Antimicrobial Evaluation of Mangiferin Analogues

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Singh, et al.: Antimicrobial Mangiferin Analogues

The naturally occurring xanthone glycoside mangiferin has been isolated by column chromatography from the ethanol extract of stem bark of Mangifera indica. Mangiferin was further converted to 5-(N-phenylaminomethyleno) mangiferin, 5-(N-p-chlorophenylaminomethyleno) mangiferin, 5-(N-p-methoxyphenylaminomethyleno) mangiferin, 5-(N,N-diphenylaminomethyleno) mangiferin, 5-(N-naphthylaminomethyleno) mangiferin and 5-(N-4-methylphenylaminomethyleno) 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 synthesized compounds were screened for antimicrobial activity.

Key words: Antifungal, antimicrobial, Mangifera indica, mangiferin

Mangiferin, C_{19}H_{18}O_{11}, a glucoxanthone (1,3,6,7-tetrahydroxyxanthone-C_{2}-β-D-glucoside) has been reported to be present in various parts of Mangifera indica viz leaves[1], fruits[2], stem bark[3], heartwood[4] and roots[5]. Mangiferin has attracted considerable interest in view of its numerous pharmacological activities, including antibacterial[6], antitumor, immunomodulatory and antiHIV[7], antidiabetic[8], antioxidative[9], anthelmintic and antiallergic[10], and antiinflammatory activity[11], antiviral[12], macrophage-inducing activity[13]. In Cuba, mangiferin is traditionally used as an antiinflammatory, analgesic and also as an antioxidant under brand name Vimang®. In Sri Lanka, mangiferin is used in the obesity treatment and particularly for diabetes type II under brand name Salaretin®. Updated literature survey reveals that many attempts have not been made to make the derivatives of mangiferin and consequently the derivatives of mangiferin have also not been subjected to the pharmacological screening. This prompted us to investigate upon mangiferin and its derivatives for their pharmacological screening.

The stem bark of Mangifera indica cultivar desi which was collected from saunda village Modinagar, Ghaziabad district of UP in the month of April 2006, 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, NMR spectra on Bruker avance II-400 MHz., spectrometer using TMS as internal reference. The bark was dried at room temperature and coarsely powdered. The fresh air-dried and coarsely powdered bark of Mangifera indica was extracted exhaustively with petroleum ether (60-80°) in Soxhlet apparatus to remove fatty matter for 56 h. Coarsely powdered bark of Mangifera indica was extracted exhaustively with ethanol (95%) in Soxhlet apparatus for 56 h. The combined alcohol extracts were concentrated under reduced pressure. Then, yellow amorphous powder was obtained.

The dried alcoholic extract was adsorbed on silica
gel (60-120 mesh) and chromatographed over silica gel column packed in petroleum ether (60-80º). 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, mp: 269-270º, Rf: 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: 3366(O-H), 2937(C-H), 1649(>C=O), 1495(C=C), 1253(-C-O), 1050(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).

The general method used for the preparation of mangiferin analogues is as follows; a mixture of equal mols of mangiferin, powdered paraformaldehyde and aromatic amine, 10 ml of 95% ethanol and 1 ml of concentrated hydrochloric acid was refluxed, cooled to room temperature and kept in a refrigerator overnight. The solid was filtered and washed with water and recrystallized from ethanol (Scheme 1).

5-(N-phenylaminomethyleno) mangiferin (PAMM); mp: 190º, Rf: 0.60, λ max: 239.6, 261.2, 317.6, 370.4 nm. IR (KBr) cm⁻¹: 3551(O-H), 3319(N-H), 2929(C-H), 1625(>C=O), 1488(C=C), 1383(-C-N), 1293(-C-O), 1037(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-CH₂-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).

5-(N-p-chlorophenylaminomethyleno) mangiferin (CPAMM); mp: 210º, Rf: 0.69, λ max: 225.2, 228.8, 261.2, 318.8, 368 nm. IR (KBr) cm⁻¹: 3410(O-H), 3360(N-H), 2926(C-H), 1625(>C=O), 1429(C=C), 1375(-C-N), 1295(-C-O), 1079(C-O-C), 715(C-Cl). 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-CH₂-N-, 2H), 4.0 (Ar-NH-, 1H), 3.8 (Ar-O-CH₃, 3H), 2.1 (-C-OH, 4H), 3.8 (-CH-O-, 2H), 3.3 (-CH-, 5H).

5-(N-4-methylphenylaminomethyleno) mangiferin (MPAMM); mp: 195º, Rf: 0.53, λ max: 230, 261.2, 317.6, 370.4 nm. IR (KBr) cm⁻¹: 3493(O-H), 3483(N-H), 2971(C-H), 1638(>C=O), 1429(C=C), 1283(-C-N), 1044(-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-CH₃, 3H), 3.7 (Ar-CH₂-N-, 2H), 4.2 (Ar-NH-, 1H), 2.3 (-C-OH, 4H), 3.7 (-CH-O-, 2H), 3.3 (-CH-, 5H).

5-(N-p-methoxyphenylaminomethyleno) mangiferin (MxPAMM); mp: 190º, Rf: 0.45, λ max: 210.8, 224, 261.2, 317.6, 370.4 nm. IR (KBr) cm⁻¹: 3536(O-H), 3445(N-H), 2941(C-H), 1646(>C=O), 1432(C=C), 1283(-C-N), 1180(Ar-O-C), 1078(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-CH₂-N-, 2H), 4.0 (Ar-NH-, 1H), 3.8 (Ar-O-CH₃, 3H), 2.1 (-C-OH, 4H), 3.8 (-CH-O-, 2H), 3.3 (-CH-, 5H).

Scheme 1: Synthesis of mangiferin analogues, 1 is mangiferin
5-(N,N-diphenylaminomethyleno) mangiferin (DAMM); mp: 210°, Rf: 0.80, λ max: 257.6, 240.8, 305.6, 364.4 nm. IR (KBr) cm⁻¹: 3371(O-H), 2931(C-H), 1647(C=O), 1405(C=C), 1297(C-N), 1253(C-O), 1031(C-O-C). NMR (δ ppm): 13.78 (ArOH intramolecurally 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-CH₂-N, 2H), 2.1 (-C-OH, 4H), 3.7 (-CH-O-, 2H), 3.3 (-CH-, 2H). 3.4 (-CH-, 3H).

5-(N-α-naphthylaminomethyleno) mangiferin (NAMM); mp: 205°, Rf: 0.60, λ max: 244.4, 297.2, 306.8 nm. IR (KBr) cm⁻¹: 3443(O-H), 3339(N-H), 2927(C-H), 1621(C=O), 1482(C=C), 1385(-C-N), 1290(-C-O), 1031(C-O-C). NMR (δ ppm): 13.78 (ArOH intramolecurally 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-CH₂-N, 2H), 4.1(Ar-NH, 1H), 2.1 (-C-OH, 4H), 3.8 (-CH₂-O-, 2H), 3.4 (-CH-, 5H).

Antimicrobial evaluation was determined using the disc diffusion method[14]. Bacterial strains of Staphylococcus aureus subsp. aureus (MTCC-737) and Escherichia coli (MTCC-1687) and fungal strains of Candida albicans (MTCC-183) and Aspergillus niger (MTCC-228) 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 5 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 h for bacteria and 30° for 48 h 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 (Table 1).

In the process of isolation of mangiferin, stem bark of Mangifera indica was defatted with petroleum ether (60-80°) prior to extraction with ethanol 95%. 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. Mangiferin analogues such as PAMM, CPAMM, MPAMM, MxPAMM, DAMM and NAMM were synthesized. The synthesized mangiferin analogues were characterized by Rf, 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[15]. Mangiferin and its derivative were also confirmed by proton NMR signals. Mangiferin and its analogues were subjected to antimicrobial study. From the extent of zone of inhibition, the activities were compared. All the analogues exhibited moderate to mild activity against Staphylococcus aureus, Escherichia coli, Candida albicans, Aspergillus niger. One of the mangiferin analogues namely MPAMM was found to be more effective than other compounds against Gram-negative organism, Escherichia coli. In the antifungal activity study, mangiferin and NAMM were found to be more effective than other compounds against Candida albicans. Good activity against Aspergillus niger was shown by 5-(N-4-methylphenylaminomethyleno) mangiferin.

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**Spectrophotometric Quantitation of Metformin in Bulk Drug and Pharmaceutical Formulations using Multivariate Technique**

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Arayne, et al.: Quantitation of Metformin

A sensitive and accurate UV spectrophotometric method with multivariate calibration technique for the determination of metformin hydrochloride in bulk drug and different pharmaceutical formulations has been described. This technique is based on the use of the linear regression equations by using relationship between concentration and absorbance at five different wavelength. The results were treated statistically and were found highly accurate, precise and reproducible. The method is accurate, precise (% recovery 102.50±0.063, CV ≤ 0.56, r =0.997) and linear within the range 1-10 µg/ml. There was no interference from the excipients i.e Povidone K 30, magnesium stearate, lactose and hydroxypropylmethylcellulose. This statistical approach gives optimum results for the eliminating fluctuations coming from instrumental or experimental conditions.

Keywords: UV spectrophotometry, metformin, pharmaceutical analysis, biguanide derivative

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Metformin hydrochloride (N,N-dimethylimidodicarbonimidicdiamide hydrochloride) is a biguanide prescribed for the treatment of type II diabetes mellitus, and is the drug of choice in obese patients. It increases glucose transport across the cell membrane in skeletal muscles and it can inhibit the formation of advanced glycosylation end-products.

The reported methods for determining metformin alone [1], in multicomponent dosage forms [2,3] in combined dosage forms [4-6], in human serum [7-10] were either by HPLC, gas chromatography [11], capillary electrophoresis [12,13], NMR spectrometry [12], fluorimetry [14], potentiometry [14], PVC membrane sensor [15,16], conductometry [17,18] and NIR spectroscopy [19]. Most of these are either time consuming; involve expensive instrumentation or the use of excess organic solvents. There is no direct UV spectrophotometric method reported in the literature...