Int J Pharm Bio Sci 2016 July; 7(3): (P) 155 - 158

**Original Research Article** **Medicinal Chemistry**

**International Journal of Pharma and Bio Sciences** **ISSN 0975-6299**

# ISOLATION OF MANGIFERIN AND IT'S ANTIVIRAL EVALUATION

**E.SHANMUGAPRIYA*[1], DR.V.RAVICHANDIRAN[2] AND DR.M.VIJEYAANANDHI[1]**

*[1]Department of Pharmaceutical chemistry, Vels University, Chennai, India.*
*[2]Director, NIPER, KOLKATA, India.*

## ABSTRACT

Mangiferin is a natural occurring xanthone C-glucoside. Mangiferin, (1, 3, 6, 7-tetrahydroxy xanthone-C2-b- D glucoside) has been isolated from various parts of *Swertia chirata*. Mangiferin is a pharmacologically active phytochemical present in large amount in bark, fruits, roots and leaves of *Mangifera indica*.The present work is aimed to isolate mangiferin by column chromatography from the ethanolic extract of various parts of *Swertia chirata* and assess its antiviral activity. The conclusive structure of the isolated compound was established using TLC, HPLC, UV/VIS, FTIR and NMR spectral analysis. *In vitro* antiviral activity of the isolated mangiferin was studied. The present study confirms the antiviral effect of the isolated mangiferin which could be further processed for its development as an antiviral agent.

**KEYWORDS**: **Mangiferin, Isolation, TLC, UV, HPLC, Antiviral activity**

**E.SHANMUGAPRIYA**
**Department of Pharmaceutical chemistry, Vels University, Chennai, India.**

## INTRODUCTION

India has a rich heritage of traditional medicine.[1] Materia medica of India provides a lot of information on the folklore claims and traditional aspects of therapeutically important natural products. India has a rich heritage of traditional medicine which constitutes different components like Ayurveda, Siddha and Unani. The development of these traditional systems of medicines with their safety, efficacy and quality will not only help to preserve the traditional heritage but also to rationalize the use of natural products in pharmaceutical sector.Natural products have been our utmost and successful source of medicine. The use of plant compounds for pharmaceutical purposes has gradually increased worldwide. The research and development thrust in the pharmaceutical sector is focused on the development of new indigenous plant-based drugs through the investigation of leads from the traditional system of medicine. Each plant is like a chemical factory capable of producing a limited number of highly complex and unusual chemical substances derived from plants that are considered as important drugs currently in use, while several other drugs are simple synthetic modifications of the natural products.[2] Mangiferin has been traditionally used in some parts of world as anti-inflammatory, antibacterial, analgesic, antipyretic, antioxidant, antitumor, antidiabetic and in obesity treatment.[3, 4, 5, 6] Mangiferin is also found to exhibit antiviral and immunomodulatory effects.[7, 8, 9]

## MATERIALS AND METHODS

*Swertia chirata* plant was purchased from Yucca enterprises, Mumbai. All the other solvents and chemicals were of analytical grades. Vero cells and DENV -2 required for the test were being maintained at the Department of Virology, King Institute of Preventive Medicine (KIPM), Guindy, Chennai – 600 032.

### *Isolation from Swertia chirata*

The coarsely powdered plant material of S.chirata were extracted exhaustively with petroleum ether(60-80°C) in soxhlet apparatus for 56 hours to remove any fatty matter. Defatted powdered plant parts were extracted by using soxhlet apparatus with required quantity of ethanol (95%) as solvent for 21 hours and concentrated under reduced pressure to yield a semisolid mass. The semisolid mass was defatted repeatedly and finally dissolved in ethanol at room temperature. The ethanolic extract was further concentrated under reduced pressure which yielded a yellow amorphous powder.

### *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°C). The column was eluted with chloroform: acetone: formic acid (8: 1.5: 0.5), which gave mangiferin as a pale yellow amorphous powder. This upon crystallization using ethanol produced pale yellow needle shaped mangiferin crystals. Lastly, the pale yellow needle-shaped crystals of mangiferin were isolated and dried.The isolated compound was further characterized using TLC, HPLC, UV/VIS, FTIR and NMR spectrophotometer. The melting point of the isolated compound was also determined.

### *Identification of the Isolated Mangiferin*

#### *Thin layer chromatography*

***Adsorbent*:** Pre-coated and pre-activated TLC plates (silica gel GF 254)
***Mobile phase***:Chloroform: acetone: formic acid (8: 1.5: 0.5)
***Sample preparation*:** 0.05% w/v of sample (mangiferin) was prepared in ethanol
***Sample Volume*:** 10 µl was applied on the TLC plate.
***Detection*:** Ammonia vapour was used as spraying agent.
***Detection wavelength*:** 366 nm.
***Development time*:** 10 minutes

#### *High Performance Liquid Chromatography*

***Method:*** Reverse phase high performance liquid chromatography (RP-HPLC).
***Detection Wavelength*:** 278 nm.
***Column*:** Hypersil™ ODS C-18 analytical column (4.6 × 100 mm; 3.5 µm).
***Mobile phase*:** Chloroform: acetone: formic acid (8: 1.5: 0.5).
***Flow rate*:** 1 mL $min^{-1}$.
***The injection volume*:** 0 µL
***Column temperature*:** 25°C.

Five (5) mg of the isolated mangiferin crystals were dissolved in 10 ml methanol, it was filtered and injected six times in an HPLC column (ODS C-18) and its retention time was determined and was compared with thereferencestandard.

### *Ultraviolet Spectroscopy*

One (1) mg of the isolated mangiferin crystals were dissolved in methanol and the maximum wavelength of absorption were determined by UV-VIS spectrophotometer (UV-1800-240V SHIMADZU). Scanning of the isolated compound was performed at the wavelength range of 200-400 nm and was compared with reference standard.

### *Fourier Transform Infrared Spectroscopy*

One (1) mg of the isolated mangiferin crystals were measured using potassium-bromide (KBr) pellet method in FTIR spectrometer (Bruker-Alpha).IR data of isolated compound was compared with the reference standard of mangiferin.

### *Nuclear Magnetic Resonance Spectroscopy*

NMR spectra of the isolated mangiferin crystals were obtained on Brukeravance II-400 MHz, spectrometer using TMS as internal reference.[11]

## RESULTS

### *Melting point*

Melting point of isolated mangiferin was 268°C.

### *Ultraviolet spectroscopy*

The UV spectrums of isolated mangiferin and reference standard in methanol showed two major peaks which were as follows: 278 nm and 216 nm.

***IR spectrum of mangiferin***

| Absorbance | Groups |
|---|---|
| 1670 | C=O keto group |
| 3367 | Phenolic OH stretching |
| 1199 | C=O Stretch |
| 2918,2850 | Aliphatic C-H Stretch |
| 1625 | Aromatic C=C Ring Stretch |
| 1050 | $RCH_2OH$ OH Stretching |

***$R_f$ value of isolated mangiferin***

| Observation | $R_f$ values | Average |
|---|---|---|
| 01 | 0.82 | 0.8 |
| 02 | 0.80 | |
| 03 | 0.81 | |

***Anti-viral activity of Swertia chirata***

Extracts of different anatomical plants were prepared by using Soxhlet extraction unit as per the standard procedure. Leaves, stems and roots of *Swertia chirata* were shade dried and powdered. Fifty grams each of respective samples were soaked in 500mL of double distilled water and 70% methanol separately. They were extracted with Soxhlet apparatus. The crude preparation was filtered through Whatman no.1 filter paper and dried at 50˚C. The drug was scrapped and stored at 4˚C. Vero cells and DENV -2 required for the test are being maintained at the Department of Virology, King Institute of Preventive Medicine (KIPM), Guindy, Chennai – 600 032. Toxicity and antiviral assays were done using 1 g of leaves, stems and roots. Aqueous extracts were prepared and dissolved in 10 mL of sterile double distilled water to give a final concentration of 30 µg - 750 µg. It was filter sterilized using 0.45 micron syringe filter. Methanol extracts were weighed and dissolved in 10 mL of 0.5% Dimethyl Sulfoxide (DMSO) and filter sterilized using 0.45 micron syringe filter. The non- toxic dilution of DMSO to Vero cell line was used to dissolve methanol extracts. The toxicity assay was performed using aqueous and methanol extracts (30 - 750 µg) of different anatomical parts of *Swertia chirata* DENV -2 was propagated in Vero cells and $10^{-7}$ $TCID_{50}$/ mL was taken for antiviral assay. This study was carried out by mixing 1ml of drug (30 - 750 µg concentration) and 1 ml of different doses of virus. The mixture was incubated at different time intervals, viz. Immediate, 30, 60, 90 and 120 min. The whole assays were observed for a period of 5 days. The cytopathogenic effect (CPE) was scored. The 50 per cent cytotoxic concentration (CTC50) was determined by the standard MTT assay. Standard Acyclovir dissolved in distilled water and used as a standard antiviral drug (1 mg/mL) with respect to test compounds at a concentration of 100µg/mL to30 µg/mL. Antiviral activity of various concentration of acyclovir was carried out against DENV-2

***Antiviral activity of Acylovir***

$IC_{50}$ µg/mL (Acyclovir)

| Virus | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 |
|---|---|---|---|---|---|---|---|---|---|---|
| Denv-2 | 0 | 0 | 0 | + | ++ | +++ | ++++ | ++++ | ++++ | ++++ |

***0, no protection; +, 25% protection; ++, 50% protection; +++, 75% protection; ++++,100% protection***

***$IC_{50}$ - Inhibitory Concentration for 50 percent of viruses***

***Antiviral activity of Mangiferin***

$IC_{50}$ µg/mL (Mangiferin)

| Virus | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 |
|---|---|---|---|---|---|---|---|---|---|---|
| Denv-2 | 0 | 0 | 0 | 0 | + | ++ | +++ | ++++ | ++++ | ++++ |

***0, no protection; +, 25% protection; ++, 50% protection; +++, 75% protection; ++++, 100% protection***

***$IC_{50}$ - Inhibitory Concentration for 50 percent of viruses***

***Antiviral activity of Swertia chirata***

| Anatomical parts used | Extracts | Cytotoxic Concentration (µg/ml) | Concentration tested (µg/ml) $IC_{50}$ | CPE inhibition Assay | | |
|---|---|---|---|---|---|---|
| | | | | $2TCID_{50}$ | $10TCID_{50}$ | $100TCID_{50}$ |
| Leaves | Aqueous | 300 | 100 | ++++ | ++++ | ++++ |
| Leaves | Methanol | 30 | 25 | ++++ | ++++ | ++++ |
| | | | 20 | 0 | 0 | 0 |
| Root | Aqueous | 300 | 100 | 0 | 0 | 0 |
| Root | Methanol | 30 | 25 | 0 | 0 | 0 |
| Stem | Aqueous | 300 | 100 | 0 | 0 | 0 |
| Stem | Methanol | 30 | 25 | 0 | 0 | 0 |

## DISCUSSION

In the present study, the plant parts of *Swertia chirata* was first defatted with petroleum ether (60-80°C) prior to extraction with 95% ethanol. Followed by this, the extract was chromatographed over silica gel and eluted with chloroform: acetone: formic acid (8:1.5:0.5) to obtain mangiferin as a pale yellow needle shaped crystals. The isolated mangiferin crystals were characterized by $R_f$, melting point, HPLC and UV, FTIR and NMR spectral analysis. The isolated mangiferin obtained from the ethanolic extract of plant parts of *Swertia chirata* showed identical TLC chromatography, HPLC and UV, FTIR and NMR spectrum to reference standard mangiferin. The absorbed maxima 278 nm and 215 nm of isolated mangiferin crystals is closely related to that of reported reference standard UV spectral data. Mangiferin was also confirmed by proton NMR signals. The available literature on structure elucidation of the isolated mangiferin reveals the conclusive structure of mangiferin ($C_{19}H_{18}O_{11}$) can be established as glucoxanthone (1,3,6,7-tetrahydroxyxanthone-$C_2$-b-D-glucoside). In vitro antiviral activity of the isolated mangiferin was evaluated by using standard $TCID_{50}$ method. The solution of the isolated mangiferin was found to exert promising antiviral activity against New Guinea strain of Dengue virus. It was observed that the aqueous extract of *Swertia chirata* have shown the inhibition of HSV-1 viral dissemination.[12] Putranjivain A, a isolate of Euphorbia jolkini Bioss showed late stage inhibition of HSV-2 replication in-vitro.[13] Various solvent extracts having different potent phytochemicals as anti-viral targets against DENV 2 has been carried out. We report here that the methanolic extract of leaves from swertia chirata was the most active anti-viral agent against DENV-2.

## CONCLUSION

The present study found a very promising new source for treating infections caused by virus. This is particularly significant because drug resistance to human pathogen has been increased not only in the developing countries but throughout the world due to indiscriminate use of antibiotics. So from the present screening it could be concluded that mangiferin possess antiviral activity and may be processed further for the development of an antiviral agent.

## REFERENCES


1. Archana, Suresh Jatawa, Rajkumar Paul and Archana Tiwari. Indian Medicinal Plants: A Rich Source of Natural Immuno-Modulator. *Int J Pharmacol,* 2011. *7: 198-205.*
2. Ye Wu Qiu, Hong gang hu, Ming juan Wang, Qing jie Zhao, Shi Chong Yu, Chao Mei Liu et al. Synthesis of mangiferin derivates and study their potent PTP1B inhibitory activity. *Chinese Chem Lett* 2007; 18 :1323-6.
3. Gong X, Zhang L, Jiang R, yeM, YinX,WanJ Antiinflammatory effects of mangiferin on sepsis induced lung injury in mice via up regulation of heme oxygenase-1 *J Nutr Biochem* 2013 June;24:1173-81.
4. Doughari JH and Manzara S In vitro antibacterial activity of crude leaf extract of *Mangifera indica* Linn. *Afr. J. Microbiol* Res. 2008; 2**:** 67-72.
5. Gabino G, Gonzalez D, Delporte C. Analgesic and anti-inflammatory effect of M. indica L. extract (Vimang). *Phytother* Res 2001; 15**:**18-20.
6. Stoilova I, Gargova S, Stoyanova A, Ho L. Antimicrobial and antioxidant activity of the polyphenol mangiferin. *Herba Polonica* 2005; 51**:**37-44.
7. Guha, S., Ghosal, S., Chattopadhyay, U., Antitumor, immunomodulatory and anti-HIV effect of mangiferin, a naturally occurring glucosylxanthone. *Chemotherapy* 1996; 42: 443–451.
8. Zheng M S, Lu Z Y. Antiviral effect of mangiferin and isomangiferin on Herpex simplex virus. *Chin Med J* 1990; 103**:** 160-65.
9. Bodhankar S, Makare N, Rangari V. Immunomodulatory activity of alcoholic extract of Mangiferaindica L. in mice.*J Ethnopharmacol* 2001; 78:133-7.
10. Gehad T, El-Sherbini, SamirM.Osman Anthelmintic activity of unripe Mangifera indica Linn, (Mango) *Strongyloides stercoralis Int. J. curr.Microbiol.App.Sci* (2013) ;295: 401-409
11. Haynes LJ, Taylor DR. Mangiferin; the nuclear magnetic resonance spectra of xanthone*s. J ChemSoc* (c) 1966; 1685-7.
12. Verma H, Patil PR, Kolhapure RM, Gopalkrishna V. Antiviral activity of Indian medicinal plant extract Swertia chirata against herpes simplex virus: A study by in vitro and molecular approach. Indian J Med Microbiol 2008; 26: 322-6.
13. Cheng HY, Lin TC, Yang CM, Wang KC, Lin LT, Lin CC. Putranjivain A from Euphorbia jolkini inhibits both virus entry and late stage replication of herpes simplex virus type 2 in vitro. J Antimicrob Chemother 2004; 53: 577-83.