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Original article

Xanthone C-glycosides isomers purified from *Dryopteris ramosa* (Hope) C. Chr. with bactericidal and cytotoxic prospects

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ABSTRACT

Xanthones C-glycosides are plants secondary metabolites with diverse biological activities. Among the C-glycoside xanthones, the mangiferin (MF) is of widespread occurrence in plants while isomangiferin (IsoMF) is not very common. For the present study mangiferin (MF) and isomangiferin (IsoMF) were isolated from *Dryopteris ramosa*. The antibacterial potential of MF and IsoMF was evaluated by using agar well diffusion method while cytotoxic properties of MF and IsoMF were assessed by brine shrimp lethality test (BSLT). The antibacterial potential of MF and IsoMF increases in dose dependent manner. The minimum inhibitory concentration (MIC) indicated strong antibacterial potential of MF against *Salmonella setubal* (125 μg/mL) and *Bacillus subtilis* (125 μg/mL) while MF showed weak antibacterial potential against *Escherichia coli* (500 μg/mL). On the other hand the IsoMF showed better antibacterial potential against all the tested strain including *Escherichia coli* (MIC = 250 μg/mL). The MF and IsoMF showed poor cytotoxicity towards Brine shrimp nauplii as indicated by their LD_{50} (969.77 ± 0.67 and 768.92 ± 0.81 μg/mL respectively). The present study has highlighted the antibacterial potential of MF and IsoMF. Further evaluation of these two isomeric compounds may prove to be the future remedies for various bacterial infections and other human ailments.

1. Introduction

Xanthones belongs to one of the important class of plants secondary metabolite and are communal in higher plant families, fungi, and lichen (Cardona et al. 1990). In plants, xanthones are limited to certain number of plant families including; Gentianaceae, Guttiferae, Moraceae, Clusiaceae, and Polygalaceae, etc. Generally Xanthones are polyhydroxylated compounds but in most cases they are found as monomethyl or polymethyl ethers or they are found as glycosides (Hostettmann and Miura, 1977). In glycosidic xanthones nucleus of xanthone is linked with sugar moiety ether by C-C linkage (C-glycosides) or by C-O-C linkage (O-glycosides). O-glycosides are much more common compare to C-glycosides (Negi et al. 2013). Mangiferin and IsoMF are the most common C-glycosides xanthones with molecular formula "$C_{19}H_{20}O_{10}$". Mangiferin (2,-C-β-D glucopyranosyl-1, 3, 6, 7-tetrahydroxyxanthone) has widespread occurrence in flowering plants and non-seeded vascular plants and was first isolated from plant *Mangifera indica* (Iseda, 1957a, 1957b; Haynes and Taylor, 1966; Bhatia et al. 1967). Various parts of mango plant (*Mangifera indica*) are rich source of MF (Iseda, 1957a, 1957b; Ajila et al. 2010). Mangiferin attracted the interest of many researchers due to its antioxidant nature (Ngo et al. 2019). An isomer, IsoMF

Abbreviations: ATCC, American type cell culture; BSLT, Brine shrimp lethality test; CFU, Colony forming units; DMSO, Dimethyl sulfoxide; IsoMF, Isomangiferin; LD, Lethal dose; MF, Mangiferin; MIC, Minimum inhibitory concentration; Nic, Nicotine.

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(4-C-β-D-glucopyranosyl-1, 3, 6, 7 - tetrahydroxy xanthone), is not very common and it has been isolated from the aerial parts of *Anemarrhena asphodeloides* (Aritomi and Kawasaki, 1970) and *Dryopteris ramosa* (Ishaque et al. 2021). Mangiferin and IsoMF were also isolated from various fern genera including Acystopteris, Cystopteris, Gymnocarpium and Woodsia (Richardson and Thaddeus, 1983).

Mangiferin and IsoMF differ from each other in respect of position of sugar moiety with xanthone nucleus (Fig. 1). The chemical structure of natural product that accomplishes the four basics have been reported to have great bioavailability by oral route: these includes; less than 500 Dalton molecular weight; fewer than five contributor functions for hydrogen bonds; less than ten acceptor utilities for hydrogen bonds; and potential log P (calculated) less than + 5 (log $P_{mangiferin}$: + 2.73) (Masibo and He, 2008). Both MF and IsoMF fulfills these requisites. Mangiferin is one of the most studied C-glycosidic xanthone and it demonstrated potent antioxidant activity and multifactorial pharmacological effects, including antidiabetic, antitumor, lipo-metabolism regulating, cardioprotective, anti-hyperuricemic, neuroprotective, antioxidant, anti-inflammatory, antipyretic, analgesic, antibacterial, antiviral and immunomodulatory effects (Du et al. 2018). On the other hand the pharmacological properties of IsoMF was not explored may be due to its uncommon occurrence status. We had isolated both MF and IsoMF from the frond of *Dryopteris ramosa* in a previous study and showed that IsoMF promised better antioxidant potential than MF (Ishaque et al. 2021).

In continuation to the search of bioactive compounds from plants and their efficacy, the present study was designed to discuss the antibacterial efficacy of MF and IsoMF and compared their potential with known antibiotic. In a previous study Biswas et al. (2015) reported antibacterial activity of MF against two bacterial strains; *Staphylococcus aureus* and *Salmonella typhi*. But we have studied antibacterial potential of MF as well as IsoMF against five bacterial strains in the present study. In addition to their antibacterial properties we also discussed cytotoxic potential of these two C-glycosidic xanthone isolated from aqueous fraction of *Dryopteris ramosa*.

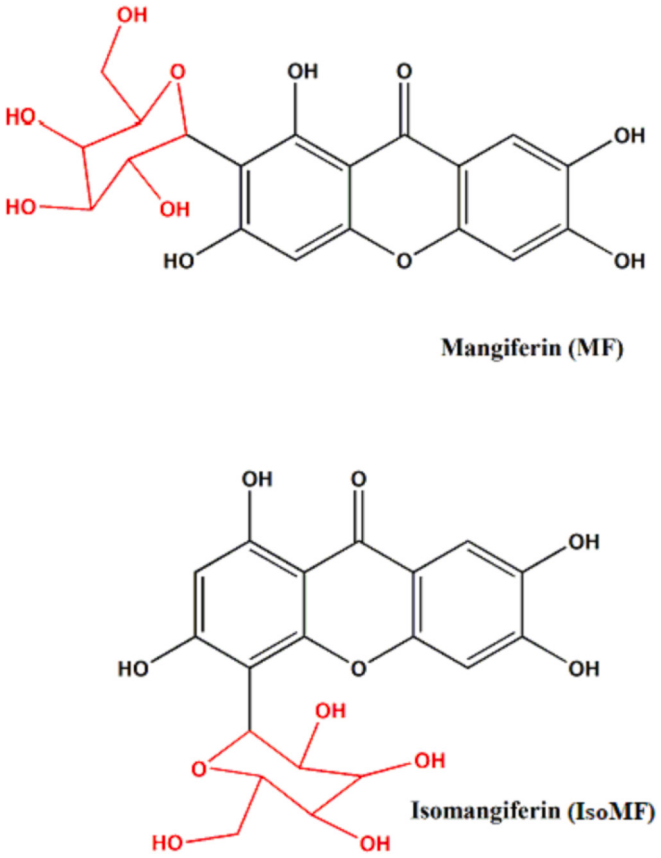

Fig. 1. Structure of mangiferin (MF) and Isomangiferin (IsoMF) isolated from frond of *Dryopteris ramosa*.

2. Experimental

2.1. Plant material and isolation of xanthones

Two isomeric xanthones (MF and IsoMF) were isolated from *Dryopteris ramosa*. The isolation of xanthones and their structure elucidation has been published (Ishaque et al. 2021). In pursue of bioactivities of isolated pure compounds from *Dryopteris ramosa*, the isolated pure compounds MF and IsoMF were used for the present study.

2.2. Evaluation of antibacterial properties

2.2.1. Bacterial strains and controls

A total five bacterial strains including *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 65380), *Klebsiella pneumonia* (ATCC 700603), *Salmonella setubal* (ATCC 19196) and *Escherichia coli* (ATCC 25922) bacteria species were tested against isolated pure compounds from aqueous fraction of *Dryopteris ramosa*. Cefixime (well-known antibiotic) and DMSO were used as positive and negative control respectively.

2.2.2. Preparation of inoculum

Genuine strains of bacteria were cultured in agar nutrient broth media at 37 ± 0.5 °C for 24–48 h and maintained in 0.85% NaCl solution [Turbidity = 0.5 McFarland standards and CFU = 10^8 ± 0.3] at 4 °C in slanted agar suspension.

2.2.3. Antibacterial bioassay

Agar well diffusion method as described by Valgas et al. (2007) was used with little amendments to estimate antibacterial prospective of MF and IsoMF. The seeded nutrient agar plates (with 1 mL of bacterial culture) were allowed to establish at 37 °C. After 10 min, equidistant wells (8 mm diameter) were cut on the surface of the agar with a sterile cork borer. From the various concentrations of MF and IsoMF, 20 μL was poured in each well. Then these nutrient agar plates were incubated at 37 °C, under aerobic environment. After 24 h, the inhibition of the bacterial growth around well was measured (in millimeters) by using scale and compared with standard and controls.

2.2.4. Minimum inhibitory concentration (MIC)

The lowest concentration (μg/mL) of every natural product that results in complete inhibition of bacterial growth was referred as MIC (Souza and Monache, 2005). The MIC was calculated as describe by Akinyemi et al. (2006) with few modifications. The MIC values of test compounds i.e. MF, IsoMF and control were calculated by nine sequential dilutions (1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.95 μg/mL). Three test tubes were used for every dilution. One tube containing test compounds, nutrient broth and inoculum (20 μL). While two control tubes were conserved i.e. one contained test compound, growth medium while second containing physiological saline and inoculum instead of test compound. After 24 h of incubation aerobically at 37 °C, MIC was determined for MF, IsoMF and controls against each bacterial strain.

2.3. Cytotoxicity evaluation

The cytotoxic prospective of MF and IsoMF was assessed by using brine shrimp lethality test (BSLT) as described by McLaughlin and Rogers (1998) with some modifications. Nicotine was used as control and standard cytotoxic compound.

2.3.1. Hatching of Artemia salina

Well aerated artificial sea water (salt 38 g/L) was used for hatching of Brine shrimp (*Artemia salina*) eggs. *Artemia salina* larvae (nauplii) 24–36 h old was used. The nauplii were attracted by a light source placed near walls of container.

2.3.2. BSLT-procedure

Stock solutions of tested compounds (MF and IsoMF) and standard were prepared in DMSO. Out of the stock solution, for each tested compound, various concentrations were prepared in artificial sea water (final volume = 5 mL) with not more than 1.25% DMSO (v/v) as suggested by Dai and Mumper (2010). Nicotine and artificial sea water were served as positive and negative controls respectively. *Artemia salina* nauplii (20 nauplii) were introduced in each concentration for 24 h at room temperature. The percentage death was determined by using equation;

$$Pd = (Tn - An / Tn) \times 100$$

Where;

Pd = percentage death,
Tn = total nauplii and,
An = Alive nauplii after 24 h.

Regression equation was used to calculate LD_{50} for MF, ISOMF and control.

2.4. Statistical analysis

All the results were presented in mean of triplicate with ± standard deviation (SD). Correlations between subject effects and main effects of test compounds were assessed by two way ANOVA at p = 0.05 by using SPSS 16.0. MS excel 2010 was used to calculate LD_{50} by regression line equation. All the charts and graphics were carried out in MS excel and MS world 2010. Chem. Draw pro 8.0 was used to draw the chemical structure of MF and IsoMF.

3. Results

3.1. Antibacterial potential of xanthones

Antibacterial potential of isolated xanthones from aqueous fraction of *Dryopteris ramosa* was compared with a well-known antibiotic commercial drug (Cefixime) by agar well diffusion method. The minimum and maximum inhibition zone for MF, IsoMF and Cefixime were ranging 6 ± 1.0 to 53 ± 1.0, 9 ± 1.0 to 51 ± 1.0 and 19 ± 0.3 to 95 ± 0.3 mm respectively (Fig. 2). The MIC values were determined against all the microorganisms (Table 1). The MIC value of MF, IsoMF, Cefixime and blank were presented in Table 1. The MIC value of MF and IsoMF ranges between 31.1 and 62.5 ± 0.7 μg/mL and 125 to 250 ± 0.5 μg/mL respectively against all the test microbes. The MIC values of isolated xanthone C-glycosides and control (Cefixime) were compared (Fig. 3).

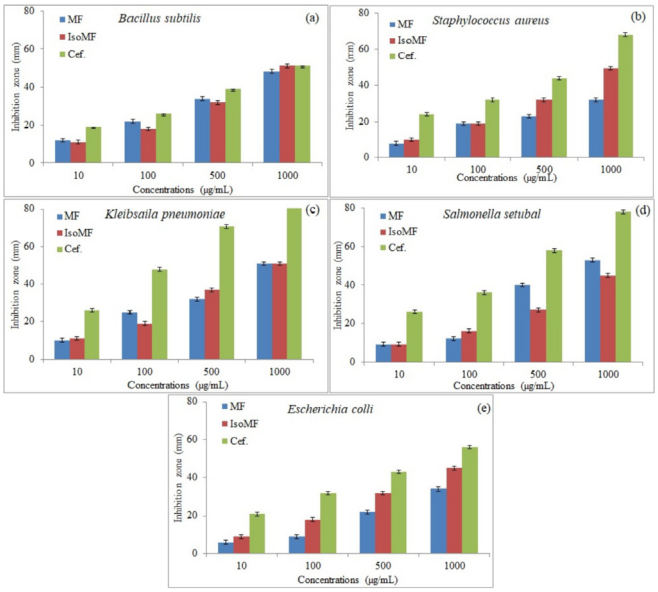

Fig. 2. Inhibition zones exhibited by isolated xanthones and Cefixime (control) against different strains of bacteria in agar well diffusion antibacterial assay. (MF = mangiferin, IsoMF = isomangiferin and Cef = Cefixime), n = 3.

3.2. Cytotoxic potential of isolated xanthones isomers from Dryopteris ramosa

The cytotoxic potential of isolated compound (MF and IsoMF) was assessed by *In- vitro* BSLT. The results were compared with strong cytotoxic compound nicotine. Brine solution was used as control. The mean percentage lethality was calculated by using the following formula;

$$\text{Mean percentage lethality} = T - T_l / T \times 100 / n$$

Where

T = Total number of larvae in each vial,
T_l = number of living larvae in each vial after 24 h and,
n = number of replicates.

The mean percentage lethality shown by MF, IsoMF and Nicotine (standard/control) has given in Fig. 4. The LD_{50} has calculated with the help of regression line equation in MS-Excel 2013 free software. The Fig. 5, showing the regression line equations for MF (Fig. 5a), IsoMF (Fig. 5b) and Nicotine (Fig. 5c) while Fig. 5d showed comparison of LD_{50} between MF, IsoMF and Nicotine which is 969.77 ± 0.67, 768.92 ± 0.81 and 46.72 ± 4.81 μg/mL respectively.

4. Discussions

In agar well diffusion method, the tested compounds inhibit the growth of microorganism around the well in agar plate. The diameter of inhibition zone so produced by compounds under analysis gives indication of their effectiveness against particular microorganism. The inhibition zone created by various concentrations of MF, IsoMF and Cefixime against different strains of bacteria were increased as dose dependent manner as presented in Fig. 2. Similar observations were also reported by Chidozie et al. (2014) in an antibacterial study on aqueous extract of *M. indica*. The MF showed least growth inhibition of *E. coli* (6 ± 1 mm) and *S. aureus* (8 ± 1 mm) while maximum inhibitions occurred in case of *S. Setubal* (53 ± 1 mm). Another previous study (Singh et al. 2009) reported

Table 1
Minimum inhibitory concentration of xanthone C-glycosides against selected bacterial strains.

Species	Minimum Inhibitory Concentration (μg/mL)								
	3.95*	7.81*	15.62*	31.25*	62.5*,†	125*,†	250*,†	500*,†	1000*,†
Mangiferin									
B. subtilis	+	+	+	+	+	a_#	–	–	–
S. aureus	+	+	+	+	+	+	a_#	–	–
K. pneumoniae	+	+	+	+	+	a_#	–	–	–
S. setubal	+	+	+	+	+	a_#	–	–	–
E. coli	+	+	+	+	+	+	+	a_#	–
Isomangiferin									
B. subtilis	+	+	+	+	+	b_♦	–	–	–
S. aureus	+	+	+	+	+	b_♦	–	–	–
K. pneumoniae	+	+	+	+	+	b_♦	–	–	–
S. setubal	+	+	+	+	+	+	b_♦	–	–
E. coli	+	+	+	+	+	+	b_♦	–	–
Cefixime									
B. subtilis	+	+	+	+	+	c_ζ	–	–	–
S. aureus	+	+	+	+	+	c_ζ	–	–	–
K. pneumoniae	+	+	+	c_ζ	–	–	–	–	–
S. setubal	+	+	+	c_ζ	–	–	–	–	–
E. coli	+	+	+	+	+	c_ζ	–	–	–
Blank/control									
B. subtilis	+	+	+	+	+	+	+	+	+
S. aureus	+	+	+	+	+	+	+	+	+
K. pneumoniae	+	+	+	+	+	+	+	+	+
S. setubal	+	+	+	+	+	+	+	+	+
E. coli	+	+	+	+	+	+	+	+	+

* (+) Represents growth of microorganism.
† (-) Represents growth inhibition of microorganism.
# (a-) MIC of Mangiferin against specific bacterial strain.
♦ (b-) MIC of Isomangiferin against specific bacterial strain.
ζ (c-) MIC of Cefixime against specific bacterial strain.

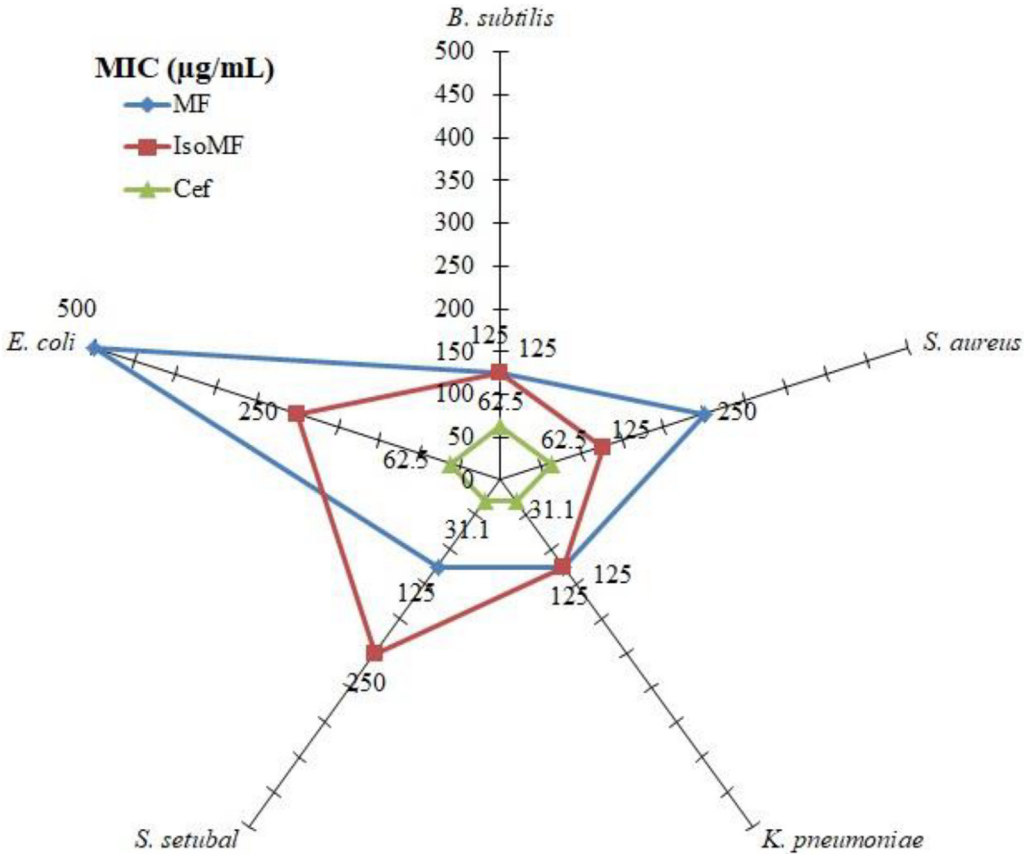

Fig. 3. Comparison between minimum inhibitory concentration (MIC) of isolated xanthones from *Dryopteris ramosa* and a well-known antibiotic (Cefixime). (MF = mangiferin, IsoMF = isomangiferin and Cef = Cefixime), n = 3.

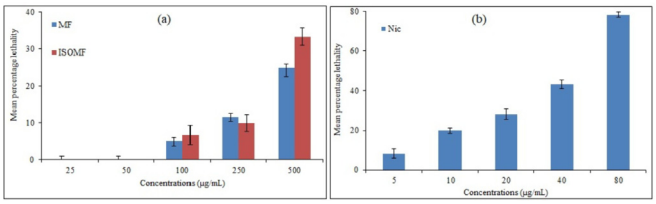

Fig. 4. Cytotoxic potential of mangiferin (MF), isomangiferin (IsoMF) and nicotine (Nic) against brine *Artemia salina* napulii. [F (4, 32) = 3.385, p = 0.020, R square 0.994, adjusted R square 0.991, n = 3] a) Mean percentage death of *Artemia salina* napulii caused by MF and IsoMF after 24 h of exposure. b) Mean percentage death of *Artemia salina* napulii caused by Nic after 24 h of exposure.

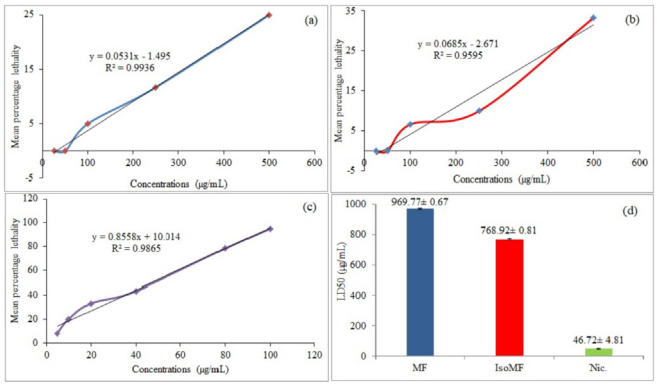

Fig. 5. Regression line equation obtained after BSLT for MF, IsoMF and Nic. a) Mangiferin (MF), b) Isomangiferin (IsoMF) and c) Nicotine (Nic) the standard-a well-known cytotoxic compound. d) LD_{50} of test compounds (isolated xanthones from *Dryopteris ramosa*) and the standard (Nic).

the similar observations. They calculated the inhibition of MF against *E. coli* (8 mm) and *S. aureus* (10 mm). Another study (Stoilova et al. 2005) about antibacterial effects of MF reported the 22 ± 0.30 mm, 0 ± 0 mm and 20 ± 0.40 mm inhibition zones against *S. aureus, E. coli and pneumoniae* respectively. The IsoMF showed strong potential against *K. pneumoniae* (51 ± 1 mm) *and B. subtilis* (51 ± 1 mm). This is the first study to the best of our knowledge in which antibacterial potential of IsoMF is being discussed separately. Although in a previous study (Thambi et al. 2016) showed antibacterial properties of mango peel (*Mangifera indica*-a major source of MF and IsoMF). In a review article Ediriweera et al. (2017) enlisted the major phyto-constituents present in mango peel. In another study Heng and Lu (1990) discussed the antiviral potential of MF and IsoMF. Biswas et al. (2015) isolated the MF from floral buds of *M. indica* and evaluate antibacterial potential against various strains of *Salmonella* and *Staphylococcus* bacteria. They proposed promising potential of MF as antibacterial agent.

The MIC is the lowest concentration of a natural product that completely inhibits the microbial growth. The MIC (µg/mL) of MF, IsoMF and Cefixime were calculated in the present study. The MIC values indicated the antibacterial potential of isolated xanthone C-glycosides. The MIC (125 µg/mL) is the same for both xanthones isomers against *S. subtilis* and *K. pneumoniae* and showed strong potential against these bacterial strains. While both xanthone isomers showed less antibacterial potential against *E. coli* but in comparison, IsoMF exhibited better anti *E. coli* potential than MF as indicated by MIC value (Fig. 3). Mangiferin showed MIC 600 µg/mL against *Helicobacter pylori* as reported by Zhang and Yue (2017). Khumpook et al. (2018) demonstrated the antibacterial potential of three varieties of mango leaves against *S. epidermidis, S. aureus,* Methicillin-resistant *S. aureus, P. acnes* and *P. aeruginosa*. The range of MIC varies from 3.91 mg/mL to 125 mg/mL. The methanolic and aqueous extracts of mango leaves were most effective against *S. epidermidis and S. aureus* (Khumpook et al. 2018). Our results also indicated that MF and IsoMF are effective against *S. aureus* with MIC 250 and 125 µg/mL respectively (Fig. 3). Just like our present study, in 2014 two separate groups of researchers (Pintu and Pal, 2014; Chidozie et al. 2014) showed that least effectiveness of Mango leaves extract against *E. coli* bacteria. On the other hand, Biswas et al. (2015) showed antibacterial potential of MF against two bacterial species, *Staphylococcus aureus* and *Salmonella typhi*. But they neither showed the antibacterial potential of IsoMF nor did they calculate MIC values. But in our present study, IsoMF showed better efficacy against *Staphylococcus aureus* than MF. We have calculated MIC for both MF and IsoMF against all the test bacterial strains. This is the first report about MIC value of IsoMF to the best of our knowledge.

Discussing mechanism of action of plant derived phenolic compounds, membrane disruption, in both Gram-positive and Gram negative bacteria, contributes to the antibacterial activity of most plant phenolics that have been mechanistically assessed. However, some phenolic compound like Quercetin showed a diverse list of antibacterial mechanism of action including cell membrane disruption, DNA intercalation, DNA gyrase inhibition, type III secretion inactivation, dehydratase inhibition (HpFabZ) and protein kinase inhibition (Rempe et al. 2017).

Among the cytotoxicity determining methods, the BSLT is the easy and inexpensive method which give rapid results (24 h). In the present study the cytotoxicity of MF and IsoMF were observed at different concentrations (Fig. 4a) and compared with known cytotoxic compound "nicotine" (Fig. 4b). It is clear from the Fig. 4a and b that the cytotoxicity of MF and IsoMF increases as dose dependent manner. Such observations are also presented by many researchers (Khan and Islam, 2012; Apu et al. 2013) working in this field. A study (Parvez and Mosaddik, 2016) on cytotoxic potential of mango peel showed strong cytotoxicity against brine shrimp larvae. It may be due to the presence of variety of compounds in mango peel. On the basis of LD_{50} (MF = 969.77 ± 0.67 and IsoMF = 768.92 ± 0.81 µg/mL) both MF and IsoMF proved to be poor in cytotoxic potential. This may be due to their strong antioxidant properties as demonstrated by Ishaque et al. 2021. The phenolic compounds have been extensively reviewed for their toxic properties by Galati and O'Brien (2004), who highlighted the pro-oxidant effects of compounds in the presence of metals and peroxidases, DNA binding of compounds with catechol groups and mouse hepatotoxicity of epicatechin gallate and propyl gallate. While most compounds derived from edible plants, including phenolics, are considered safe at common levels of consumption. Rigorous toxicity testing must be done to ensure safety at different concentrations and in different conditions.

5. Conclusion

The isolated C-glycosidic xanthones showed strong antibacterial efficacy. MF showed strong bactericidal potential against *Bacillus subtilis* and *Klebsiella pneumonia* while IsoMF was active against *Bacillus subtilis*, *Staphylococcus aureus* and *Klebsiella pneumonia*. Both MF and IsoMF showed mild or minimal cytotoxicity towards brine shrimp larvae. Further evaluation of these two isomeric compounds may prove to be the future remedies for various bacterial infections and other human ailments.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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