IJPT
0000-0000/03/21-22-24
IRANIAN JOURNAL OF PHARMACOLOGY & THERAPEUTICS
Copyright © 2003 by Razi Institute for Drug Research (RIDR)
IJPT 2:22-24, 2003

RIDR

Evaluation of Antibacterial Activity of Tribulus terrestris L. Growing in Iran

SAIED KIANBAKHT and FERESHTEH JAHANIANI

Department of Pharmacology, Faculty of Medicine, Arak University of Medical Sciences, Arak, Iran (S.K.); Department of Pharmacology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran (F.J.)

Received May 6, 2003; Revised June 10, 2003; Accepted June 25, 2003

This paper is available online at *http://ijpt.iums.ac.ir*

ABSTRACT

Tribulus terrestris is used as a urinary anti-infective in folk medicine. To validate this use, the *in vitro* antibacterial activity of methanolic extracts of different parts (fruits, stems plus leaves and roots) of *T. terrestris* L. growing in Iran was evaluated against four reference bacteria by broth dilution assay and agar diffusion assay. The MIC value of the methanolic extracts of fruits and stems plus leaves against all bacteria was 2 mg/mL and the MIC value of roots against *S. aureus*, *E. faecalis* and *E. coli* was 4 mg/mL and the MIC value of roots against *P. aeruginosa* was 2 mg/mL. In agar diffusion assay, the methanolic extracts of all parts of the plant showed considerable activity against all bacteria.

Keywords: *Tribulus terrestris, Antibacterial activity*

The traditional medicinal methods, especially the use of medicinal plants, still play a vital role to cover the basic health needs in the developing countries and moreover the use of herbal remedies has risen in the developed countries in the last decades. In this connection, plants continue to be a rich source of therapeutic agents. The active principles of many drugs are found in plants or are produced as secondary metabolites. The remarkable contribution of plants to the drug industry was possible, because of the large number of phytochemical and biological studies all over the world. Herbal remedies used in folk medicine provide an interesting and still largely unexplored source for the creation and development of potentially new drugs for chemotherapy which might help overcome the growing problem of resistance and also the toxicity of the currently available commercial antibiotics. Therefore, it is of great interest to carry out a screening of these plants in order to validate their use in folk medicine and to reveal the active principle by isolation and characterization of their constituents.

Tribulus terrestris L. (Zygophllaceae) is an annual plant distributed in warm regions of Asia, Africa, Europe, America and Australia [1-3]. *T. terrestris* is used in folk medicine as tonic, aphrodisiac, analgesic, astringent, stomachic, anti-hypertensive, diuretic, lithontriptic and urinary anti-infective [4].

The main constituents of *T. terrestris* are saponins, diosgenins, alkaloids and amides [5-7].

One of the uses of *T. terrestris* is in urinary infections. The ethanolic extracts of Yemeni *T. terrestris* has demonstrated no detectable antibacterial activity against any of the reference bacteria [8]. However, the ethanolic extracts of all parts (fruits, stems plus leaves and roots) of Turkish *T. terrestris* showed activity against all reference bacteria [9]. Moreover, ethanolic extracts of the fruit and leaf of Indian *T. terrestris* were active against *Escherichia coli* and *Staphylococcus aureus* [10] Regarding that the antibacterial activity of Iranian *T. terrestris* has not been studied, the in vitro antibacterial activity of methanolic extracts of different parts of *T. terrestris* growing in Iran was evaluated by broth dilution assay and agar diffusion assay.

MATERIALS AND METHODS

Chemicals. Methanol (99.5%) (Merck); Ampicillin sodium (Merck); Gentamicin hydrochloride (Merck).

Plant material. Tribulus terrestris used in this study was collected at the end of November from lands around the Qom–Arak highway 10 km away from Arak in the Markazi province in Iran. Herbarium specimen of *T. terrestris* (voucher number 1237) is preserved in the herbarium of department of botany of Arak University, Arak, Iran.

Extraction procedure. Dried and powdered fruits, stems plus leaves and roots (100 g) were extracted with 85% methanol in a Soxhlet apparatus and the extracts

were dried in vacuo by a Heidolph model Laborta 4001 rotary evaporator.

Media. The media used in broth dilution assays were brain-heart infusion broth (BHI broth) (Merck) and Mueller-Hinton broth (MH broth) (Merck) and the medium used in agar diffusion assay was Mueller- Hinton agar (MH agar) (Merck).

Test bacteria. *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853.

ANTIBACTERIAL ACTIVITY TESTS

Broth dilution assay. Broth dilution assay was carried out according to Murray et al [11]. A loopful of the bacterial culture from the slant was inoculated in the nutrient broth (BHI broth as well as MH broth) and incubated at 37±1ºC for 24 hours. The fresh broth (20 mL) was seeded with 0.25 mL of the 24-hour broth cultures and a two-fold serial dilution method was followed as below. The dried plant extracts were dissolved in 85% methanol to obtain an 80 mg/mL solution and sterilized by filtration through a 0.45 µm membrane filter. A 0.2 mL solution of the material was added to 1.8 mL of the seeded broth and this formed the first dilution. 1 mL of this dilution was diluted further with 1 mL of the seeded broth to produce the second dilution, and the process was repeated until six dilutions were obtained. A set of tubes containing only seeded broth was kept as control and 85% methanol controls were also maintained. After incubation for 24 h at 37±1ºC the last tube with no visible growth of the bacteria was taken to represent the minimum inhibitory concentration (MIC) of the test sample which is expressed in mg/mL. Moreover, the broth dilution assay was carried out with ampicillin and gentamicin in BHI broth as well as MH broth in the same way as the extracts and the MIC values of ampicillin and gentamicin were determined.

Agar diffusion assay. The dried plant extracts were dissolved in 85% methanol to a final concentration of 40 mg/mL and sterilized by filtration through a 0.45 µm membrane filter. Agar disc diffusion assay was then carried out according to Murray et al [11] using an inoculum containing 106 bacterial cells on MH agar plates (1 mL inoculum/plate). The discs (diameter, 6 mm) were each impregnated with 50 µl of extract (2 mg/disc) at a concentration of 40 mg/mL and placed on the inoculated agar and incubated at 37ºC for 24 h. Each test was carried out in triplicate with controls. Moreover, filter paper discs containing the antibiotics ampicillin and gentamicin were used as positive controls.

RESULTS

The MIC values of all extracts in BHI broth were identical to the MIC values of extracts in MH broth. The MIC value of the methanolic extracts of fruits and stems plus leaves against all bacteria was 2 mg/mL and the MIC value of roots against *S. aureus*, *E. faecalis* and *E. coli* was 4 mg/mL and the MIC value of roots against *P. aeruginosa* was 2 mg/mL (Table 1 and Table 2). The MIC values of ampicillin and gentamicin in BHI broth were identical to the MIC values of ampicillin and gentamicin in MH broth. The MIC value of ampicillin against *S. aureus* was 0.25 µg/mL and the MIC value of ampicillin against *E. faecalis* and *E. coli* was 8 µg/mL. The MIC value of gentamicin against *S. aureus* and *E. coli* were 0.5 µg/mL and 1 µg/mL respectively, while the MIC value of gentamicin against both *E. faecalis* and *P. aeruginosa* was 10 µg/mL (Table 3 and Table 4).

In agar diffusion assay, the methanolic extracts of all parts of the plant showed considerable activity against all bacteria (Table 5). Further, in agar diffusion assays, *S. aureus*, *E. faecalis* and *E. coli* were sensitive to both ampicillin and gentamicin and *P. aeruginosa* was sensitive to gentamicin (Table 6).

Table 1. The MIC values in mg/mL of *T. terrestris* extracts in brain-heart infusion broth.

Methanolic extract	S. aureus	E. faecalis	E. coli	P. aeruginosa
Fruits	2	2	2	2
Stems plus leaves	2	2	2	2
Roots	4	4	4	2

Table 2. The MIC values in mg/mL of *T. terrestris* extracts in Mueller-Hinton broth.

Methanolic extract	S. aureus	E. faecalis	E. coli	P. aeruginosa
Fruits	2	2	2	2
Stems plus leaves	2	2	2	2
Roots	4	4	4	2

Table 3. The MIC values in µg/mL of ampicillin and gentamicin in brain-heart

Antibiotics	S. aureus	E. faecalis	E. coli	P. aeruginosa
Ampicillin	0.25	8	8	
Gentamicin	0.5	10	1	10

Table 4. The MIC values in µg/mL of ampicillin and gentamicin in Mueller-Hinton broth.

Antibiotics	S. aureus	E. faecalis	E. coli	P. aeruginosa
Ampicillin	0.25	8	8	
Gentamicin	0.5	10	1	10

Table 5. Antibacterial activity of *T. terrestris* extracts against bacteria in Mueller-Hinton agar disc diffusion assay (disc diameter, 6 mm; disc potency, 2mg/disc).

Methanolic extract	Inhibition zone diameter (mm)			
	S. aureus	E. faecalis	E. coli	P. aeruginosa
Fruits	27.8	14.6	25.7	26.2
Stems plus leaves	28.2	15.7	22.3	23.8
Roots	26.8	14.3	20.9	21.3

Table 6. Antibacterial activity of ampicillin and gentamicin against bacteria in Mueller-Hinton agar disc diffusion assay (disc diameter, 6 mm; disc potency, 10 µg/disc)

Antibiotics	Inhibition zone diameter (mm)			
	S. aureus	E. faecalis	E. coli	P. aeruginosa
Ampicillin	29	31	21	
Gentamicin	19	15	18	17

DISCUSSION

It seems that the MIC values of the extracts as well as ampicillin and gentamicin do not depend on the type of the media used and also the antibacterial activity of roots is lower than fruits and stems plus leaves. The MIC values of the extracts are in the mg/mL range, while the MIC values of ampicillin and gentamicin are in the µg/mL range. Furthermore, the potencies of the discs of extracts are 2 mg/mL, but the potencies of the discs of ampicillin and gentamicin are 10 µg/mL. Thus, the extracts of all parts of the plant have activity against all reference bacteria, but their antibacterial activities are much lower than ampicillin and gentamicin.

As the Yemeni *T. terrestris* had no detectable antibacterial activity against any of the reference bacteria [8] and all parts (fruits, stems plus leaves and roots) of Turkish *T. terrestris* showed activity against all reference bacteria [9], but only fruit and leaf of Indian *T. terrestris* were active against exclusively *E. coli* and *S. aureus* [10], it can be argued that antibacterial activity of Iranian *T. terrestris* against the reference bacteria is similar to Turkish *T. terrestris*.

The activity of the plant against both gram-positive and gram-negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds or simply general metabolic toxins in the plant. Since Iranian *T. terrestris* demonstrates activity against the most prevalent gram-negative bacteria in urinary infections namely *E. coli*, the use of the plant as a urinary antiinfective is validated. Furthermore, it may help to discover new chemical classes of antibiotics that could serve as selective agents for the maintenance of animal or human health and provide biochemical tools for the study of infectious diseases.

REFERENCES

1. Topia MO, Giordano MA, Gueper HG. An Outbreak of Hepatogenous Photosensitization in Sheep Grazing Tribulus terrestris in Argentina. *Vet Hum Toxicol* 1994;**36(4):**311-3.
2. Abeywickrama K, Bean GA. Toxigenic Aspergillus Flavus and Aflatoxins in Sri Lankan Medicinal Plant Material. *Mycopathologia* 1991;**113:**187-90.
3. Kostova I, Dinchev D, Rentsch GH, Dimitrov V, Ivanova A. Two New Sulfated Furostanol Saponins from Tribulus terrestris. *Z Naturforsch [C]* 2002;57(1-2):33-8.
4. Ody P. The Complete Guide Medicinal Herbal. London: Dorling Kindersley; 2000. p. 223.
5. Evans WC. Trease and Evans Pharmacognosy. 15th ed. Edinburgh: W.B. Saunders; 2002. p. 481.
6. Yan W, Ohtani K, Kasai R, Yamasaki K. Steroidal Saponins from Fruits of Tribulus terrestris. *Phytochemistry* 1996;**42:**1417-22.
7. Borke CA, Stevens GR, Garriqan MJ. Locomotor Effects in Sheeps of Alkaloids Identified in Australian Tribulus terrestris. *Aust Vet J* 1992;**69:**163-5.
8. Awadh-Ali NA, Jülich WD, Kusnick C, Lindequist U. Screening of Yemeni Medicinal Plants for Antibacterial and Cytotoxic Activities. *J Ethnopharmacol* 2001;**74:**173-9.
9. Abbasoglu U, Tosun F. Antimicrobial Activity of Tribulus terrestris L. Growing in Turkey. *Hacettepe Universitesi Eczacilik Fakultesi Dergisi* 1994;**14(2):**81-5.
10. Williamson EM. Major Herbs of Ayurveda. China: Churchill-Livingstone; 2002. p. 312.
11. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolke RH. Manual of Clinical Microbiology. 7th ed. Washington: ASM; 1999. p. 1527-39.

Address correspondence to: Saied Kianbakht, PhD, Arak Faculty of Medicine, Kooye Golestan, Arak, Iran. E-mail: saiedkianbakht@hotmail.com