Antimicrobial and antioxidant activity of the polyphenol mangiferin

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Summary

The antimicrobial effect of the polyphenol mangiferin obtained from leaves of mango trees was studied according to the diffusion method. The solutions of mangiferin in polyethylene glycol-400 showed an activity with regard to seven bacterial and five fungal species - *Bacillus pumilus, Bacillus cereus, Staphylococcus aureus, Staphylococcus citreus, Escherichia coli, Salmonella agona, Klebsiella pneumoniae, Saccharomyces cerevisiae, Thermoascus aurantiacus, Trichoderma reesei, Aspergillus flavus* and *Aspergillus fumigatus*. Its antioxidant activity with regard to the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was also studied, as the concentration of mangiferin causing 50% inhibition of the free radical (IC_{50}) was assayed to be 7.5 ± 0.0075 μg/ml.

Key words: mangiferin, antimicrobial effect, antioxidant activity, DPPH

Mango (*Mangifera indica* L.) is one of the most popular tropical fruit-bearing trees in the world, as some 69 varieties of the species are known.

The aqueous extract from the bark of mango, obtained under the name Vimang, contains polyphenols whose major ingredient is mangiferin, [C-glucosyl-xanthone (1,3,6,7-tetrahydroxyxanthone-C2-β-D-glucoside)] [1]. Mangiferin is a normal metabolite also to be found in mango leaves. In the Republic of Vietnam a technology has been elaborated, and subsequently improved, for obtaining mangiferin from mango leaves [2].

Mangiferin, traditionally used by native inhabitants of Bolivia, Southern Guiana, the Antilles, Columbia, the Philippines and India in the treatment of a number of diseases, has subsequently been proved to have a varied pharmacological activity, such as antiviral and antitumor [3, 4, 5], spasmolytic [6], antidiabetic [7, 8], and immunostimulating [9]. It is biologically healthy for a number of organs, including liver and brain tissues, with its analgetic and antiphlogistic effects [10]. The antioxidant activity of mangiferin is attributed to its being a polyphenol [11], as polyphenols have a protective effect against the development of cardio-vascular diseases, since they prevent the oxidation of low-density lipoprotein and the ensuing development of atherosclerotic harms [12].

Regardless of the large number of studies on the properties of mangiferin, there are no specific data in the literature concerning its antimicrobial effect or its antioxidant activity with regard to the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). These studies were aimed at establishing the antimicrobial activity of mangiferin, as well as its antiradical effect with regard to the free radical DPPH.

EXPERIMENTAL

Plant material

The mangiferin under study was provided by the company VIMEDIMEX II, Ho Chi Minh City, Vietnam. Mangiferin is a yellow odorless powder, degree of purity 97%. It was used to prepare solutions in polyethileneglycol-400 with concentrations in % (w/v): 8, 10, 15, 20, 25, 30, 35.

Study of the antibacterial effect

The following bacterial species were used: *Bacillus pumilus* ATCC 14884, *Bacillus cereus* ATCC 11778, *Staphylococcus aureus* ATCC 6538 P, *Staphylococcus citreus* BTCC, *Escherichia coli* ATCC 8739, *Klebsiella pneumoniae* 1056, *Pseudomonas aeruginosa* ATCC 28G, obtained from the National Bank for Industrial Microorganisms and Cellular Cultures (NBIMCC), Sofia, and *Salmonella agona* - from the Medical Academy, Plovdiv. The strains were kept on mesopeptone agar with 1% glucose. Suspensions of 24 h cultures with concentration 10^8 cfu/cm^3 were used. In order to establish the antibacterial effect the diffusion method was applied and performed as follows: in Petri dishes with diameter 90 mm the above medium was poured, inoculated with 0.1 cm^3 of the tested microorganism, after which sterile filter disks were placed (diameter 6 mm). 0.04 cm^3 of the mangiferin solutions were dropped on the disks in three repetitions for each concentration, and the solvent polyethylene glycol-400 was used for reference. The zones of inhibition of the growth of the bacteria around the disks were measured after a 24-48 h incubation at 37°C.

Study of the antifungal effect

The yeast *Candida albicans* ATCC 10231 (NBIMCC), *Saccharomyces cerevisiae* and the mycelial fungi *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Fusarium moniliforme, Fusarium oxysporum, Trichoderma reesei, Thermoascus aurantiacus* were used. The cultures were from the collection of the Department of Biotechnology at the University of Food Technology in Plovdiv, Bulgaria, and were kept on malt agar at 4°C. The suspension was prepared from 14-day cultures with concentration 10^7 cfu/cm^3. Inoculation of the tested microorganisms and depositing mangiferin was implemented as with the bacteria. The zones of inhibition around the disks were measured after a 7-day incubation at 28°C.

The antimicrobial activity of mangiferin was assessed by the diameter (mm) of the obtained sterile zones around the disks.

Study of the antiradical activity

The antiradical activity was determined according to the method described by Mensor et al. [13].

From the initial concentration 10^{-4} M mangiferin in absolute ethanol concentrations 2, 4, 6, 8 and 10 μg/cm^3 were obtained by means of dilution and were driven to a final volume of 2.5 cm^3. One ml of 0.3 mM alcohol solution of DPPH was added to 2.5 cm^3 from the samples with different concentrations of mangiferin. The samples were kept at room temperature in the dark and after 30 minutes the optic density was measured at 518 nm.

The antiradical activity (AA) was determined by the following formula:

$$AA\,\% = 100 - \{[Abs._{sample} - Abs._{empty\ sample}) \times 100]/Abs._{control}\},$$

where: empty samples: 1 cm^3 ethanol + 2.5 cm^3 from various concentrations of mangiferin;

control sample: 1 cm^3 0.3 mM DPPH + 2.5 cm^3 ethanol.

The optic density of the samples, the control and the empty samples was measured in comparison with ethanol.

With the results obtained, a linear dependence was plotted between the concentrations of mangiferin and the average value of antioxidant activity from three experiments. It was on the basis of this dependence that the concentration of mangiferin, causing a 50% inhibition of free radical (IC_{50}), was determined.

The antioxidant activity of mangiferin was determined likewise, at 0.1 mM concentration of DPPH in ethanol.

Statistical analysis

The statistical processing of the data obtained from all the studies was implemented by means of dispersion analysis with the Sigma Plot 8.0 software. The data are expressed as means ± standard deviation (SD). A statistical analysis was performed with Student's *t*-test. A difference was considered statistically significant when $p \leq 0.05$.

RESULTS AND DISCUSSION

The antibacterial effect of the solutions with different concentration of mangiferin is presented in Table 1. The results from this study showed the antibacterial effect of mangiferin with regard to seven bacterial species: *Bacillus pumilus, Bacillus cereus, Staphylococcus aureus, Staphylococcus citreus, Escherichia coli, Salmonella agona, Klebsiella pneumoniae*, i.e. it showed a wide range of effects - both with regard to Gram-positive, and Gram-negative bacteria. As for the antibacterial effect with regard to the Gram-positive microorganisms, lower concentrations of mangiferin - up to 20% - were needed, as the diameters of the obtained sterile zones were from 12 mm to 17 mm. The species most sensitive to mangiferin among the Gram-positive microorganisms was *Bacillus pumilus*. Concerning the antibacterial effect with regard to the Gram-negative microorganisms, higher concentrations of mangiferin were necessary (30%-35%), as the sterile zones were from 12 mm to 24 mm. Among the Gram-negative species, the one most sensitive to mangiferin was *Salmonella agona*. Mangiferin did not show any activity with regard to *Pseudomonas aeruginosa*. Polyethylene glycol-400, used for reference, did not show antibacterial activity.

Table 1.

Antibacterial effect of mangiferin.

tested microorganisms	concentration of mangiferin, %						
	8	10	15	20	25	30	35
	inhibition zones (mm) ± SD						
B. pumilus	0	12 ± 0.20	18 ± 0.50	20 ± 0.40	nt*	nt	nt
B. cereus	0	0	15 ± 0.30	17 ± 0.54	18 ± 0.30	nt	nt
S. aureus	0	0	0	17 ± 0.40	20 ± 0.20	22 ± 0.30	nt
S. citreus	0	0	0	15 ± 0.20	18 ± 0.60	20 ± 0.30	nt
E. coli	0	0	0	0	0	0	12 ± 0.20
S. agona	0	0	22 ± 0.64	26 ± 0.30	29 ± 0.70	nt	nt
K. pneumoniae	0	0	0	0	0	20 ± 0.40	24 ± 0.60
P. aeruginosa	0	0	0	0	0	0	0

*nt – not tested

The antifungal effect of mangiferin in various concentrations is presented in Table 2. The strongest antifungal effect was shown by mangiferin with regard to *Thermoascus aurantiacus*, as the zone of inhibition was 18 mm. More weakly expressed was its effect with regard to *Saccharomyces cerevisiae, Trichoderma reesei, Aspergillus flavus, Aspergillus fumigatus* – it was shown at higher concentrations and with a smaller zone. No zones of inhibition were found with regard to *Candida albicans, Aspergillus niger, Fusarium moniliforme* and *Fusarium oxysporum*. The solvent used for reference did not show antifungal activity.

Table 2.

Antifungal effect of mangiferin.

tested microorganism	concentration of mangiferin, %		
	25	30	35
	inhibition zones (mm) ± SD		
C. albicans	0	0	0
S. cerevisiae	0	0	15 ± 0.60
A. niger	0	0	0
A. flavus	0	0	15 ± 0.40
A. fumigatus	0	0	15 ± 0.64
F. moniliforme	0	0	0
F. oxysporum	0	0	0
T. aurantiacus	0	18 ± 0.50	nt*
T. reesei	0	0	15 ± 0.30

*nt – not tested

Aspergillus niger and *Fusarium moniliforme* are species which cause infection of leaves, fruits, branches, stem and blossom of mango. The sick racemes cannot yield fruits. Chakrabarti and Ghosal [14] have found that the fungus *Fusarium moniliforme* var. *subglutinans* transforms mangiferin into polymerous quinone, possibly due to the phenoloxidase it releases. It is possible that the resistance to mangiferin of the other mycelial fungi under study is due to the same mechanism.

In search for new antimicrobial substances amongst various plant sources in most cases only an antibacterial effect is established, as only few of them have been reported to have an antifungal effect [15, 16]. The results obtained characterised mangiferin as a product with a broad antimicrobial effect, not only with regard to bacteria, but also with regard to fungi.

The stable free radical DPPH is widely used for an assay of the radical-scavenging activity both of plant extracts, and of pure substances. The method is based on the reduction of DPPH in an alcohol solution by an antioxidant, donor of hydrogen.

The dependence between the concentration of mangiferin and the inhibition of the free radical DPPH is presented in Figure 1. 7.5 ± 0.0075 μg/ml of mangiferi causing a 50% inhibition of the free radical (r = 0.9991) was assayed on the ba-

sis of the obtained linear dependence. The lower value of IC_{50}, the stronger the antioxidant effect of the substance under study. IC_{50} for mangiferin was 1.8 times lower than that determined for rutin (14.16 μg/ml) [13] and 3.2 times higher than that for ascorbic acid (2.34 μg/ml) [17].

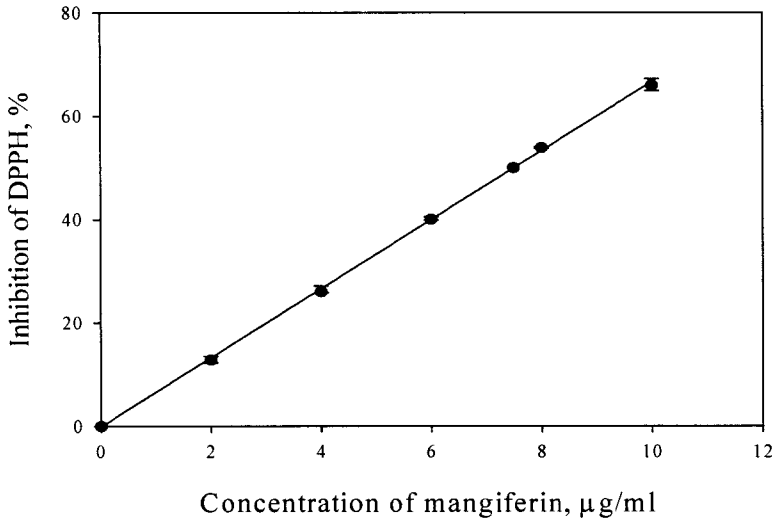

Fig. 1. Antioxidant activity of mangiferin.

The maximum values of the radical scavenging activity of mangiferin, obtained by two concentrations of DPPH (0.1 mM and 0.3 mM), are shown in Table 3. The studies on the activity of mangiferin at the lower concentration of DPPH (0.1 mM) showed the highest percentage of inhibition at a ratio of 0.2%–99.98%. The activity of mangiferin obtained at that concentration was comparable with that determined for caffeic acid [18] and rosmarinic acid [19]. When 0.3 mM DPPH was used, the antiradical activity reached 92.73% at a considerably higher ratio – 0.83.

Table 3.

Antioxidant activity of mangiferin, determined using two different concentrations of DPPH.

mg antioxidant per mg DPPH (0.1mM)	radical scavenging activity, %	mg antioxidant per mg DPPH (0.3 mM)	radical scavenging activity, %
0.10	72.45 ± 0.63	0.16	72.90 ± 0.60
0.15	86.60 ± 1.24	0.50	87.59 ± 1.04
0.20	99.98 ± 0.10	0.83	92.73 ± 0.74

The results obtained determined mangiferin as a strong antioxidant. According to some authors [17], the high radical scavenging activity of plant substances is the cause of their antimicrobial effect.

In conclusion, our studies characterised mangiferin as a product with a high antiradical activity combined with a wide-range antimicrobial effect.

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PRZECIWDROBNOUSTROJOWE I PRZECIWUTLENIAJĄCE DZIAŁANIE MANGIFERYNY

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Streszczenie

Działanie przeciwdrobnoustrojowe mangiferyny pozyskiwanej z liści mangowca badano za pomocą metody dyfuzji. Roztwory mangiferyny w glikolu polietylenowym 400 okazały się skuteczne w wypadku siedmiu gatunków bakterii i pięciu gatunków grzybów - *Bacillus pumilus, Bacillus cereus, Staphylococcus aureus, Staphylococcus citreus, Escherichia coli, Salmonella agona, Klebsiella pneumoniae, Saccharomyces cerevisiae, Thermoascus aurantiacus, Trichoderma reesei, Aspergillus flavus* oraz *Aspergillus fumigatus*. Badano także działanie przeciwutleniające w stosunku do wolnego rodnika 2,2-difenylo-2-pikrylohydrazyłu (DPPH). Wartość stężenia mangiferyny powodującego 50-procentową inhibicję wolnego rodnika (IC_{50}) wyniosła 7,5 ± 0,0075 μg/ml.

Słowa kluczowe: mangiferyna, działanie przeciwdrobnoustrojowe, działanie przeciwutleniające, DPPH