

Original Article

IN VITRO SUSCEPTIBILITY OF ISOLATED *SHIGELLA FLEXNERI* AND *SHIGELLA DYSENTERIAE* TO THE ETHANOLIC EXTRACTS OF *TRACHYSPERMUM AMMI* AND *PEGANUM HARMALA*

IBRAHIM SIDDIG HAMID¹, IKRAM MOHAMED ELTAYEB^{*}

University of medical sciences and technology (UMST)
Email: kramela_07@yahoo.com

Received: 29 Aug 2018 Revised and Accepted: 04 Dec 2018

ABSTRACT

Objective: This study represents the first attempt to investigate the antimicrobial activity of *Peganum harmala*, and *Trachyspermum ammi* seeds extract against the isolated bacillary dysentery-causing microorganisms.

Methods: *T. ammi* and *P. harmala* were extracted by 96% ethanol using Soxhlet apparatus. The extracts were screened for their phytochemical constituents. Their antimicrobial activity against the isolated dysentery-causing microorganisms was evaluated using the agar diffusion method.

Results: The antimicrobial activity result showed that, the two isolated bacteria, *Shigella flexneri*, and *Shigella dysenteriae* were found to be sensitive to the extract of *T. ammi* seed with inhibition zones up to 25 mm, compared to the inhibition zone of 20 mm produced by Gentamycin standard drug, this is mainly due to the presence of the different phytochemical in the extract such as tannin, flavonoids, terpenoids which are well known for their antimicrobial effects. The two isolated bacteria were found to be insensitive (zero mm) to *P. harmala* extract, Amoxicillin, and Amoclan (Amoxicillin+clavulanic acid) standard drugs, this is due to the fact that, the phytochemicals constituents of *P. harmala* possess the antagonistic effect to each other's. Addition to; these bacteria became resistant to both Amoxicillin and Amoclan.

Conclusion: From the results it concludes, *T. ammi* seeds extract had a considerable level of antimicrobial activity against bacillary dysentery-causing microorganisms resistant to Amoxicillin and Amoxicillin+clavulanic acid drugs.

Keywords: Trachyspermu, ammi, Peganum, Harmala, Shigella, Flexneri, Dysentery

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DOI: <http://dx.doi.org/10.22159/ijpps.2019v11i1.29411>

INTRODUCTION

The medicinal plants are very important to the health of individuals and communities worldwide; this is mainly due to that, most of the drugs derived from herbs are free of side effects or reactions [1, 2]. The herbs have medicinal quality provide rational means for the treatment of many diseases, which are considered of difficult cure [3].

In Sudan the mixtures of *Peganum harmala* and *Trachyspermum ammi* seeds are traditionally used for the treatment of bacillary dysentery. *Trachyspermum ammi* commonly known as Ajwain is a very valued medicinally plant belongs to Apiaceae family. It is an abundantly branched annual herb [4]. It used traditionally for treatment of gastrointestinal ailments, lack of appetite and bronchial problems [5] as well used as antiseptic, antipyretic, febrifugal and in the treatment of typhoid fever [6, 7] as antimicrobial agent [8], digestive stimulant [9], antihypertensive, hepatoprotective, antispasmodic, broncho-dilating [10], antilithiasis, diuretic [11], abortifacient [12], galactogogic [13], antiplatelet-aggregatory [14], anti-inflammatory [15], antitussive [16], antimalarial [17], gastro-protective [18], anthelmintic [19], detoxification of aflatoxins [20], and ameliorative effects [21].

Peganum harmala belongs to the family Zygophyllaceae is a perennial plant growing up to 0.8m [22] has been reported to have an antibacterial activity [23], an abortifacient [24], and in large quantities, it can reduce spermatogenesis and male fertility in rats [25]. It also used to treat depression, recurring fevers [26], kill algae, bacteria, intestinal parasites and molds [27]. Its endogenous alkaloids have been proven to be involved in Parkinson's disease [28].

Bacillary dysentery, which is caused by invasive bacteria mainly by one or more types of *Shigella* species such as *Shigella dysenteriae* and *shigella flexneri* [29] is mainly found in hot countries like Sudan. Poor hygiene and sanitation increase incidence of dysentery by spreading microorganism causing it through food and water which are contaminated by human feces [30].

Bacillary dysentery symptoms include sudden onset of high fever and chills, abdominal pain, cramps and bloating, the urgency to pass stool, weight loss, and dehydration. The symptoms start within 2 to 10 d, and if left untreated it can lead to serious complications include delirium, convulsions, and coma. A serious infection can be fatal within 24 h [31]. The principal aim of this study was to investigate the antimicrobial activity of *Peganum harmala* and *Trachyspermum ammi* seeds extracts against isolated *Shigella flexneri* and *Shigella dysenteriae*, bacillary dysentery-causing microorganism (Shigellosis) whereas these microorganisms are becoming resistant to antimicrobial drugs, addition too many side effects caused by synthetic drugs.

MATERIALS AND METHODS

Plant material collection and preparation

Dried seeds of *P. harmala* and *T. ammi* were collected from the botanical garden at Omdurman area, Sudan, and authenticated by taxonomist at the Department of Aromatic and Medicinal Plants Research in the Sudan National Research Center. The voucher specimens identified as IBRAHIM (3), December-2017) and; IBRAHIM (4), December-2017) were deposited at the Department of Pharmacognosy, Faculty of Pharmacy, University of Medical Science and Technology.

Preparation of *P. harmala* and *T. ammi* extracts

The coarsely powdered seeds of *P. harmala* and *T. ammi* were extracted with 96% ethanol using Soxhlet extractor apparatus. Extraction was carried for six to eight hours till the color of solvent at the last siphoning time returned colorless. The solvent was evaporated under reduced pressure using a rotary evaporator apparatus, then the extracts were complete dryness, and the percentage yields were calculated. The extracts stored at 4 °C for further investigation.

Phytochemical screening test

The phytochemical constituents of the extracts were detected using the standard procedure as described by Trease and Evans [32] and Sofowora [33].

Collection and preparation of the isolated bacteria

Feces samples were collected from Yastabsheron Hospital/Sudan and the bacteria were isolated and identified by a microbiologist at the National Centre for Research/Sudan. The isolated bacteria were cultured in agar media and incubated for 24 h to allow bacterial growth and then the antimicrobial test was conducted.

Antimicrobial bioassay

Antimicrobial testing was carried out using the agar diffusion method with some minor modification. Nutrient agar medium was used as a growth medium and was placed in an autoclave using the following conditions 37 °C and 15 lbs pressure for 15 min for sterilization. The medium (20 ml) was then poured in a Petri dish under aseptic conditions and (0.2 ml) of the intended microorganism was introduced into the prepared medium. After the medium had solidified, discs containing the prepared extracts were added in each plate. The diameters (mm) of the inhibition zones were measured [34]. The experiment was repeated with serial dilution of plant extracts (100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml and 6.25% mg/ml) to determine the minimum inhibitory concentration (MIC) of the extracts. All experiments were conducted in triplicate.

RESULTS

Percentage Yields

T. ammi and *P. harmala* seeds extract produced by using of 96% ethanol were found to be of slightly difference in their percentages yields (table 1).

Phytochemical screening

The phytochemical screening of *T. ammi* and *P. harmala* extracts (table 2) revealed the presence of saponins, tannins, cardiac glycosides, terpenoids, steroids and reducing sugars in both plants. The alkaloids are present in the extract of and *P. harmala*.

Antimicrobial activity

The antimicrobial activity of *T. ammi* seeds extracts, against the isolated microorganisms (fig. 1) showed the highest activity (25, 24 mm) against the two tested bacteria, it was slightly highest against *Shigella flexneri* than *Shigella dysenteriae*. Whereas, the two bacteria were found to be insensitive to the *P. harmala* extract, Amoxicillin, and Amoxicillin+Clavulanic acid. Gentamycin showed the same and high activity (20 mm) against both bacteria.

Table 1: Percentage yield of *T. ammi* and *P. harmala* seeds extracts

Plant extract	Weight of sample	Weight of extract	Yield %
<i>T. ammi</i>	100g	33.07	33.07%
<i>P. harmala</i>	100g	32.78	32.78%

Table 2: Qualitative phytochemical screening of *T. ammi* and *P. harmala* seeds extracts

Secondary metabolites	<i>T. ammi</i>	<i>P. harmala</i>
Alkaloids	-ve	+ve
Saponins	+ve	+ve
Tannins	+ve	+ve
Cardiac glycoside	+ve	+ve
Flavonoids	-ve	-ve
Sterols	+ve	+ve
Terpenoids	+ve	+ve
Reducing sugars	+ve	+ve
Compound reducing sugars	-ve	-ve

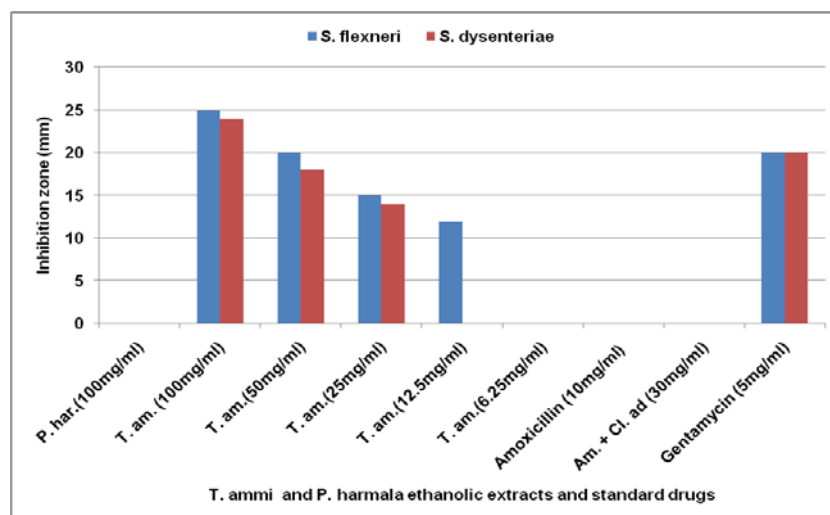


Fig. 1: Antimicrobial activity of *T. ammi* and *P. harmala* seeds ethanolic extracts against isolated bacteria, *P. har* = *P. harmala*, *T. am.* = *T. ammit*, *Am.+Cl. ad* = Amoxicillin+Clavulanic acid. The values represent the mean of the three determinants

DISCUSSION

The medicinal plants contain a wide range of active secondary metabolites which may include synergies one another to give a

beneficial pharmacological effect [1]. The preliminary phytochemical analysis of *T. ammi* and *P. harmala* seeds (table 2) revealed the presence of saponins, tannins, cardiac glycosides, terpenoids, steroids and reducing sugars in both assessed plants as reported in

these two plants [35, 36]. Additions to alkaloids were found in the extract of *P. harmala* compatible with their present in plant [36]. Flavonoids show the negative result which is incompatible with the previous study [36], due to different in climate and cultivation process of the plant sample.

Regarding the antimicrobial activity results of *T. ammi* and *P. harmala* seeds extracts against the isolated bacillary dysentery-causing bacteria (fig. 1), both isolated bacteria; *Shigella dysenteriae* and *Shigella flexneri* were found to be highly sensitive (24-25 mm) towards the extract of *T. ammi* with MIC value of 25 mg/ml against *Sh. dysenteriae* and 25 mg/ml against *Sh. flexneri*. On the other hand, *P. harmala* seeds extract was found to be inactive against the two tested bacteria

It is noteworthy that the isolated *Sh. dysenteriae* and *Sh. flexneri* were found to be resistant towards both Amoxicillin and Amoxicillin+clavulanic acid standard drugs, and highly sensitive towards Gentamycin (fig. 1). The activity order was higher in *T. ammi* extract (24-25 mm) than that of Gentamycin standard drug (20 mm) against both bacteria.

The results of *T. ammi* extract against two assessed bacteria, which were well known for their causing of bacillary dysentery [29] is comply with antimicrobial activity of the plant [9] and confirm it is traditionally used for the treatment of bacillary dysentery [5, 6]. However, the result of *P. harmala* extract against both bacteria is incompatible with it is an antibacterial effect [24] which is in line with the specify effect of the plant against microorganisms and agrees with the report that the effect of an agent varies with target species [37].

Although the two assessed plants were nearly having similarity in general phytochemicals (table 2); they appeared to be of highly different in their activity towards the tested bacteria (fig. 1), this is mainly due to the fact that, compounds found in each plant are the difference. Addition to that, the tested bacteria were also found to differ in their susceptibility towards the three standard drugs used (fig. 1), this is in agreement with the report that the effect of an agent varies with target species [37].

CONCLUSION

The study has revealed that *T. ammi* ethanolic extract possessed antimicrobial activity as stated in the literature. From the results, it appeared that Amoxicillin and Amoxicillin+clavulanic acid were completely insensitive for both *Shigella flexneri* and *Shigella dysenteriae*. *T. ammi* extract exhibited the highest antimicrobial activity, which was higher than that of Gentamycin. This justified their use as antimicrobial in Sudanese traditional medicine.

ACKNOWLEDGMENT

Authors are thankful to Yastabshiron Hospital/Sudan for providing the stool infected sample.

AUTHORS CONTRIBUTIONS

The experimental part of the work and writing of the manuscript was done by the first author Mr. Ibrahim Siddig Hamid. Design of the work, interpretation of data and correction of the manuscript was done by Dr. Ikram Mohamed Eltayeb.

CONFLICT OF INTERESTS

Declared none

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