Antibacterial and Antifungal Activity of *Corchorus olitorius* L. (Molokhia) Extracts

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Received: 15 March 2007
Accepted: 26 May 2007

Abstract

The present study describes the antimicrobial activity of 3 extracts of *Corchorus olitorius* L. (Molokhia) (Tiliaceae), collected from Doğancı, Güzelyurt, North Cyprus. Successive petroleum ether, methanol and ethyl acetate+water extracts of *C. olitorius* leaves were tested (in vitro) for their antibacterial and antifungal activities by agar-well diffusion assay. All extracts displayed varied levels of antibacterial or antifungal activity. The petroleum ether extract exhibited antibacterial effect against all of the bacteria tested and the diameter of zones varied between 14-20 mm. The petroleum ether extract of *C. olitorius* leaves presented a good activity against *Escherichia coli, Staphylococcus aureus* and *Yersinia enterocolitica*, 20 mm, 19 mm and 19 mm, respectively. The ethyl acetate+water extract presented a good activity against *Geotrichum candidum* and *Botrytis cinerea*, 20 mm and 12 mm, respectively. The results obtained in this study appear to confirm the antibacterial and antifungal potential of *C. olitorius* leaves, as well as its usefulness in the treatment of diseases that may be as a result of infection.

Key Words: antimicrobial activity, *Corchorus olitorius* L. (Molokhia), disc diffusion method

INTRODUCTION

There is an ever continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action due to the alarming increase that has been witnessed in the incidence of both new and re-emerging infectious diseases. A further big concern is the development of resistance to the antibiotics in current clinical use.

Corchorus olitorius Linn. (Tiliaceae) is an annual herb with slender stems. *C. olitorius* (Jute) is an important green leafy vegetable in many tropical areas including Egypt, Sudan, India, Bangladesh, in tropical Asia in such countries as the Philippines and Malaysia, as well as in tropical Africa, Japan, South America, the Caribbean and Cyprus. In West African countries particularly Ghana, Nigeria and Sierra Leone, where staple diets consist of starchy food-stuffs such as rice, cassava, maize and yams, leafy vegetables are used to complement such staple foods [1]. It is cultivated to provide bark for the production of fibres (Jute) and its mucilaginous leaves are used in food as a vegetable [2, 3].

Jute is commonly known as Long-fruited jute, Tossa jute, Jute mallow, Jew' s mallow, Bush okra and West African sorrel. It is also called Moroheiya in Japan, Molehiya in Cyprus and Saluyot in the Philippines [1].

The crop is an excellent source of vitamins A and C, fiber, minerals including calcium and iron and other micronutrients. *C. olitorius* L. is extensively consumed as a "healthy vegetable" in Japan, because it contains abundant carotenoids, vitamin B_1, B_2, C and E, and minerals [4].

Jute contains high levels of all essential amino acids except methionine which is at marginal concentrations [1]. It has high protein levels and is, along with other leafy species, the main source of dietary protein in many tropical countries [1].

The seeds are used as a purgative and leaves as demulscent, diuretic, febrifuge (infusion) and in chronic cystitis and dysuria. On preliminary analysis, seeds have been found to contain cardenolide glycosides [5]. The methanol extracts of *C. olitorius* seeds have shown a broad spectrum of antibacterial activity [6].

The cardenolide glycosides of *Corchorus* spp have already been reported; erysimoside, olitoriside, corchoroside A and coroliside were isolated as constituents of the seeds of tossa jute by Nakamura et al. [7].

Based on the fact that there is no scientific research reporting on the antifungal activity of this plant, we decided to take this opportunity to screen for both its potential both antibacterial and antifungal activity. The aim of this study was to evaluate the in vitro antibacterial and antifungal activity of three different extracts from *C. olitorius* leaves, collected from Cyprus.

MATERIAL AND METHOD

Plant material

Plant material was collected from the Doğancı Village, (Güzelyurt, North Cyprus) in July 2005 when it harvested period. The voucher specimen was deposited in the Herbarium of the Faculty of Science and Arts, Eskişehir Osmangazi University, Eskişehir-Turkey (ESOGU).

The plant samples were transported in polypropylene bags. Plant samples were dried under shade at room temperature and then ground in a mortar into fine powders. These were then stored in airtight containers at room temperature.

Plant extracts

The extraction process was completed at three steps. First, 30 g of sample in powder was extracted with 250 ml of petroleum ether by using Soxhlet equipment for 8 h Following evaporation of the petroleum ether, 5 g of plant material whose lipids had previously been removed, was again extracted with 50 ml of 70% methanol at 40 °C in a shaker (Gerhardt Germany) at room temperature for 2 h and then filtered. Next, 200 ml of water + ethyl acetate (1:3) was added to the remaining solid material and after shaking for 1 h filtered. Following filtration with Whatman filter paper (No 1), all extracts were concentrated and evaporated to dryness *in vacuo* at 55 °C using a rotary evaporator [8]. The yields from the different extracts were weighed, recorded and dissolved in dimethyl sulphoxide (DMSO) to a final concentration of 100 mg/ml. The extracts were then stored at 4 °C and further used for an antimicrobial activity test.

Test Microorganisms

The extracts inhibitory effects on a total of 22 microbial species including 12 bacteria, 8 molds and 2 yeasts, were used as test organisms in this study. These microbial strains were obtained from the American Type Culture Collection (ATCC; Rock-ville, MD,USA); Northern Regional Research Laboratory (NRRL, USDA, Peoria, Illinois/USA), Anadolu University, Department of Biology Eskişehir/TURKEY and Eskişehir Osmangazi University, Department of Biology Eskişehir/TURKEY. They included gram-positive bacteria: *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* NRRL B-3711, *Bacillus subtilis* NRRL B-209, *Micrococcus luteus* NRRL-B 1018, *Enterococcus faecium* NRRL B-3502, *E. faecalis* ATCC 29212; and gram-negative bacteria: *Proteus vulgaris* NRRL B-123, *Pseudomonas gingeri* 3146, *Escherichia coli* ATCC 25922, *Enterobacter aerogenes* NRRL-B 3567, *Salmonella typhimurium* ATCC 14028, *Yersinia enterocolitica*. The following nine fungal strains were also tested, *Candida albicans* Y- 12983, *C. glabrata*, *Aspergillus flavus* NRRL 1957, *A. niger* ATCC 10949, *A. fumigatus* NRRL 163, *A. parasiticus* NRRL 465, *Botrytis cinerea* (AHU 9424), *Geotrichum candidum*, *Fusarium graminarum*, *F. solani* (wild types).

Determination of antimicrobial activity

The antimicrobial activities of the petroleum ether, methanol and ethyl acetate + water extracts from the plant sample were evaluated by means of agar-well diffusion assay [9, 10] with some modifications. Fifteen milliliters of the molten agar (45 °C) were poured into sterile petri dishes (Ø 90 mm). Cell suspensions containing 10^8 CFU/ml cells for bacteria, 10^7 CFU/ml cells for yeasts, and 10^5 spore/ml of fungi were prepared and evenly spread onto the surface of the agar plates of Mueller-Hinton agar (Oxoid, UK) for bacteria, or Sabouraud dextrose agar (Oxoid, UK) medium for yeasts and fungi using sterile swab sticks. Once the plates had been aseptically dried, 10 mm wells were bored using a sterile cork borer. Extracts (100 µl) were placed into the wells and the plates were incubated at 37°C for 24 h for bacterial strains, 48 h for yeasts and 72 h for fungi at room temperature. Vancomycine and tetracycline (30 mg/ml) for bacteria and amphotericine (10 mg/ml) for yeasts and fungi were used as positive controls. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. The tests were performed in triplicate.

RESULTS AND DISCUSSION

The plant material was subjected to an extraction process, with petroleum ether, methanol and ethyl acetate+water. The yields were 8% for the petroleum ether extract, 3.8% for methanol extract and 2.2% for the ethyl acetate+water extract.

As shown in Table 1, the extracts from the *C. olitorius* plant displayed antibacterial and antifungal activity against all/or some of the tested gram positive and gram negative bacterial and fungal strains, yeasts and molds, with the diameters of zone inhibition ranging between 11 and 20 mm. The most active extract was that obtained from petroleum ether and this extract inhibited the growth of all the bacterial strains tested, specifically *E. coli* (20 mm) *S. aureus* (19 mm) and *Y. enterocolitica* (19 mm). Furthermore, among the fungi studied, *G. candidum* and *B. cinerea* were susceptible to all extracts while *A. flavus, A. parasiticus*, *A. fumigatus, F. solani* and *C. glabrata* were resistant against all extracts.

Thornes, working in 1954, sought an agent to treat vaginal candidiasis in his pregnant patients. Coumarins have been found to inhibit *C. albicans in vitro.* Hydroxycinnamic acids, related to coumarins, have been seen to be inhibitory on gram positive bacteria. Phytoalexins, hydroxylated derivatives of coumarin, are usually uniform within a plant family but diverse within the plant kingdom. They are antimicrobial compounds synthesized by a plant in response to infection or stress. They are produced in carrots, for example, in response to fungal infection and can be presumed to have antifungal activity. All in all, data about the specific antibiotic properties of coumarins are scarce, although many reports give reason to believe that some utility may reside in these phytochemicals [3, 11].

The petroleum ether extract of investigated species had an *in vitro* potential antimicrobial activity against all bacteria. The data indicated that yeast *C. albicans* extracts of *C. olitorius* leaves exhibited the strongest inhibition effect. The presence of coumarin compounds in *C. olitorius* leaves may be responsible for the inhibition activity.

The coumarin compounds and additional volatile components were obtained from the leaves of *C. olitorius* by GC/MS analysis, with 49 components identified [3]. We know that many of these determined volatile components are effective against gram positive and gram negative bacteria [12]. However, in this study, the extraction method applied wasn't suitable for isolation of volatile components.

The plant *C. olitorius*, popularly known as Molehiya, is found in wide distribution throughout the tropics and subtropics. The leaves of plant are used commonly by the people in food as a vegetable. The human body is able to directly absorb the different compounds and due to the low concentration of these compounds, it has transpired that the plant particularly provides effective protection against infection. This protective property, either through the plant itself or by the compounds contained within, also has the effect of prolonging the shelf life of food in which it is used or prevents it being spoilt by microorganisms.

Table 1. Antibacterial and antifungal activity of three extracts from *Corchorus olitorius* L. as inhibition zones (mm) (well Ø 10 mm)

Tested bacterial strains	Petroleum ether	MeOH	Ethyl-acetate+water	Vancomycine (disc Ø 6 mm)	Tetracycline (disc Ø 6mm)
Bacillus cereus NRRL 3711	14±0.1	15±0.1	12±0.1	24±0.6	30±0.1
Bacillus subtilis NRRL B-209	18±0.1	19±0.1	15±0.1	23±0.1	34±0.1
Enterobacter aerogenes NRRL B-3567	18±0.5	15±0.4	12±0.1	25±0.1	12±0.1
Enterococcus faecalis ATCC 29212	18±0.2	17±0.2	18±0.1	19±0.1	12±0.1
Escherichia coli ATCC 25922	20±0.4	10±0.7	-	-	30±0.1
Micrococcus luteus NRRL B-1018	18±0.1	15±0.1	14±0.3	24±0.1	40±0.1
Proteus vulgaris NRRL B-123	17±0.1	16±0.1	14±0.1	-	15±0.1
Pseudomonas gingeri 3146	16±0.4	-	13±0.1	23±0.1	28±0.1
Salmonella typhimurium ATCC 14028	18±0.1	-	15±0.2	-	14±0.1
Staphylococcus aureus ATCC 25923	19±0.1	18±0.1	19±0.1	20±0.1	29±0.1
Streptococcus faecium NRRL B-3502	17±0.1	15±0.1	13±0.5	18±0.1	25±0.1
Yersinia enterocolitica	19±1.1	16±0.1	17±0.1	20±0.1	29±0.1
Tested fungal strains	**Petroleum ether**	**MeOH**	**Ethyl-acetate+water**	**Amphotericine (well Ø 10 mm)**	
Aspergillus flavus NRRL 1957	-	-	-	-	
A. fumigatus NRRL 163	-	-	-	-	
A. niger ATCC 10949	-	12±0.1	11±0.6	12±0.1	
A. parasiticus NRRL 465	-	-	-	-	
Botrytis cinerea 9492	18±0.4	14±0.1	12±0.1	-	
Candida albicans NRRL Y-12983	15±0.1	-	-	-	
C. glabrata	-	-	-	-	
Fusarium graminearum (wild type)	-	12±0.1	-	12±0.1	
F. solani (wild type)	-	-	-	-	
Geotrichum candidum (wild type)	14±0.3	18±0.8	20±0.1	14±0.1	

–: absence of inhibition

Its current use as a plant additive to other food and consumed by the public may be due to its health-giving properties and its taste. There is a definite need for more research to determine the metabolite synthesis that occurs in this species of plant, as well as the construction of new molecules.

REFERENCES

[1] Tulio Jr AZ, Ose K, Chachin K, Ueda Y. 2002. Effects of storage temperatures on the postharvest quality of jute leaves (*Corchorus olitorius* L.). Postharvest Biology and Technology. 26: 329-338.

[2] Meikle RD. 1977. Flora of Cyprus. Vol.1, pp 832. Bentham-Moxon Trust.

[3] Abou Zeid AHS. 2002. Stress metabolites from *Corchorus olitorius* L. leaves in response to certain stress agents. Food Chemistry. 76: 187-195.

[4] Matsufuji H, Sakai S, Chino M, Goda Y, Toyoda M, Takeda M. 2001. Relationship Between Cardiac Glycoside Contents and Color of *Corchorus olitorius* Seeds. Journal of Health Science. 47(2): 89-93.

[5] Gupta M, Mazumder UK, Pal DK, Bhattacharya S. 2003. Onset of puberty and ovarian steroidogenesis following adminstration of methanolic extract of *Cuscuta reflexa* Roxb. stem and *Corchorus olitorius* Linn. seed in mice. Journal of Ethnopharmacology. 89: 55-59.

[6] Pal DK, Mandal M, Senthilkumar GP, Padhiari A. 2006. Antibacterial activity of *Cuscuta reflexa* stem and *Corchorus olitorius* seed. Fitoterapia. 77(7-8): 589-91.

[7] Nakamura T, Goda Y, Sakai S, Kondo K, Akiyama H, Toyoda M. 1998. Cardenolide Glycosides from Seeds of *Corchorus olitorius*. Phytochemistry. 49: 2097-2101.

[8] Koşar M, Bozan B, Temelli F, Başer KHC. 2002. Sumak (*Rhus coriaria*)'ın fenolik bileşikleri ve antioksidan etkileri (in Turkish), 14. Bitkisel İlaç Hammaddeleri Toplantısı. Bildiriler, 29-31 May, Eskisehir.

[9] Güven K, Yücel E, Çetintaş F. 2006. Antimicrobial activities of fruits of *Crataegus* and *Pyrus* species. Pharmaceutical Biology. 44(2): 79-83.

[10] National Committee For Clinical Laboratory Standards, 1993. Performance standards for antimicrobial disk susceptibility tests. Approved Standards NCCLS, Publication M2-A5, Villanova. PA, USA.

[11] Cowan MM. 1999. Plant Products as Antimicrobial Agents. Clinical Microbiology Reviews. 12: 564-582.

[12] Burt S. 2004. Essential oils: their antibacterial properties and potential applications in foods-a review. International Journal of Food Microbiology. 94: 223-253.