccharide matrix of the biofilm. Nosocomial UTIs frequently involve organisms selected for by their antibiotic resistance mechanisms, such as vancomycin-resistant enterococci and extended-spectrum β-lactamase-producing *Klebsiella pneumoniae.*[4]

The incidence of multidrug resistance among pathogenic microbes has necessitated the need to search for newer antibiotics. In recent years, pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants to produce cost-effective remedies that are affordable to the population. Garlic (*A. sativum*) is one of the twenty most important medicinal plants with various uses throughout the world. It has been proposed as one of the richest sources of phenolic compounds and has been highly ranked regarding its contribution of phenolic compounds to human diet. Allicin, one of the active components of freshly crushed garlic homogenates, in its pure form was found to exhibit antibacterial activity against Gram-negative and Gram-positive bacteria, including multidrug resistance to enterotoxicogenic strains of *Escherichia coli.*[5]

Findings on antimicrobial activity of garlic showed that allicin (allyl-2-propene thiosulfinate), a notable flavonoid in garlic, is formed when garlic cloves are crushed and the main antimicrobial effect of allicin is due to its chemical reaction with thiol groups of various enzymes, e.g., alcohol dehydrogenase and thioredoxin reductase, which can affect essential metabolism of cysteine proteinase activity in the virulence of microbes. Garlic also contains some sulfur-containing compounds such As ajoene, diallysulfide, S-allyl-cystein, enzymes, and other nonsulfur-containing compounds including Vitamin B, proteins, minerals, saponins and flavonoids.[6]

In view of the worrisome resistance of uropathogens to many standard antibiotics, there is therefore an obvious need to search for the plant-based product that can serve as an alternative therapeutic option. This study determined the antimicrobial property of garlic (*A. sativum*) on clinical isolates of uropathogens from cases of UTI.

Materials and Methods

Collection of bacterial isolates

A total number of six clinical isolates of uropathogens (*E. coli*, *Staphylococcus saprophyticus*, *P. aeruginosa*, *Klebsiella* spp., *Proteus* spp., and *S. marcescens*) and *E. coli* ATCC-25922 control strain were obtained from the routine microbiology laboratory, University College Hospital, Ibadan. The isolates were authenticated through subculturing on to selective media, *E. coli*, *S. saprophyticus*, *P. aeruginosa*, subcultured on eosin methylene blue agar, mannitol salt agar, and cetrimide nutrient agar, while *Klebsiella* spp., *Proteus* spp., and *S. marcescens* were plated on MacConkey agar medium, followed by some confirmatory biochemical tests on the isolates as described by Bharti Arora.[7]

Collection of garlic

The cloves of garlic in its entirety were purchased from Sabo market located at Ojoo Ibadan in Oyo state and authenticated with voucher number *G123* at the Department of Pharmacognosy herbarium, University of Ibadan. The raw *A. sativum* was sliced, crushed, dried at 60°C for 7 days, in a dryer and then pulverized to coarse powder using a mechanical grinder.

Extraction of garlic (*Allium sativum*) for phytochemicals analysis

Aqueous extract

The extraction was performed by soaking 100 g of the pulverized garlic in 600 mL of distilled water for 72 h; the residue and the filtrate were obtained by filtering the aqueous mixture using Whatman No. 1 filter paper. The residue was dried on a cardboard paper and the filtrate was concentrated using rotary evaporator at 40°C to yield aqueous extract of *A. sativum*.

Methanol extract

The extraction was performed by soaking 100 g of the pulverized garlic in 600 mL of 99.5% methanol at a ratio of 1:5 for 72 h at room temperature. The residue and the filtrate were obtained by filtering the aqueous mixture using Whatman No. 1 filter paper. The residue was dried on a cardboard paper and the filtrate was concentrated using a rotary evaporator at 40°C to yield methanol extract of *A. sativum*.

Phytochemicals (qualitative tests)

Test for alkaloids

Ten milliliters of methanol was added to 0.2 g of dried garlic powder in a test tube, and after few minutes, it was filtered with Whatman filter paper no 1. Two milliliters of filtrate of 2% H_2SO_4 was taken and steam heated for 2 min. Few drops of Draggendorff reagent were added to the filtrate. An orange color or red precipitate infers the presence of alkaloids.

Test for saponin

The method described by Harbone (1998) was used. One milliliter of the extract filtrate was measured into test tube and

5 mL of distilled water was added and boiled. The presence of persistent froths after standing for few minutes indicated the presence of saponin.[8]

Test for flavonoid

A volume of 4 mL of extract solution was treated with 1.5 mL of methanol solution, the solution was warmed and metal magnesium was added to this solution, 5–6 drops of concentrated hydrochloric acid were added, and yellow color observed was an indicative of flavonoids.

Test for glycosides

A volume of 5 mL of distilled water was added to 2 g of garlic powder in a test tube, and then, it was boiled for 2 min in water bath at 100°C. The solution was filtered through No. 1 filter paper. The 1mL of extract and 0.5mL of glacial acetate was added in another test tube. Few drops of 5% ferric chloride and a few drops of concentrated H_2SO_4 were added. Appearance of greenish blue color was observed as an indication of glycoside.

Test for reducing sugars

A volume of 1 mL of Fehling's solution A and B was added to aqueous extract of *Allium sativum*. The solution was boiled in water bath for 5–10 min. The presence of nonreducing sugar was indicated by formation of brick red precipitates

Test for tannins

About 0.5 g of the extract was boiled in 10 mL of water in a test tube and then filtered. A few drop of 0.1% ferric chloride was added and observed for brownish green or a blue black colorations; blue black color was observed for garlic tannins.

Phytochemical (quantitative analysis)

Alkaloids

Quantitative determination of alkaloid was according to the methodology by Harbone. A volume of 200 cm^3 of 10% acetic acid in ethanol was added *Allium sativum* sample (2.50 g) in a 250 cm^3 beaker and allowed to stand for 4 h. The extract was concentrated on a water bath to one-quarter of the original volume followed by addition of few drops of concentrated ammonium hydroxide to the extract until the precipitation was complete immediately after filtration. After 3 h of mixture sedimentation, the supernatant was discarded and the precipitates were washed with 20 cm^3 of 0.1 M of ammonium hydroxide and then filtered using Whatman filter paper (12.5 cm). The residue was dried in an oven and the percentage of alkaloid was expressed mathematically. %Alkaloid = Weight of alkaloid/weight of sample × 100

Saponins

Saponin quantitative determination was carried out using the method reported by Ejikeme *et al*.[9] A volume of 100 cm^3 of 20% aqueous ethanol was added to 5 g of *Allium sativum* sample in a 250 cm^3 conical flask. The mixture was heated over a hot water bath for 4 h with continuous stirring at a temperature of 55°C. The residue of the mixture was re-extracted with another 100 cm^3 of 20% aqueous ethanol after filtration and heated for 4 h at a constant temperature of 55°C with constant stirring. The combined extract was evaporated to 40 cm^3 over water bath at 90°C. A volume of 20 cm^3 of diethyl ether was added to the concentrate in a 250 cm^3 separator funnel and vigorously agitated, from which the aqueous layer was recovered while the ether layer was discarded. This purification process was repeated twice. A volume of 60 cm^3 of n-butanol was added and extracted twice with 10 cm^3 of 5% sodium chloride. After discarding the sodium chloride layer, the remaining solution was heated in a water bath for 30 min, after which the solution was transferred into a crucible and was dried in an oven to a constant weight. The saponin content was calculated as a percentage: % Saponin = Weight of saponin × 100 Weight of sample.

Flavonoids

The total flavonoid content (mg/mL) was determined using aluminum chloride ($AlCl_3$) method. The assay mixture consisting of 0.5 mL of the garlic extract, 0.5 mL distilled water, and 0.3 mL of 5% $NaNO_2$ was incubated for 5 min at 25°C. This was followed by addition of 0.3 mL of 10% $AlCl_3$ immediately. Two milliliters of 1 M NaOH was then added to the reaction mixture, and the absorbance was measured at 510 nm. Quercetin was used as a standard.

Glycosides

Glycoside quantitative determination was carried out using the method reported by Amadi *et al*.[10] 2 g of *A. sativum* sample was weighed into a round bottom flask and about 200 cm^3 of distilled water was added to the sample and allowed to stand for 2 h for autolysis to occur. Full distillation was carried out in a conical flask containing 20 cm^3 of 2.5% NaOH (sodium hydroxide) in the sample after adding an antifoaming agent (tannic acid). Cyanogenic glycoside (100 cm^3), 8 cm^3 of 6 M NH4OH (ammonium hydroxide), and 2 cm^3 of 5% KI (potassium iodide) were added to the distillate(s), mixed, and titrated with 0.02 M $AgNO_3$ (silver nitrate) using a microburette against a black background. Turbidity which was continuous indicates the endpoint which determined quantity of glycoside.

Tannin

Tannin content was determined using an insoluble polyvinyl-polypyrrolidone (PVPP), which binds tannins. Concentration of 1 mg/mL of extracts was prepared in methanol and 1 mL of each extract of *A. sativum* in which the total phenolics was determined, mixed with 100 mg of PVPP, vortexed, kept at 40°C for 15 min, and then centrifuged at 3000 rpm for 10 min. In the clear supernatant, nontannin phenolics were determined the same way as that of total phenolics. Tannin content was calculated as a difference between total phenolic and nontannin content.

Screening for antibacterial activity (agar cup diffusion method)

Susceptibilities of the test organisms to the plant extract were assayed as described by Rosina *et al*. (2009). The test organisms from growth on nutrient agar incubated at 37°C for 18 h were

suspended in Normal saline (0.85% NaCl) and adjusted to match a turbidity of 0.5 (10^6 cells/ml) McFarland standard. The standardized suspension was used to inoculate the surfaces of Mueller Hinton agar plates using sterile cotton swab. 6-mm diameter wells were punched using cork borer size 6 on agar medium and filled with the desired concentrations (200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL) of the aqueous and methanol extracts. Gentamicin (10µg/mL) was used as reference standard to determine the sensitivity of the isolates while water was used as negative control for aqueous extract and methanol for methanol extract.

The plates were allowed to stand for 1 h at room temperature for extract and standard antibiotic (gentamicin) to diffuse into the agar medium and then incubated at 37°C overnight. The test was conducted in triplicate. Antibacterial activities were evaluated by measuring diameter of zones of growth inhibition.[11]

Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was also determined using broth dilution method ranging from 25mg/mL to 100mg/mL. This was achieved by weighing 2 g of each dried extract into 10 ml of sterile distilled water to attain a stock concentration of 200 mg/mL. Thereafter, double-fold dilution was done to attain different concentrations (200 mg/mL, 100 mg/mL, 50 mg/mL, and 25 mg/mL). A volume of 2 mL of each diluted extract (aqueous and ethanol) of the aforementioned concentrations was dispensed into 2 mL of sterile Mueller Hinton broth in a set of 4 test tubes to obtain a final concentrations of 100 mg/mL, 50 mg/mL, 25 mg/mL, and 12.5 mg/mL, respectively.

Each test organism was inoculated into the labeled tube by adding 0.1 mL of the standardized bacterial suspension except the control; the tubes were incubated at 37°C for 18 h. The MIC was taken as the lowest concentration that prevented visible growth.

Determination of minimum bactericidal concentration

From the test tubes used in the determination of MIC, the tubes that showed no visible growth including the tube that gave MIC were subcultured onto freshly prepared Mueller Hinton agar and incubated at 37°C for 48 h. The least concentration at which the organisms did not recover and grow was taken as the MBC.

Results

Table 1 shows the results of the qualitative phytochemical screening of garlic (*A. sativum*) for secondary metabolites.

Alkaloids were found to be the highest in quantity of phytochemical (7.1%), followed by tannin (4.6%), saponin (4.2%), flavonoids (2.14%), and glycoside (1.1%) as shown in Table 2.

The aqueous and methanol extracts exhibited varied antimicrobial activities against the six uropathogens tested as shown in [Figures 1 and 2]. Both aqueous and methanol extracts had maximum zones of growth inhibitions of 22 mm at 200 mg/mL and 25 mm at 200 mg/mL against *E. coli* followed by *P. aeruginosa* with zones of growth inhibitions of 21 mm and 25 mm at the same concentration. *Proteus* spp. exhibited the lowest zones of growth inhibition of 16mm at 200mg/mL and 10 mm at 25 mg/mL for aqeous extract.The six isolates and control strain were susceptible to both aqueous and methanol extract of *A. sativum* as shown in Table 3.

Table 1: Qualitative phytochemical profile of aqueous and methanol extracts of *Allium sativum*

Phytochemical constituents	Aqueous extract	Methanol extract
Alkaloids	+	+
Saponins	+	+
Flavonoids	+	+
Glycosides	+	+
Reducing sugar	−	−
Tannins	+	+

+: Present, −: Absent

Table 2: Quantitative determination of phytochemical constituents of *Allium sativum*

Phytochemical constituents	Quantitative analysis
Alkaloids	7.10±0.04
Saponins	4.20±0.03
Flavonoids	2.14±0.04
Glycosides	1.08±0.02
Reducing sugar	Absent
Tannins	4.60±0.04

Values are expressed as mean±SEM (*n*=3). SEM: Standard error of mean

The MICs of the aqueous and methanol extracts of garlic varied for each organism *E. coli* had 100 mg/mL and 220 mg/mL, respectively, while *Klebsiella spp*., were 200 mg/mL and 80 mg/mL and Proteus spp. had 100 mg/mL and 180 mg/mL, respectively. The MBCs recorded were relatively higher in comparison to the MIC values as shown in Table 4.

Discussion

Antibacterial evaluation of aqueous and ethanol extracts of garlic elicited a significant antibacterial potency against the test organisms. The phytoconstituents of garlic have long been known and its antibacterial properties have been widely reported (Gibbons, 2010) and occur as secondary metabolites. Their presence in garlic was confirmed in this study reflecting different chemical classes, namely, glycoside, alkaloids, saponins, tannins, and flavonoids. They exerted antibacterial activity as bioactive compounds working synergistically to inhibit the uropathogens tested.[12]

The susceptibility of these uropathogens to garlic extracts in this study is an indication of the therapeutic potentials of garlic against the etiologic agents of UTI. Alkaloids were found to be the highest in quantity of phytochemicals (7.1%), followed by tannin (4.6%), saponin (4.2%), flavonoids (2.14%), and

Table 3: The diameter of zone of growth inhibition produced by the aqueous and methanol extracts of *Allium sativum* at different concentrations against the test organisms

Test organisms	200		100		50		25		+control water	−ve control gentamicin
	AE	ME	AE	ME	AE	ME	AE	ME		
Escherichia coli	22	25	18	21	16	19	10	12	-	18
Serratia marcescens	20	23	17	20	14	18	10	16	-	14
Pseudomonas aeruginosa	21	25	17	22	12	19	10	15	-	-
Staphylococcus saprophyticus	19	20	17	18	12	16	10	12	-	20
Klebsiella spp.	18	22	18	19	12	16	10	13	-	18
Proteus spp.	16	27	18	22	11	16	10	12	-	20
Escherichia coli ATCC-25922	24	28	18	22	14	16	10	12	-	24

AE: Aqueous extract, ME: Methanol extract

Table 4: Minimum inhibitory concentration and minimum bactericidal concentration of garlic extract against the test organisms (mg/mL)

Test organisms	MIC (mg/mL) aqueous	Methanol	MBC (mg/mL) aqueous	Methanol
Escherichia coli	100	50	220	100
Serratia marcescens	160	100	200	120
Pseudomonas aeruginosa	120	80	200	125
Staphylococcus saprophyticus	120	50	250	100
Klebsiella spp.	140	40	200	80
Proteus spp.	180	100	320	240
Escherichia coli ATCC-25922	200	160	240	200

MIC: Minimum inhibitory concentration, MBC: Minimum bactericidal concentration

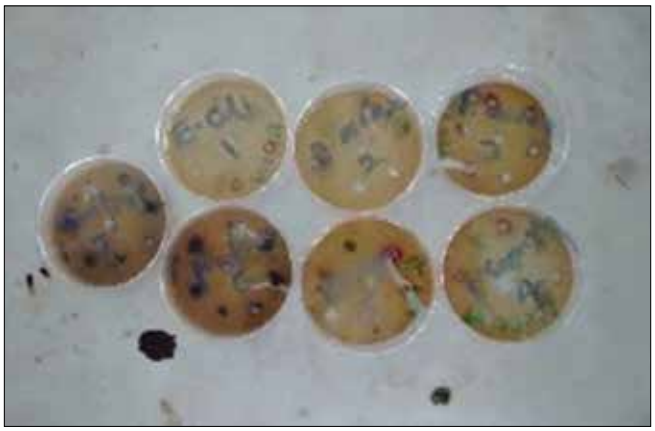

Figure 1: The sensitivity of the isolates of uropathogens tested against aqueous extract of *Allium sativum*

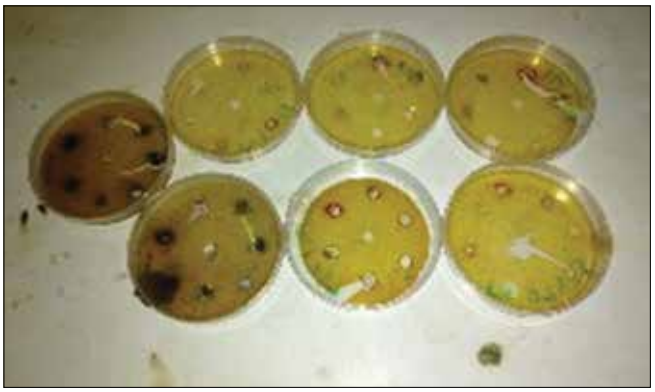

Figure 2: The sensitivity of the isolates of uropathogens tested against methanol extract of *Allium sativum*

glycoside (1.1%). These secondary metabolites, singly or in combination, could be attributed to the antimicrobial and pharmacologic that have been associated with garlic. Both aqueous and methanol extracts had maximum zones of growth inhibitions of 22 mm at 200 mg/mL and 25 mm at 200 mg/mL against *E. coli,* followed by *P. aeruginosa* with zones of growth inhibitions of 21 mm and 25 mm at the same concentration. *Proteus* spp. exhibited the lowest zones of growth inhibition of 16mm at 200mg/mL and 10 mm at 25 mg/mL for aqeous extract. It was observed that standard strain *E. coli ATCC-25922* was more sensitive to the garlic extract studied at varied concentrations. The extract showed broad spectrum of activity; both Gram-negative and Gram-positive bacteria tested were sensitive to aqueous and methanol extract of garlic at varied concentrations. This is in consonance with the study of Mukhtar *et. al.* (2001) on the antibacterial activities of aqueous and methanol extract of *Pistia stratiotes*.[13]

The varied MICs of the garlic extract on the uropathogens tested correlate with the reports of Debnath that microorganisms do exhibit varied level of susceptibility to plant extract.[14] The methanol extract has been observed to be more potent than the aqueous extract, which is in conformity with the findings by El-Mahmood and Amey on antibacterial activity of *Parkia biglobosa* bark extract against some microorganisms associated with urinary infections.[15] This accounts for the influence of the solvent system, which also affects the

antibacterial activity of the crude extract. The varied zones of inhibition produced by the garlic extract against the test organisms indicated the potency of the active ingredient in them. The MBCs recorded were relatively higher than the MIC values, suggesting that the extract inhibited growth of the test organisms while being bactericidal at higher concentrations. The isolates' variation in their sensitivity to aqueous and methanol extracts could be attributed to the inherent strain variation, metabolic mechanisms, intrinsic properties that are related to the permeability of their cell surface to the extract, polarity of solvents of extractions and prevalent immediate environmental milieu. Turmeric (*Curcuma longa)* had also been reported to have relatively similar antimicrobial potential in comparison with garlic in traditional Indian medicine, but its pharmacological potential is still under investigation. This agrees the study of Jaber and Al-Mossawi on susceptibility of some resistant bacteria to garlic extract.[16] From this study point of view, the renewed interest in exploiting herbal medicine as sources of potentially important new pharmaceutical substances is a step in the right direction.

Conclusion

This study justifies the antibacterial property of garlic(Allium sativum) on UTI caused by the uropathogens tested. There is a need, therefore, for the isolation and purification of the active compound in the extracts that could be formulated into antibacterial drug.

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Conflicts of interest

There are no conflicts of interest.

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