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Antimicrobial evaluation of mangiferin and its synthesized analogues

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ABSTRACT

Objective: To isolate the mangiferin from *Mangifera indica* (*M. indica*) and assess the antimicrobial activity of different analogues synthesized from mangiferin. **Methods:** Mangiferin was isolated by column chromatography from the ethanolic extract of stem bark of *M. indica.* Mangiferin was further converted to 5-(N-phenylamino methyleno) mangiferin, 5-(N-p-chlorophenylamino methyleno) mangiferin, 5-(N-2-methyl phenylamino methyleno) mangiferin, 5-(N-p-methoxy phenylamino methyleno) mangiferin, 5-(N, N-diphenylamino methyleno) mangiferin, 5-(N- α -napthylamino methyleno) mangiferin and 5-(N-4-methyl phenylamino methyleno) mangiferin. Mangiferin and its analogues were characterized by melting point and Rf value determination and through spectral technique like UV, IR, and NMR spectral analysis. The antimicrobial effect of mangiferin and its derivatives was studied according to the disc diffusion method. **Results:** The solutions of mangiferin and its derivatives in polyethylene glycol-400 showed an activity with regard to four bacterial species-*Bacillus pumilus* (*B. pumilus*), *Bacillus cereus* (*B. cereus*) and *Salmonella virchow* (*S. virchow*) and two fungal species-*Thermoascus aurantiacus* (*T. aurantiacus*) and *Aspergillus flavus* (*A. flavus*). **Conclusion:** The present study confirms the antimicrobial activity of different analogues of mangiferin which could be further processed for the development of a potential antimicrobial agent.

1. Introduction

Mangiferin, $C_{19}H_{18}O_{11}$, a glucoxanthone (1,3,6,7-tetrahydroxyxanthone- C2- β -D-glucoside) is reported to be a principal constituent of *Mangifera indica* (*M. indica*) L. and is present in various parts of plants viz leaves[1], fruits, stem bark, roots and heartwood[2]. Mangiferin has been evaluated for numerous pharmacological activities such as antibacterial[3], antitumor, immunomodulatory, antiHIV[4], antidiabetic[5], antioxidative[6], anthelminthic, antiallergic[7], antiinflammatory[8], antiviral[9] and as a inducer of macrophage activation[10]. In Cuba, mangiferin is traditionally used under brand name Vimang® and is known to have potential antiinflammatory, analgesic and also antioxidant activities, whereas in Sri Lanka, mangiferin is used in the treatment of obesity, particularly for type II diabetes under brand name Salaretin® . Even though mangiferin has been evaluated for various pharmacological activities but still very few attempts have been made to make its derivatives and further evaluate their pharmacological activities. Therefore, the present investigation includes preparation of some analogues of mangiferin which were later screened for their antimicrobial activity.

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2. Materials and methods

The stem bark of *M. indica* was collected from saunda village Modinagar, Ghaziabad district of Uttar Pradesh in the month of April 2006 and was authenticated at the Department of Botany, M.M.P.G. College, Modinagar. Bacterial and fungal strains were obtained from the Institute of Microbial Technology, Chandigarh, India. Melting points were determined in open capillary tubes and purity of the compounds was checked by TLC on silica gel G. UV spectra were recorded on Systronics double beam UV spectrophotometer 2202, IR spectra were recorded in KBr on Jasco FTIR 4100 spectrophotometer while NMR spectra were obtained on Bruker avance II-400 MHz., spectrometer using TMS as internal reference.

2.1. Defatting of stem bark

The stem bark of *M. indica* was first dried at room temperature and then was further coarsely powdered.

The coarsely powdered bark of *M. indica* was extracted exhaustively with petroleum ether (60-80 ℃) in Soxhlet apparatus to remove fatty matter for 56 h.

2.2. Extraction of mangiferin

Coarsely powdered stem bark of *M. indica* was extracted exhaustively using ethanol (95%) as a solvent in Soxhlet apparatus for 56 h. The combined alcoholic extract was further concentrated under reduced pressure which yielded a yellow amorphous powder.

2.3. Isolation of mangiferin

The dried alcoholic extract was adsorbed on silica gel (60-120 mesh) and chromatographed over silica gel column packed in petroleum ether (60-80 ℃). The column was eluted with chloroform: methanol (1:1) which gave mangiferin as a pale yellow amorphous powder. This upon crystallization from ethanol produced pale yellow needle shaped mangiferin crystals.

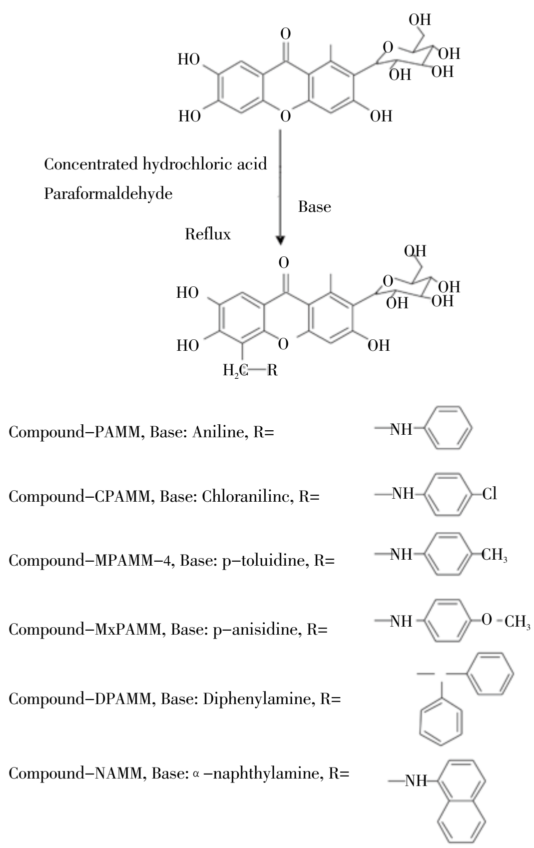

Figure 1. Synthesis of mangiferin analogues where 1= Mangiferin.

2.4. General method for the preparation of Mangiferin analogues

The method includes mixing equal mol. of mangiferin, powdered paraformaldehyde and aromatic amine, 10 mL of 95% ethanol and 1 mL of concentrated hydrochloric acid which was refluxed, cooled to room temperature and kept in a refrigerator overnight. The obtained solid crystals were filtered and washed with water and recrystallized from ethanol (Figure 1).

2.5. Antimicrobial evaluation

Antimicrobial study was determined by disc diffusion method[11]. Bacterial strains of *Bacillus pumilus* (*B. pumilus*) (MTCC-1607), *Bacillus cereus* (*B. cereus*) (MTCC-430), *Salmonella virchow* (*S. virchow*) (MTCC-1163) and *Pseudomonas aeruginosa* (*P. aeruginosa*) (MTCC-741), and fungal strains of *Aspergillus flavus (A. flavus)* (MTCC-277) and *Thermoascus aurantiacus* (*T. aurantiacus*) (MTCC-375) were used. The nutrient agar plates were prepared by pouring 15 mL of molten media into sterile Petri plates. The plates were allowed to solidify for 5 min and 0.1% inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 min. The compounds were loaded on 6 mm discs. The loaded discs were placed on the surface of medium and the compounds were allowed to diffuse for 5 min and the plates were kept for incubation at 37 ℃ for 24-48 h for bacteria and 28 ℃ for 7 d for fungi with yeast peptone dextrose agar and czapek yeast agar media. At the end of incubation, inhibition zones formed around the discs were measured.

3. Results

3.1. Characterization of mangiferin (MG)

Melting point: 269-270 ℃, R_f: 0.77 using *n*-butanol: acetic acid: water (4:1:2.2) as a Solvent system, λ_{max}: 205.6, 256.8, 238.4, 315.2, 367.2 nm. IR (KBr) cm^{-1}: 3 366 (O-H), 2 937 (C-H), 1 649 (>C=O), 1 495 (C=C), 1 253 (-C-O), 1 050 (C-O-C). NMR (δ ppm): 13.81 (ArOH intramolecularly bonded, 1H), 7.9 (ArOH, 3H), 6.82 (Ar-H, 1H), 6.36 (Ar-H, 1H), 7.4 (ArH, 1H), 2.5 (-C-OH, 4H), 3.7 (-CH-O-, 2H), 3.3 (-CH-, 2H), 3.5 (-CH-, 3H)

3.1.1. 5-(N-phenylamino methyleno) mangiferin (PAMM)

Melting point: 190 ℃, R_f: 0.60, λ_{max}: 239.6, 261.2, 317.6, 370.4 nm. IR (KBr) cm^{-1}: 3 551 (O-H), 3 319 (N-H), 2 929 (C-H), 1 625 (>C=O), 1 488 (C=C), 1 383 (-C-N), 1 293 (-C-O), 1 037 (C-O-C). NMR (δ ppm): 13.70 (ArOH intramolecularly bonded, 1H), 8 (ArOH, 3H), 6.82 (Ar-H, 6H), 7.39 (Ar-H, 1H), 3.7 (Ar-CH_2-N-, 2H), 4.1 (Ar-NH-, 1H), 2.9 (-C-OH, 4H), 3.7 (-CH-O-, 2H), 3.4 (-CH-, 1H), 3.5 (-CH-, 4H).

3.1.2. 5-(N-p-chlorophenylamino methyleno) mangiferin (CPAMM)

Melting point: 210 ℃, R_f: 0.69, λ_{max}: 225.2, 228.8, 261.2, 318.8, 368 nm. IR (KBr) cm^{-1}: 3 410 (O-H), 3 360 (N-H), 2 926 (C-H), 1 625 (>C=O), 1 429 (C=C), 1 375 (-C-N), 1 295 (-C-O), 1 079 (C-O-C), 715 (C-Cl). NMR (δ ppm): 13.66 (ArOH intramolecularly bonded, 1H), 7.9 (ArOH, 3H), 6.82 (Ar-H, 5H), 7.36 (Ar-H, 1H), 4.2 (Ar-CH_2-N-, 2H), 4.0 (Ar-NH-, 1H), 2.1 (-C-OH, 4H), 3.7 (-CH-O-, 2H), 3.4 (-CH-, 5H).

3.1.3. 5-(N-4-methyl phenylamino methyleno) mangiferin

Table 1.
Antibacterial and antifungal activity of mangiferin and its analogues.

Compound	Concentration (%)	Antibacterial activity Inhibition zones (mm)				Antifungal activity Inhibition zones (mm)	
		B.p.	B.c.	S.v.	P.a.	A.f.	T.a.
MG	15	18	15	22	0	0	0
	20	20	17	26	0	0	0
	25	23	18	29	0	0	0
	30	nt*	nt*	nt*	0	12	18
PAMM	15	16	12	19	0	0	0
	20	19	15	22	0	0	0
	25	22	16	23	0	0	0
	30	nt*	nt*	nt*	10	11	14
CPAMM	15	15	12	20	0	0	0
	20	17	14	21	0	0	0
	25	18	15	23	0	0	0
	30	nt*	nt*	nt*	8	11	13
MPAMM-4	15	17	15	20	0	0	0
	20	19	17	22	0	0	0
	25	22	19	25	0	0	0
	30	nt*	nt*	nt*	10	14	15
MxPAMM	15	18	14	20	0	0	0
	20	19	17	21	0	0	0
	25	22	19	23	0	0	0
	30	nt*	nt*	nt*	9	11	16
DPAMM	15	17	14	19	0	0	0
	20	18	15	20	0	0	0
	25	20	18	22	0	0	0
	30	nt*	nt*	nt*	9	12	14
NAMM	15	18	13	18	0	0	0
	20	19	15	20	0	0	0
	25	21	18	23	0	0	0
	30	nt*	nt*	nt*	10	11	15

B.p .is *B. pumilus*, B.c is *B. cereus*, S.v. is *S. virchow*, P.a. is *P. aeruginosa*, A.f. is *A. flavus* and T.a. is *T. aurantiacus*. *nt – not tested.

(MPAMM-4)

Melting point: 195 ℃, R_f: 0.53, λ_{max}: 230, 261.2, 317.6, 370.4 nm. IR (KBr) cm^{-1}: 3 493 (O-H), 3 483 (N-H), 2 971 (C-H), 1 638 (>C=O), 1 429 (C=C), 1 283 (-C-N), 1 044 (C-O-C), 713. NMR (δ ppm): 13.66 (ArOH intramolecularly bonded, 1H), 7.9 (ArOH, 3H), 6.82 (Ar-H, 5H), 7.36 (Ar-H, 1H), 2.3 (Ar-CH_3, 3H), 3.7 (Ar-CH_2-N-, 2H), 4.2 (Ar-NH-, 1H), 2.3 (-C-OH, 4H), 3.7 (-CH-O-, 2H), 3.3 (-CH-, 5H).

3.1.4. 5-(N-p-methoxy phenylamino methyleno) mangiferin (MxPAMM)

Melting point: 190 ℃, R_f: 0.45, λ_{max}: 210.8, 224, 261.2, 317.6, 370.4 nm. IR (KBr) cm^{-1}: 3 536 (O-H), 3 445 (N-H), 2 941 (C-H), 1 646 (>C=O), 1 432 (C=C), 1 283 (-C-N), 1 180 (Ar-O-C), 1 078 (C-O-C). NMR (δ ppm): 13.66 (ArOH intramolecularly bonded, 1H), 7.9 (ArOH, 3H), 6.8 (Ar-H, 1H), 6.9 (Ar-H, 4H), 7.36 (Ar-H, 1H), 4.2 (Ar-CH_2-N-, 2H), 4.0 (Ar-NH, 1H), 3.8(Ar-O-CH_3, 3H), 2.1 (-C-OH, 4H), 3.8 (-CH-O-, 2H), 3.3 (-CH-, 5H).

3.1.5. 5-(N, N-diphenylamino methyleno) mangiferin (DPAMM)

Melting point: 210 ℃, R_f: 0.82, λ_{max}: 257.6, 240.8, 305.6, 364.4 nm. IR (KBr) cm^{-1}: 3 371 (O-H), 2 931 (C-H), 1 647 (>C=O), 1 405 (C=C), 1 297 (-C-N), 1 253 (-C-O), 1 031 (C-O-C). NMR (δ ppm): 13.78 (ArOH intramolecularly bonded, 1H), 7.87 (ArOH, 3H), 6.84 (Ar-H, 2H), 7.4 (Ar-H, 2H), 7.04 (Ar-H, 4H), 7.02 (Ar-H, 4H), 3.9 (Ar-CH_2-N-, 2H), 2.1 (-C-OH, 4H), 3.7 (-CH-O-, 2H), 3.3 (-CH-, 2H), 3.4 (-CH-, 3H).

3.1.6. 5-(N- α -napthylamino methyleno)-mangiferin (NAMM)

Melting point: 205 ℃, R_f: 0.60, λ_{max}: 244.4, 297.2, 306.8 nm. IR (KBr) cm^{-1}: 3 443 (O-H), 3 339 (N-H), 2 927 (C-H), 1 621 (>C=O), 1 482 (C=C), 1 385 (-C-N), 1 290 (-C-O), 1 038 (C-O-C). NMR (δ ppm): 13.78 (ArOH intramolecularly bonded, 1H), 7.9 (ArOH, 3H), 6.87 (Ar-H, 1H), 7.36 (Ar-H, 1H), 7.4 (napth-H, 5H), 7.5 (napth-H, 2H), 4.29 (Ar-CH_2-N-, 2H), 4.1(Ar-NH-, 1H), 2.1 (-C-OH, 4H), 3.8 (-CH_2-O-, 2H), 3.4 (-CH-, 5H).

3.2. Antimicrobial evaluation

The antimicrobial activity of solutions having different concentrations of mangiferin and its analogues are represented in Table 1. The results depicted a higher activity at higher concentration in all bacterial as well as fungal stains tested. The maximum inhibition for bacterial stains i.e. *B. pumilus*, *B. cereus* and *S. Virchow* was observed at concentration of 25% for all mangiferin analogues, whereas maximum inhibition against *P. aeruginosa* was observed at 30%. In case of fungal stains *T. aurantiacus* and *A. flavus* tested, the maximum effect of mangiferin analogues was found to be at 30% concentration (Table 1).

4. Discussion

In the present study stem bark of *M. indica* was first defatted with petroleum ether (60–80 ℃) prior to extraction with ethanol 95%. Followed by this the extract was chromatographed over silica gel and eluted with chloroform: methanol (1:1) to afford the parent mangiferin as pale yellow needle shaped crystals. The obtained mangiferin was than processed for the synthesis of different analogues which resulted in the synthesis of analogues such as 5–(N–phenylamino methyleno) mangiferin, 5–(N–p–chlorophenylamino methyleno) mangiferin, 5–(N–4–methyl phenylamino methyleno) mangiferin, 5–(N–p–methoxy phenylamino methyleno) mangiferin, 5–(N, N–diphenylamino methyleno) mangiferin, 5–(N–α–napthylamino methyleno) mangiferin. The synthesized mangiferin analogues were characterized by R_f, mp, UV, IR and NMR spectral analyses. The absorbed maxima 205.6, 256.8, 238.4, 315.2 and 367.2 nm of mangiferin is closely related to that of reported UV spectral data. Mangiferin and its derivative were also confirmed by proton NMR signals[12].

The antibacterial effect was evaluated taking four bacterial species: *B. pumilus*, *B. cereus*, *S. virchow* and *P. aeruginosa*. It showed a wide range of effects both with regard to Gram–positive as well as Gram–negative bacteria. Antibacterial effects with Gram–positive microorganisms were obtained using low concentrations of mangiferin and its derivatives. *B. pumilus* was found to be most sensitive to mangiferin and its derivatives. Mangiferin and its derivatives, when used in high concentrations exerted antibacterial effect against Gram–negative microorganisms. *S. virchow* was found to be most sensitive to mangiferin and its derivatives. Mangiferin did not show any activity with regard to *P. aeruginosa* while its derivatives showed activity at high concentrations only. Mangiferin and its derivatives, when used in high concentrations exhibited antifungal activity against *T. aurantiacus* and *A. flavus*. Polyethylene glycol–400 which was used as a solvent for reference did not show any antibacterial and antifungal activity.

The present study confirms the antimicrobial activity of mangiferin analogues. Thus, the present study may act as a referential source for the development of potent antimicrobial agents.

Conflict of interest statement

We declare that we have no conflict of interest.

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