International Journal of Multidisciplinary and Current Research

Research Article

ISSN: 2321-3124
Available at: http://ijmcr.com

Biological Activity of Modified Chrysin

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Accepted 15 January 2014, Available online 10 February 2014, **Vol.2 (Jan/Feb 2014 issue)**

Abstract

The aim of the present study was to determine the effect of chrysin and its metal conjugates chrysin-copper and chrysin-manganese on auxotrophic strains of Escherichia coli for lysine and vitamin B12. Further studies were also done on Lactobacillus delbrueckii for histidine. The auxotrophic strains were supplemented with chrysin metal conjugates along with their respective growth limiting factor. Gradient was created by increasing the concentration of chrysin conjugates and gradually decreasing the concentration of growth limiting factor sequence. At 0.15 μg/ml concentration of chrysin metal conjugates auxotrophic strains were reverted to prototrophs. The reverted strains were grown in minimal agar with Ethidium Bromide and organisms were able to grow in the range of 0.10μg/ml up to 0.34μg/ml concentration. From Reverse Mutation assay Chrysin and its metal conjugates proved to be mutagenic agent but not a carcinogen. The assumption of Ames test that when a compound is mutagen there is 90% chance of it being carcinogen but chrysin comes in remaining 10% i.e. it is a mutagen but not a carcinogen.

Keywords: *flavonoids, chrysin, anticarcinogen, metal complex*

Abbreviations: *FTIR,-Fourier Transform Infrared, DMSO-Dimethyl sulfoxide*

1. Introduction

Flavonoids are a broad class of polyphenolic secondary metabolites abundant in plants and in various common foods such as apples, onions, tea, red wine etc. Apart from their important biological roles in nitrogen fixation and chemical defence, flavonoids possess a broad range of pharmacological properties.[1]

Chrysin (5,7-dihydroxyflavone), a natural, widely distributed flavonoid, has diverse biologically active properties, including anti oxidative, [1,2] anticancer,[3] anxiolytic,[4] anti inflammatory,[5,6] anti-diabetic,[7] and anti-glucosidase characteristics.[8] Recently several researchers attempted to modify chrysin and found that some chrysin derivatives have diverse activities including anti-diabetic effects.[9–15]

Detailed studies related to characterisation and synthesis of metal complexes of chrysin has not yet been done.

Chrysin is one of the lesser-known flavonoids [16,] (Z Barghouthi -2013). It has been synthesized and characterized as solid complexes of several metal ions: Co (II), Ni (II), Cu (II), Cd (II), Pb (II) and Fe (III) etc.

In this study commercially available chrysin (Sigma Aldrich) was used to synthesize chrysin metal conjugates using copper and manganese. Later the synthesized products were characterized and used for investigation on studies with Auxotrophic strains. Our aim was to study reverse mutation effect of chrysin and its metal conjugates on few bacterial strains. For furthermore studies we exposed the strains to chrysin, its metal complexes and ethidium bromide, a known carcinogen.

2. Materials and Methods

Chemicals- Chrysin purchased from Sigma Aldrich, Copper acetate from Merck chemicals and manganous acetate S M Fine Chemicals Ltd. of LR grade. All other chemicals were analytical grade.

Synthesis of Copper and Manganese Complexes of Chrysin: It involves interaction of the methanolic solutions of copper acetate and manganous acetate with chrysin in 1:2 metal ligand stoichiometries and maintaining the reaction mixture at pH 8 with the help of 0.2 M sodium acetate (CH_3COONa), at room temperature with constant stirring for 6 hrs using a magnetic stirrer. The precipitating metal conjugates (green in case of Copper and brown in case of Manganese) were collected by centrifugation and washed with methanol. Finally, all compounds were dried under vacuum.

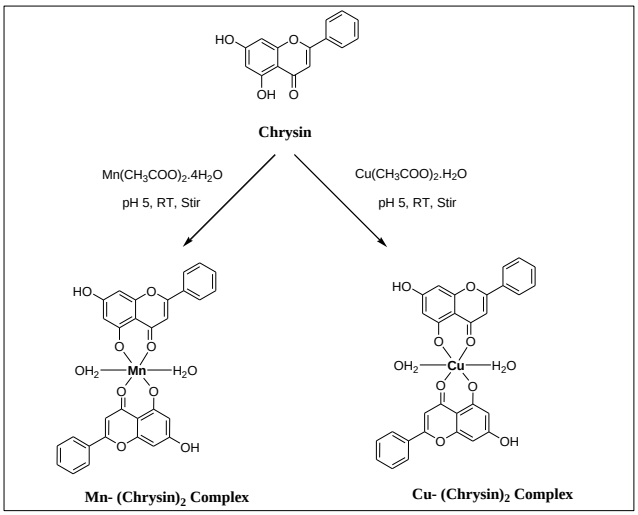


Fig 1: Schematic representation of Preparation of Copper and Manganese complexes of Chrysin

Characterization of isolated compounds

I. Thin layer chromatography:

Steps involved in performing TLC of isolated compound:
TLC plate: Precoated Silica TLC plate (Merck) was used for sample application.
Activation of TLC plate: oven for 10 min. at 50^{O}C.
Mobile Phase: Ethyl acetate: Methanol: Water: Formic acid; 10:1:1:0.5(Reference 17)

Sample application: Capillary tubes were dipped into the sample solution to be examined and spotting was done on the TLC plate at a point about 2 cm from the bottom. The spot was then air-dried. Chamber saturation: The glass chamber used for TLC was saturated with mobile phase for about 30 mins.

Chromatogram development: samples were spotted on the plate, and were kept in the chamber. The solvent level was maintained in the chamber. The solvent was allowed to run up to 3/4th of the silica plate.

Visualization: Plates were removed and were examined visually, under UV light. R_f value was calculated by following formula [Wagner and Bladt, 1996]

R_f = Distance travelled by solute from origin line/ Distance travelled by solvent from origin line

II. FT-Infra red spectroscopy:

Infra red spectra were recorded as potassium bromide pellets on (Shimadzu 8400S Infra red spectrophotometer) at Department of Chemistry, University of Pune. Potassium bromide was heated for 30 minutes to remove moisture. Sample was mixed thoroughly with potassium bromide in proportion of 1:9. The pellets were prepared from the above mixture and a spectrum was measured under FTIR instrument by plotting graph of % transmittance vs. wave number.

III. UV-Visible spectroscopy

Electronic absorption spectra were recorded on UV-Visible spectroscope (Jasco V630) using a matched pair of 1cm^2 quartz cells at Allana College of Pharmacy, Pune .Chrysin is easily soluble in methanol; hence samples of chrysin were prepared in methanol. When chrysin is complexed with metal its solubility decreases and the metal complexes are insoluble in methanol. So, in order to fully solubilise and analyse metal complexes samples were prepared in DMSO.

1. Reverse mutation assay:

Sample preparation:

Stock solutions of chrysin and its metal complexes were prepared by dissolving in 1M DMSO solution and stock solution of 0.05mM histidine; lysine & Vitamin B_{12} were prepared in double distilled water.
Media: Minimal Broth
Strains used: Auxotrophic

- *Lactobacillus delbrueckii NCIM 2025 (lysine)*
- *Escherichia coli NCIM 2089 (histidine)*
- *Escherichia coli NCIM 1133 (vitamin B 12)*

Procedure:

Chrysin and its metal complexes were tested against auxotrophic strains.Chrysin was taken in concentration of 300μg/ml. Culture suspension was provided with required supplement i.e. 0.05mM histidine, lysine and vitamin B12.Chrysin solution with respective supplement was added to sterile 10 ml minimal broth. A loopful of culture suspension was added and kept for incubation for 24 hours at room temperature.

Later the concentration of chrysin was increased and concentration of respective supplement was decreased in fresh sterile 10 ml minimal broth and 100ul culture of grown culture was transferred from previous day flask.

Increasing gradient of chrysin concentration and decreasing gradient of supplement concentration was done till the concentration of supplement was zero as shown in the table below. The auxotrophic cultures grow in presence of chrysin and their respective amino acid supplement which they are unable to synthesize.

2. Anticarcinogenic Activity:

Anti carcinogenic property of chrysin was tested against reverted *E.coli* 2089 culture in presence of a known carcinogen ethidium bromide. The reverted culture of *E.coli* 2089 was exposed to ethidium bromide in 10ml

sterile minimal broth with equal amount of chrysin. The amount of ethidium bromide and chrysin exposed to *E.coli* 2089 were in different concentrations viz- 0.10μg, 0.20μg, 0.30μg and 0. 40μg Growth was observed up to 0.30 μg but no growth was seen at 0.40μg concentration of ethidium bromide.

The concentration was further narrowed down between 0.30 to 0.40 μg viz-0.32, 0.34, 0.36, 0.38 & 0.40μg. Growth was observed up to 0.34μg.The concentration was further narrowed down to 0.33, 0.34 & 0.35μg. Chrysin inhibited activity of ethidium bromide to a concentration of 0.35μg.

Results and Discussion

Table I: Electronic spectral assignments of chrysin and chrysin metal complexes

Compound	π- π* (nm)	n- π* (nm)	d-d transitions	Charge transfer
CHRY	212, 268	313	_____	_____
CRCU	_____	_____	672.5	_____
CRMN	_____	_____	_____	405

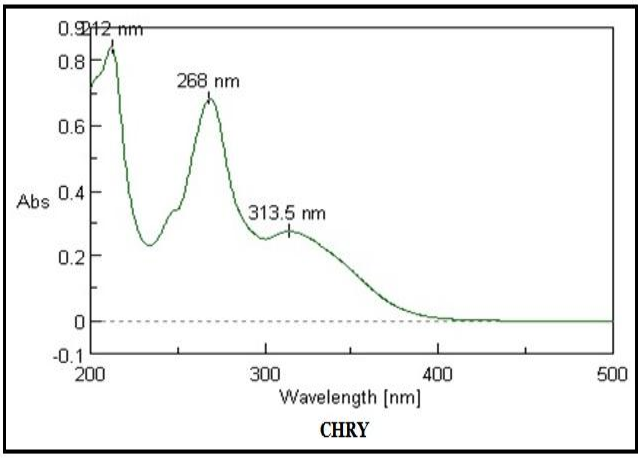
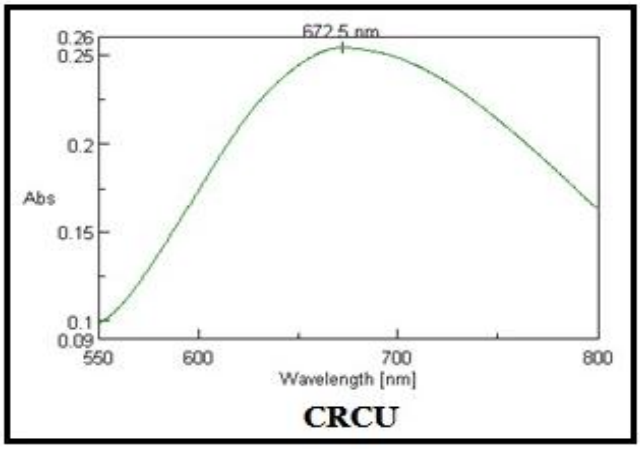


Fig 2: Electronic spectrum of Chrysin



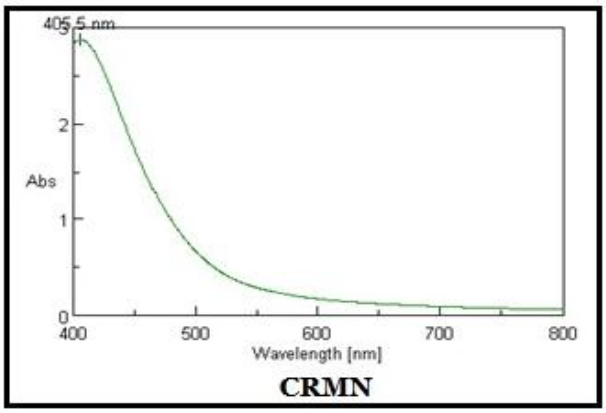


Fig 3: Visible Spectra of Copper and Manganese Complexes of Chrysin

IR Spectrum of Chrysin and its Metal Complexes:

The characteristic IR absorption frequencies in the spectral range 4000-400 cm^{-1} were measured for free Chrysin and its metal complexes and is summarized in Table 02. The IR absorption spectra of copper and manganese complexes clearly indicate that the free chrysin molecule loses its original characteristics and participates in coordination with the metal ions.

Table II: Important IR absorption ranges of chrysin and its metal complexes

Compound	O-H	C-H	C=O	C=C
CHRYSIN	3009, 3080	2945	1655	1610
CRCU	3383	2897	1643	1608
CRMN	3410	3076	1629	1593

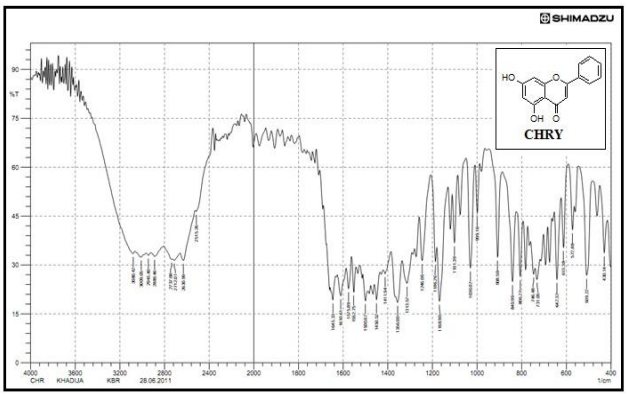


Fig 4: Infrared Spectra of Chrysin

An intense band in the region 3500-3000 cm^{-1} appeared in the spectrum of the ligand which is attributed to the symmetrical and asymmetrical stretching modes of O-H

which undergo change in intensity in the spectra of the complex. This reduction in intensity suggests the loss of one OH group during the coordination to the metal ions. A strong band at about 1655 cm^{-1} detected in the spectrum of the ligand is assigned to C=O which was shifted in the spectra of the metal complexes which indicates that the coordination occurs through the C=O oxygen atom.

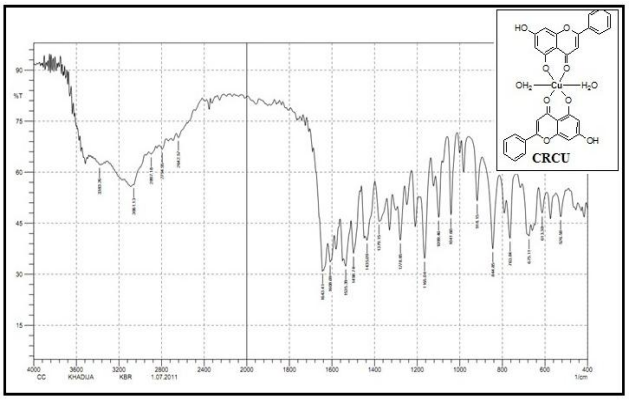


Fig 5: Infrared Spectra of Copper Complex of Chrysin

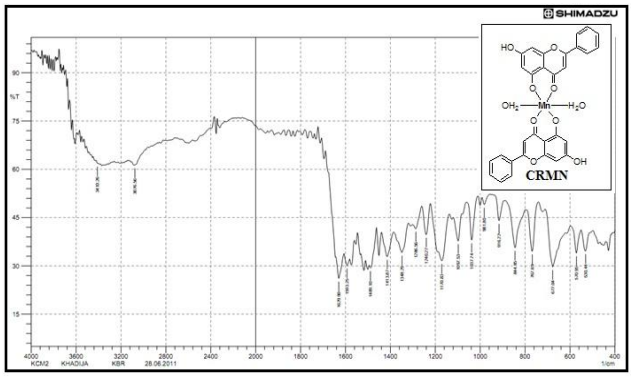


Fig 6: Infrared Spectra of Manganese Complex of Chrysin

IV. Mass spectroscopy:

MS Spectrum of chrysin metal conjugates showed m/z charge value 561.8 for chrysin-copper complex and 607.8 for chrysin-manganese complex.

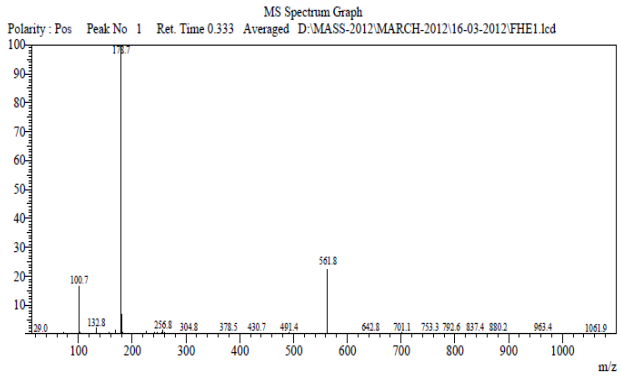


Fig 7: Mass Spectra of Chrysin-Copper complex

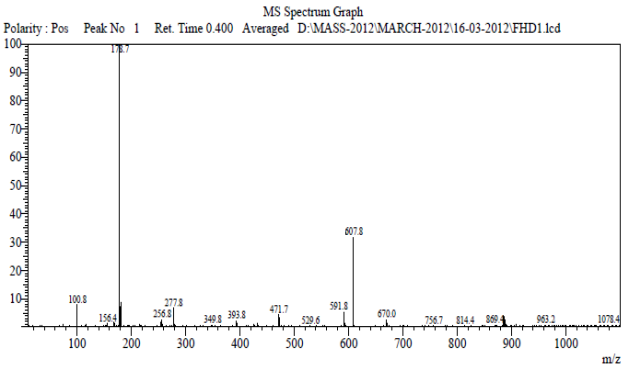


Fig 8: Mass Spectra of Chrysin-Manganese complex

Biological studies

From Reverse Mutation assay chrysin and its metal conjugates proved to be mutagenic agents but not a carcinogen. The assumption of AMES test that when a compound is mutagen there is 90% chance of it being a carcinogen was not applicable but chrysin seems to be in the remaining 10% that it is a mutagen but not a carcinogen.

Conclusion

Chrysin is reported to be an anticancer agent in a number of studies .It was complexed with manganese for the first time to enhance its activity. The formation of metal complexes of chrysin was confirmed by the UV-VIS, FTIR and Mass spectroscopy studies. In biological studies it is proved to be a mutagen but not a carcinogenic compound. The metal complexes showed more potency than the lead compound. Chrysin and its metal conjugates act as mutagenic agents in conversion of auxotrophs to prototrophs. Chrysin acts against known carcinogen i.e. ethidium bromide. Thus it can be claimed that metal complex formation is a useful strategy for enhancing the activity of the lead compound.

Acknowledgement

We are thankful to Dr. E.M Khan Principal and the Management of Abeda Inamdar Senior College, Azam Campus, Pune, for their constant support and motivation.We also appreciate and thank Dr. S B Padhye (Director, ISTRA) for his kind support and guidance in conducting the experimental work.

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