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Antibacterial Properties of Methanolic and Aqueous Extracts of Some Plants against Some Enterobacteriaceae Species

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Abstract

The extracts of six medicinal plants namely *Terminalia chebula* fruits, *Commiphora myrrha* gum, *Solenostemma argel* leaves, *Rutagraveolens* aerial parts, *Cistanche phelypaea* aerial parts and *Striga hermonthica* stem used in traditional Sudanese medicine for the treatment of gastrointestinal tract infections were selected to evaluate their potential antibacterial activity. The antibacterial activity of methanolic and aqueous extracts of these plants were determined by agar diffusion technique *in vitro* against 20 clinical isolates (2 were *Salmonella typhi*, *5 Proteus mirabilis*, 4 *Escherichia coli*, 5 *Pseudomonas aeruginosa*, 3 *Staphylococcus aureus*, one was *Salmonella paratyphi B*) and 5 standard bacterial strains (*Staphylococcus aureus* ATCC 25923), *Bacillus subtilis* (NCTC 8236), *Escherichia coli* (ATCC 25922), *Salmonella typhi* (ATCC1319106) and *Klebsiella pneumoniae* (ATCC 35657) at a concentration of 100 mg/ml. Of all plants methanolic and aqueous extracts of *T. chebula* fruits were the most active with clinical isolates and standard bacterial strains showed relatively high antibacterial activity against most of the tested microorganisms with the diameter of inhibition zones ranging between 20 and 24 mm, whereas the methanolic extract of *Commiphora myrrha* showed high antibacterial activity against *Proteus mirabilis* and *Escherichia coli* clinical isolate (1Z = 20 mm). *Solenostemma argel leaves* was found moderately effective against *S. aureus* (ATCC 25923 ((1Z = 18 mm) but did not show any activity against all tested clinical isolates bacteria. Most susceptible Gram-negative clinical Isolates bacteria were *Escherichia* coli and *Proteus mirabilis*. Most susceptible Gram negative standard bacteria were *Bacillus subtilis* (NCTC 8236) and *Escherichia coli* (ATCC 25922) and least susceptible Gram negative bacterium was *Klebsiella pneumoniae* (ATCC35657). In Gram positive standard bacteria, most susceptible was *S. aureus* (ATCC 25923). Antibiotics was used as standards drug for antibacterial assay. The present study reveals potential use of these plants for developing new antibacterial compounds against gastrointestinal tract pathogenic microorganisms.

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Antibacterial Activity, Medicinal Plants, Clinical Isolates and Standard Pathogenic Bacteria

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1. Introduction

The use of medicinal plants to treat human diseases has its roots in pre-historical times. Despite of the modern advances achieved in the field of synthetic chemistry, the most efficient drugs available had derived directly or indirectly, related from plant kingdom. Indigenous communities had long used plant extracts to treat illness. Many of these extracts had shown effective action with new bioactive compounds being extracted and screened every year. These extracts had also proven to be good sources of therapeutic agents to the treatment of infectious diseases [1] [2]. Sudan throughout its long history has accumulated a rich body of empirical knowledge of the use of medicinal plants for the treatment of various diseases.

The antimicrobial activity of *Terminalia chebula* against a variety of bacterial and fungal strains has been well investigated by several researchers [3]-[20].

The antibacterial and fungal activities of *Solenostemma argel* [21] [22], *Comiphora myrrha* [24]-[27] and *Cistanche phelypaea* [28] have been received considerable.

The main aim of this study is that due to increasing concerns about the development of antimicrobial resistance among pathogenic bacteria, so alternative strategies are sought that do not use antibiotics to reduce pathogenic bacteria from foods and patients.

2. Materials and Methods

The fruits of *Terminalia chebula*, *Commiphora myrrha* gum, *Cistanche phelypaea* root, *Striga hermonthica* stem, and *Solenostemma argel* leaves were purchased from Omdurman market on the basis of undocumented reports for antibacterial activity. The plants were identified by one author Prof. Hatil H. Elkamali and by comparison with herbarium of the Department of the Botany Department, Faculty of Science and Technology, Omdurman Islamic University. The dried plants were pulverized with a mechanical grinder.

Two hundred gramsofall plants was macerated separately with 50% methanol (MeOH) in a conical flask for 24 hours. Mother liquor (crude MeOH extract) was filtered and evaporated to dryness. The dry crude extract was sterilized. All extracts were stored dry in sterilized containers at room temperature until used for antibacterial testing. At the time of testing, the extracts were prepared at a concentration of 100 mg/ ml in methanol.

Dried plant material (100 g) was ground to a fine powder. It was macerated with distilled water (1 L), and left for 24 hours at room temperature. The mother liquor was filtered. The filtrate, thus obtained was evaporated to complete dryness at room temperature. The residue thus obtained was aqueous plant extract.

The antibacterial activity was tested by well-agar diffusion method [29] [30]. 250 ml of sterilized nutrient agar was used for testing. The inoculum size of each test organism was adjusted to suspension of 10^6 cells. 2 ml of 24 hours old culture of bacteria were added to 250 ml of melted cooled test agar and after through mixing, approximately 20 ml of this seeded agar were poured into 10 cm diameter presterilized petri dishes and allowed to solidify. Four wells (10 mm in diameter) were bored in the agar using a sterile cork borer and the agar discs were removed. 0.1 ml aliquots of the prepared extract was placed into a well with a pipette and the plate was held for 2 hours at room temperature for diffusion of extract into agar. Subsequently, the plate was incubated at 37˚C for 24 hours. After incubation, the diameter of the zones of inhibition were measured to the nearest mm.

The minimum inhibitory concentration (MIC) was determined by a modification of the agar diffusion method [29]. A two-fold serial dilution of each extract was prepared in methanol to achieve a decreasing range of extract concentrations from 100 mg/ml to approximately 3.125 mg/ml. A 0.1 ml sample of each dilution was introduced into duplicate wells in a nutrient agar plate already seeded with bacterial cells as described above. Incubation was at 37˚C for 24 hours. The lowest concentration of extract showing a zone of inhibition was taken as the MIC.

Multidisc for antimicrobial susceptibility testing from Axiom laboratories, New Delhi, India for were used as

positive control and methanol as a negative control.

3. Results and Discussion

Some bacterial strains showed a fairly high degree of sensitivity to the methanolic extracts of *T. chebula* fruits such as *P. mirabilis* isolates no. (3, 4), *E. coli* isolates no. (5, 10), *P. aeruginosa* (7, 8, 13) and *S. aureus* isolate no. (12) (1Z = range between 20 - 28 mm). Standard bacteria, *S. aureus* (ATCC 25923) and *B. subtilis* (NCTC 8236) showed promising result (1Z = range between 18 - 24 mm), bacterial species: *S.typhi* (ATCC1319106), *K. pneumoniae* (ATCC 35657) and *E. coli* (ATCC 25922) were found to be resistant (**Table 1**). Methanol extracts of *Commiphora myrrha* gum showed high antibacterial activity against *P. mirabilis* isolate no. (3) and *E. coli* isolate no. (6) (1Z = 20 mm). *Solenostemma argel leaves* was found effective against *S. aureus* (ATCC 25923 ((1Z = 18 mm) (**Table 1**). Methanol extract of *R.graveolens* aerial parts, *Cistanche phelypaea* roots, *Striga hermonthica* stem, were found ineffective against all tested Gram-positive and Gram-negative bacteria.

All bacterial species were found to be resistant against aqueous extracts of all plants except *T. chebula* fruits (**Table 1**). Aqueous extract of *Terminalia chebula* fruits was found moderately active to *S. typhi B* isolate no. (17), *S. typhi* isolate no. (1), *E.coli* isolate no. (6), *P. aeruginosa* isolate no. (8) and *P.mirabilis* isolate no. (19) (1Z = range between 16 - 14 mm) (**Table 1**). *B. subtilis* (NCTC 8236) and *S. aureus* (ATCC 25923) showed moderate result (1Z = range between 16 - 14 mm). Bacterial species: *E. coli* (ATCC 25922), *S. typhi* (ATCC1319106), *K. pneumonia* (ATCC 35657) were found to be resistant (**Table 1**).

Solenostemma argel leaves was found effective against *S.aureus* (ATCC 25923) (1Z = 18 mm), but did not showed any activity against all tested clinical isolates bacteria (**Table 1**). Methanolic and aqueous extracts of *R.graveolens* aerial parts, *Cistanche phelypaea* roots and *Striga hermonthica* stem, were found ineffective against all tested bacteria (**Table 1**).

The antibacterial activity related to known antibiotics was calculated. The results are shown in **Table 2**.

Ceftizoxime was found effective against *P. mirabilis* isolate no. (2) and *P. aeruginosa* isolate no. (13) at concentration 30 mcg, and moderate effectively was observed against *P. aeruginosa* isolate no. (15) and *E. coli* (ATCC 25922). Cefotaxime (CF) showed promising result against *P. mirabilis* isolate no. (4) at concentration 30 mcg (**Table 2**). It was showed no antibacterial activity against *S. aureus* (ATCC 25923) and *S. typhi* (ATCC-1319106). Ciprofloxacin (CP) showed a fairly high degree of sensitivity to *E. coli* isolate no. (6), *P. aeruginosa* isolate no. (7), *S. typhi B* isolate no. (7) and *B. subtilis* (NCTC 8236) at concentration 5 mcg, and demonstrated antibacterial activity against *E.coli* (ATCC 25922), *K. pneumoniae* (ATCC 35657) (**Table 3**).

Tetracycline (TE) showed good results against *E. coli* isolate no. (6) and *E. coli* (ATCC 25922) at concentration 30 mcg. Amikacin (AK) showed high antibacterial activity against *E. coli* isolate no. (10) at concentration 30 mcg, and moderate effectively was observed against *E. coli* isolate no. (5), *P. aeruginosa* isolate no. (7) and *E. coli* (ATCC 25922). It was showed no antibacterial activity against *S. aureus* (ATCC 25923) and *S. typhi* (ATCC1319106). *S. aureus* (ATCC 25923), *B. subtilis* (NCTC 8236), *E. coli* (ATCC 25922), *S. typhi* (ATCC-1319106) and *K. pneumoniae* (ATCC 35657) was found to be co-trimoxazole resistant. Co-Trimoxazole was found effective against *S. typhi B* isolate no. (17) at concentration 25 mcg and showed good results against *E. coli* isolate no. (9), *S. aureus* (14) and *P. aeruginosa* isolate no. (15).

Piperacillin/Tazobactam (TZP) showed good results against *E. coli* isolate no. (6) at concentration 100/10 mcg, and found to be ineffective against all standard bacteria except *B. subtilis* (NCTC 8236). Chloramphenicol (CH) showed promising result against *B. subtilis* (NCTC 8236) at concentration 30 mcg, and moderate effectively was observed against *E. coli* isolate no. (9) *S. aureus* isolate no. (14), *P. aeruginosa* isolate no. (15) and *P. mirabilis* isolate no. (19), whereas *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *S. typhi* (ATCC1319106) and *K. pneumoniae* (ATCC 35657) was found to be chloramphenicol resistant.

Methanolic extracts of *T. chebula* fruits showed high antibacterial activity against clinical isolates and moderate to some of standard bacteria, about 60% of *P. mirabilis* revealed promising sensitivity, 50% of *S. aureus* clinical isolates showed good activity and surprise sensitivity of standard *S. aureus* (ATCC 25923) has become resistant to all known antibiotics has posed a threat already for a number of years. It has thus become apparent that new antimicrobial agents will continue to select for resistant strains from the pool of bacteria which continuously undergo genetic change [31]. *T. chebula* can serve as a starting point in future drug development aimed at the production of a new safe, effective and bio-accessible therapeutic agent.

The polar extracts of *T. chebula* fruits, (methanol) exhibited promising antibacterial activity against most *Pro-*

Table 1. Antibacterial activity of methanolic and aqueous extracts of studied plants against clinical isolates and standard bacteria.

No	Clinical isolates bacteria	Extract	*Terminalia chebula*	*Commiphora myrrha*	*Rutagraveolens*	*Cistanche phelypaea*	*Striga hermonthica*	*Solenostemma argel*
1	*Salmonella typhi*	MeOH	16	2	-	-	-	2
		H_2O	14	-	-	-	-	4
2	*Proteus mirabilis*	MeOH	18	2	2	-	2	2
		H_2O	8	-	-	-	-	6
3	*Proteus mirabilis*	MeOH	20	20	12	2	2	2
		H_2O	8	6	-	8	-	2
4	*Proteus mirabilis*	MeOH	28	2	2	2	2	4
		H_2O	4	-	-	-	-	4
5	*Escherichia coli*	MeOH	24	2	-	-	2	2
		H_2O	10	-	-	-	-	6
6	*Escherichia coli*	MeOH	10	20	-	2	4	-
		H_2O	14	6	-	6	-	4
7	*Pseudomonas aeruginosa*	MeOH	20	-	-	2	-	2
		H_2O	6	-	-	4	-	10
8	*Pseudomonas aeruginosa*	MeOH	20	4	2	2	4	-
		H_2O	14	-	-	4	-	6
9	*Escherichia coli*	MeOH	2	2	2	2	2	-
		H_2O	4	-	-	6	-	4
10	*Escherichia coli*	MeOH	28	-	-	2	2	-
		H_2O	10	-	-	4	-	-
11	*Staphylococcus aureus*	MeOH	4	12	6	2	-	-
		H_2O	8	6	-	10	-	6
12	*Staphylococcus aureus*	MeOH	20	2	-	-	2	-
		H_2O	4	-	-	-	-	4
13	*Pseudomonas aeruginosa*	MeOH	20	6	6	4	6	4
		H_2O	-	2	-	-	-	-
14	*Staphylococcus aureus*	MeOH	2	2	6	-	2	-
		H_2O	4	6	-	-	-	4
15	*Pseudomonas aeruginosa*	MeOH	10	-	2	-	2	-
		H_2O	12	-	-	-	-	-
16	*Proteus mirabilis*	MeOH	2	-	2	-	-	-
		H_2O	12	6	4	-	-	-
17	*Salmonella paratyphi B*	MeOH	2	2	11	2	2	-
		H_2O	16	6	-	8	-	4
18	*Salmonella typhi*	MeOH	10	2	-	-	2	-
		H_2O	12	6	-	-	-	2
19	*Proteus mirabilis*	MeOH	2	-	-	2	2	2
		H_2O	14	6	-	-	-	6
20	*Pseudomonas aeruginosa*	MeOH	-	-	-	-	2	-
		H_2O	12	6	-	2	-	8
			Standard bacteria					
1	*Staphylococcus aureus* (ATCC 25923)	MeOH	24	-	8	10	8	18
		H_2O	14	-	-	-	-	6
2	*Bacillus subtilis* (NCTC 8236)	MeOH	18	8	10	8	8	4
		H_2O	16	6	-	-	-	-
3	*Escherichia coli* (ATCC 25922)	MeOH	6	6	8	2	4	4
		H_2O	6	-	-	-	-	-
4	*Klebsiella pneumonia* (ATCC 35657)	MeOH	4	2	2	2	2	2
		H_2O	10	-	-	-	-	4
5	*Salmonella typhi* (ATCC1319106)	MeOH	12	2	4	2	2	2
		H_2O	10	6	-	-	-	2

Values are the mean of four replicates; -: no inhibition. Tested concentration of extracts: 100 mg/ml (0.1 ml/well). Methanol did not show any inhibitory activity. Sensitive: >18, intermediate: 14 - 18 mm, Resistant: <14 mm, -: No inhibition zone. ATCC: American Type Collection Culture-NCTC: National Collection Type Culture.

Table 2. Antibacterial activity of antibiotics-Gram (-ve) against clinical isolates.

	Antibiotics Clinical isolates	AS 20 mcg	BA 25 mcg	CF 30 mcg	TZP 100/10 mcg	CH 30 mcg	CP 5 mcg	CI 30 mcg	TE 30 mcg	OF 5 mcg	GM 10 mcg	AK 30 mcg	GF 5 mcg
1	*Salmonella typhi*	2	-	14	10	8	10	14	2	10	10	10	10
2	*Proteus mirabilis*	8	10	2	12	8	10	20	2	10	10	10	10
3	*Proteus mirabilis*	-	-	-	-	-	-	-	-	-	-	-	-
4	*Proteus mirabilis*	-	-	20	-	-	10	-	-	-	-	-	-
5	*Escherichia coli*	6	-	12	12	10	-	-	2	8	14	14	14
6	*Escherichia coli*	-	6	8	16	-	20	-	20	10	12	10	8
7	*Pseudomonas aeruginosa*	-	10	4	12	-	24	-	4	18	10	14	18
8	*Pseudomonas aeruginosa*	-	-	-	-	-	-	-	-	-	-	-	-
9	*Escherichia coli*	4	16	6	12	14	6	12	-	6	-	-	12
10	*Escherichia coli*	-	-	-	12	8	14	-	10	14	18	20	14
11	*Staphylococcus aureus*	-	-	-	-	-	-	-	-	-	-	-	-
12	*Staphylococcus aureus*	2	-	-	6	-	-	-	-	6	-	-	16
13	*Pseudomonas aeruginosa*	10	10	10	10	10	12	20	6	10	8	8	10
14	*Staphylococcus aureus*	-	14	2	-	16	8	12	-	4	-	-	10
15	*Pseudomonas aeruginosa*	-	14	4	-	16	10	16	-	6	16	8	14
16	*Proteus mirabilis*	-	-	-	-	-	-	-	-	-	-	-	-
17	*Salmonella paratyphi B*	6	26	8	12	4	30	-	12	18	14	8	16
18	*Salmonella typhi*	-	-	-	-	-	-	6	-	-	10	6	-
19	*Proteus mirabilis*	2	12	2	12	16	4	10	-	4	-	-	10
20	*Pseudomonas aeruginosa*	-	-	-	-	-	-	-	-	-	-	-	-

Values are the mean of four replicates; -: no inhibition. Tested concentration of extracts: 100 mg/ml (0.1 ml/well); Methanol did not show any inhibitory activity. Sensitive: >18, intermediate: 14 - 18 mm, Resistant: <14 mm, -: No inhibition zone; Multidisk for Antimicrobial Susceptibility Testing For Gram Negative Isolates: -Ampicillin/sulbactam AS 20 mcg, Co-Trimoxazole BA 25 mcg, Cefotaxime CF 30 mcg; Piperacillin/Tazobactam TZP 100/10 mcg, Chloramphenicol CH 30 mcg, Ciprofloxacin CP 5 mcg, Ceftizoxime CI 30 mcg, Tetracycline TE 30 mcg, Ofloxacin OF 5 mcg, Gentamicin GM 10 mcg, Amikacin AK 30 mcg, Gatifloxacin GF 5 mcg.

Table 3. Antibacterial activity of antibiotics-Gram (-ve) against standard bacteria.

Antibiotics Standard bacteria	AS 20 mcg	BA 25 mcg	CF 30 mcg	TZP 100/10 mcg	CH 30 mcg	CP 5 mcg	CI 30 mcg	TE 30 mcg	OF 5 mcg	GM 10 mcg	AK 30 mcg	GF 5 mcg
Salmonella typhi (ATCC1319106)	4	-	-	-	6	-	-	10	-	-	-	-
Klebsiella pneumonia (ATCC 35657)	4	10	4	10	10	16	-	10	10	10	10	10
Bacillus subtilis (NCTC 8236)	6	-	8	20	20	20	-	10	10	10	10	10
Escherichia coli (ATCC 25922)	-	-	6	-	-	14	16	18	12	14	16	14
Staphylococcus aureus (ATCC 25923)	-	-	-	-	-	-	-	-	-	-	-	-

ATCC: American Type Collection Culture. NCTC: National Collection Type Culture; Values are the mean of four replicates; -: no inhibition. Tested concentration of extracts: 100 mg/ml (0.1 ml/well); Methanol did not show any inhibitory activity. Sensitive: >18, intermediate: 14 - 18 mm, Resistant: <14 mm, -: No inhibition zone; Multidisk for Antimicrobial Susceptibility Testing For Gram Negative Isolates: -Ampicillin/sulbactam AS 20 mcg-Co-Trimoxazole BA 25 mcg-Cephaexin PR 30 mcg-Piperacillin/Tazobactam TZP 100/10 mcg-Chloramphenicol CH 30 mcg-Ciprofloxacin CP 5 mcg-Ceftizoxime CI 30 mcg-Tetracycline TE 30 mcg-Ofloxacin OF 5 mcg-Gentamicin GM 10 mcg-Amikacin AK 30 mcg-Gatifloxacin GF 5 mcg.

teus mirabilis and *P. aeruginosa* species and the aqueous extract showed the lowest activity. The most polar solvent results in a greater yield extract of natural antioxidant compounds because most of them are polar compounds such as flavonoids. Solvent with higher polarity are effective for extraction of natural antioxidants [32]. The antibacterial activity of *T. chebula*, does not come as a surprise, since sesquiteprene lactones, flavonoids and essential oils in general have been associated several times with antibacterial effect [33] [34].

The aqueous extracts of all plants except *T. chebula* do not possess significant antibacterial activity both against standard and clinical strains. These results account for why the Sudanese people do not frequently use these plants as a remedy, but may possess anti-inflammatory activities.

T. chebula can serve as a starting point in future drug development aimed at the production of a new safe, effective and bio-accessible therapeutic agent. Plants were able to develop new, faster and natural antimicrobials and then man-made remedies, and that is explaining why plants succeed in its fighting against microbes since millions of years while human failed [35].

Methanolic extracts of *Commiphora myrrha* gumshowed potent antibacterial activity against clinical isolates *P. mirabilis* isolate no. (3) and *E. coli* isolate no. (6). Furanosesquiterpenes, the active compounds in *C. myrrh* essential oil, possessed a significant antiseptic property, also be the characteristic components of pharmaceutical myrrh [26] [36]. All standard bacteria showed resistant to methanolic and aqueous extracts. Most of standard bacteria showed antibiotic-resistant. The reason for different sensitivity between Gram-positive and Gram negative bacteria could be ascribed to the morphological differences between these microorganisms. Gram-negative bacteria have an outer phospholipids membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes, while porins constitute a selective barrier to the hydrophilic solutes. The Gram-positive bacteria should be more susceptible since they have only an outer peptidoglycan layer which is not an effective permeability barrier [37].

Antibiotic-resistant *S. aureus* (ATCC 25923) showed promising activity against *S. argel* leaves. *Staphylococcal* disease has been a perennial problem in the hospital environment since the beginning of the antibiotic era, hospital strains of *S. aureus* were usually resistant to a variety of different antibiotics. A few strains were resistant to all clinically useful antibiotics except vancomycin, and vancomycin-resistant strains were increasingly reported [38].

Methanolic extracts of *T. chebula* fruits showed high antibacterial activity against *E. coli* and *S. aureus* which showed antibiotic-resistant to most antibiotic and similarly to amikacin, and *S. aureus* (12) showed antibiotic-resistant to most antibiotic and similarly to gatifloxacin. These plants could serve as useful sources for new antimicrobial agents and the fruits extracts of *T. chebula* may be used as remedy against various diseases without any side effects [39].

Staphylococcus has changed from the status of a non-pathogen to that of an opportunistic pathogen. Although once regarded as an important opportunistic innocuous member of the normal skin flora, *Staphylococcus* now recognized as an important opportunistic pathogen. It is routinely found on the skin and in the hospital environment, with prevalence on the skin surface of 85% - 100%. *Staphylococcus* was found excessively on damaged skin surface in normal persons [40].

Of the all *S. aureus* isolates, only one was more sensitive to themethanolic extract of *T. chebula* fruits and effective more than Gram negative antibiotics, also *T. chebula* showed high activity against standard *S. aureus* (ATCC 25923) were it showed antibiotic-resistant (**Table 1**). Study of the synergistic interaction of active phyto-compounds with antibiotics is required to exploit these potential plant extracts in the combination therapy of infectious diseases caused by multi drug-resistant organisms [41].

75% of *P. aeruginosa* showed high sensitivity toward methanolic extract, *Escherichia coli* which showed antibiotic-resistant to most antibiotic and similarly to amikacin. In the last decades, prevalence and outbreaks of the multi-drug resistant bacterial strains has been increasingly documented throughout the world. At present most clinical isolates of *E. coli* areconsidered as highly resistant to most commercially known antibiotics [35]. Out of the 20 clinical isolates from the infected stool, 2 were *S. typhi*, 5 *P. mirabilis*, 4 *E. coli*, 5 *P. aeruginosa*, 3 *S. aureus*, one was *S typhi B*. This is in line with fact that *P. mirabilis*, *E. coli* and *P. aeruginosa* are the most commonly taxa encountered contaminants of stool in foods. All bacterial species were found to be resistant against aqueous extracts of all studied plant species (except *T. chebula*). Compared to the most reference antibiotics, the spectrum of antibacterial activity of *T.chebula* was found to be clearly superior (**Table 4** & **Table 5**).

The phytochemistry of *Terminalia chebula*, *Solenostemma argel*, *Commiphora myrrha*, *Rutagraveolens* and *Cistanche phelypaea* have been received considerable interest [42]-[46]. Hexane and dichloromethane extracts

Table 4. Minimum inhibition zone (MIC) and antibacterial activity of crude methanolic extractives—clinical isolates and STD.

Plants	Clinical Isolates	Concentrations					
		0.5 mg	0.3 mg	0.15 mg	0.05 mg	0.03 mg	0.01 mg
Terminalia chebula	*Proteus mirabilis* 3	20	16	20	18	4	4
	Proteus mirabilis 4	28	10	10	10	-	-
	Escherichia coli 5	24	4	2	2	-	-
	Escherichia coli 10	28	4	4	2	-	-
	Pseudomonas aeruginosa 13	20	14	14	14	4	2
	Pseudomonas aeruginosa 7	20	14	14	12	4	2
	Pseudomonas aeruginosa 8	20	-	-	-	-	-
	Staphylococcus aureus 12	20	6	6	2	-	-
Commiphora myrrha	*Proteus mirabilis* 3	20	4	4	4	-	-
	Escherichia coli 6	20	6	4	2	-	-
Standard bacteria							
Terminalia chebul	*Bacillus subtilis* (NCTC 8236)	18	12	8	8	-	-
	Escherichia coli (ATCC 25922)	6	2	-	-	-	-
	Staphylococcus aureus (ATCC 25923)	24	4	4	4	-	-
Soleno stemma argelarge	*Staphylococcus aureus* (ATCC 25923)	24	4	4	4	-	-

Values are the mean of four replicates. Tested concentration of extracts: 100 mg/ml (0.1 ml/well).methanol did not show any inhibitory activity. Sensitive: >18, intermediate: 14 - 18 mm, Resistant: <14 mm, -: No inhibition zone; *Standard bacteria; S.a: *Staphylococcus aureus* (ATCC 25923) B.s: *Bacillus subtilis* (NCTC 8236), E.c: *Escherichia coli* (ATCC 25922), K.n: *Klebsiella pneumoniae* (ATCC 35657), Sa.t: *Salmonella typhi* (ATCC-1319106); Sensitive: >18, intermediate: 14 - 18 mm, Resistant: <14 mm, -: No inhibition zone; ATCC: American Type Collection Culture; NCTC: National Collection Type Culture.

Table 5. Minimum Inhibition zone (MIC) and antibacterial activity of crude aqueous extractives—clinical isolates and STD.

Plants	Clinical Isolates	Concentrations		
		100%	50%	25%
Terminalia chebula	*Salmonella typhi*.No 1	14	-	-
	Escherichia coli .No 6	14	10	8
	Pseudomonas aeruginosa. No 8	14	14	12
	Salmonella paratyphi B. No 17	16	8	4
	Proteus mirabilis. No 19	14	-	-
Standard bacteria				
Terminalia chebula	*Bacillus subtilis* (NCTC 8236)	16	20	10
	Salmonella typhi (ATCC1319106)	10		
	Klebsiella pneumonia (ATCC 35657)	10	-	-
	Staphylococcus aureus (ATCC 25923)	14	6	2

Values are the mean of four replicates. Tested concentration of extracts: 100 mg/ml (0.1 ml/well).methanol did not show any inhibitory activity. Sensitive: >18, intermediate: 14 - 18 mm, Resistant: <14 mm, -: No inhibition zone; ***Standard bacteria;** S.a: *Staphylococcus aureus* (ATCC 25923) B.s: *Bacillus subtilis* (NCTC 8236), E.c: *Escherichia coli* (ATCC 25922), K.n: *Klebsiella pneumoniae* (ATCC 35657), Sa.t: *Salmonella typhi* (ATCC-1319106); Sensitive: >18, intermediate: 14 - 18 mm, Resistant: <14 mm, -: No inhibition zone; ATCC: American Type Collection Culture; NCTC: National Collection Type Culture.

of *Terminalia chebula* have shown more antibacterial compounds than acetone extract indicating the non-polar character of the antibacterial compounds [42]. Nine flavonoidal compounds were extracted from stem aqueous extract of *Solenostemma argel*. The kaempferol was more effective as antibacterial agent [43]. Three compounds known for their antibacterial effects of *Commiphora myrrha*: 2-fluorodiphenylmethane, tribenzo-1, 2,3,4,5,6anthracene and 2-bromo-1-(4-bromophenyl)ethanone [44]. Orlanda and Nascimento mention that *Rutagraveolens* could be used as a natural source for antibacterial compounds and possible applications in the pharmaceutical industry [45]. Several phenylethanoid glycosides isolated from *Cistanche* spp. showed an antibacterial activity against *Staphylococcus aureus* [46]. Therefore, the results obtained in our research work match the results obtained by other researches.

4. Conclusions

The most antibacterial active plant was *T. chebula* fruits. Of all extracts the methanolic and aqueous extracts of *T. chebula* fruits was the most active, whereas, the aqueous extracts of all plants do not possess significant antibacterial activity both against standard and clinical strains.

Methanol extracts of *C. myrrha* gumand *T. chebula* fruits showed high antibacterial activity against *P. mirabilis, E. coli, P. aeruginosa, S. aureus, S. typhi and S. typhi B* clinical isolates, whereas the aqueous extracts of all plants were found to be ineffective against all tested bacteria Gram-positive and Gram-negative *T. chebula* fruitswhich showed moderate effect against *P. mirabilis, E. coli, P. aeruginosa S. typhi and S. typhi B.*

Some standard bacterial species showed a fairly high degree of sensitivity to the methanolic extracts of *T. chebula* fruits against *B. subtilis* (NCTC 8236) and *S. aureus* (ATCC 25923), whereas the aqueous extracts of all plants were found to be ineffective against all tested bacteria except *T. chebula* fruits showed moderate potency against *B. subtilis* (NCTC 8236).

The antibacterial screening of the different extracts (methanol and aqueous) was performed against standard and clinically isolated bacterial strains. The highest antibacterial activity was found in methanolic extracts, the lowest one was found in aqueous extract.

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