Biointerface Research in Applied Chemistry
Platinum Open Access Journal (ISSN: 2069-5837)

Article
Volume 11, Issue 1, 2021, 7921 - 7931
https://doi.org/10.33263/BRIAC111.79217931

Screening of Plants Hydro-Alcoholic Extracts from Kerman for their Inhibition of β-Glucuronidase Activity

Ahmad Gholamhoseinian [1,2], Fariba Sharififar [3], Hamide Jalaeeian [1,4], Beydolah Shahouzehi [4,5,*]

1 Department of Clinical Biochemistry, Kerman University of Medical Sciences, Kerman, Iran
2 Physiology Research Center, Institute of Basic and Clinical Physiology Sciences, Kerman University of Medical Sciences, Kerman, Iran
3 Department of Pharmacognosy, School of Pharmacy, Kerman University of Medical Science, Kerman, Iran
4 Student Research Committee, Kerman University of Medical Science, Kerman, Iran
5 Cardiovascular Research Center, Institute of Basic and Clinical Physiology Sciences, Kerman University of Medical Sciences, Kerman, Iran
* Correspondence: b.shahozehi@kmu.ac.ir; bshahouzehi@gmail.com;

Scopus Author ID 36470992900
Received: 15.06.2020; Revised: 4.07.2020; Accepted: 5.07.2020; Published: 9.07.2020

Abstract: In neonatal jaundice, β-glucuronidase converts conjugated bilirubin into the unconjugated form and increases its level in the blood. Many natural compounds have been identified as β-glucuronidase inhibitors. The aim of this study was to evaluate the effect of hydro-methanolic extracts of 100 plants on β-glucuronidase. The β-glucuronidase activity was measured by a spectrophotometric method using Phenolphthalein glucuronide and 4-nitrophenyl β-D-glucuronide. Kinetic study of the enzyme was performed in the presence and absence of the plant extract. It was revealed that from hydro-methanolic (70%) extracts, *Rosa damascena* and *Ipomoea tricolor* showed more than 85% inhibitory effect on β-glucuronidase. *Rosa damascena* showed competitive inhibition, and *Ipomoea tricolor* showed non-competitive inhibition. The K_m and V_{max} values for β-glucuronidase were 23.32 mM and 0.814 mM min^{-1}, respectively. When using 4-nitrophenyl β-D-glucuronide, *Stevia* and *Cerasus avium* showed more than 65% inhibitory effect on β-glucuronidase. Both *Stevia* and *Cerasus avium* showed non-competitive inhibition. The K_m and V_{max} values for β-glucuronidase were 16.98 mM and 0.936 mM min^{-1}, respectively. None of the plant extracts showed an activation effect on the enzyme. The data suggest that these plants might be good candidates for the treatment of neonatal jaundice and its related diseases.

Keywords: β-glucuronidase; Neonatal jaundice; Phenolphthalein Glucuronide; 4-Nitrophenyl β-D-glucuronide.

1. Introduction

Jaundice is caused by high levels of bilirubin in the blood due to infection, genetic factors, and preterm delivery [1-4]. The jaundice is derived from the Greek word *jaune*, which is recognized by yellowing the skin and eye scrubs due to the increased amount of serum bilirubin pigments [3,5]. Jaundice can be detected with a wide variety of benign or threatening disorders. Bilirubin level above 2.5-5 mg/dL is a symptom of jaundice [3,6,7]. However, the central nervous system is at high risk when the unconjugated bilirubin level is between 10-20 mg/dL, which requires treatment [2,4,7,8]. Jaundice may be due to various diseases, including genetic syndrome, liver diseases, autoimmune disorders, gallstones, hepatitis, pancreatic

cancer, papillary carcinoma, and medicines [3,9]. Severe jaundice can cause deafness, cerebral palsy, kernicterus, and other types of brain damage [10].

In neonatal jaundice, the level of unconjugated bilirubin increases. In other words, β-glucuronidase (EC 3.2.1.31), with its hydrolase activity, converts the conjugated bilirubin to unconjugated, which plays an important role in the development of neonatal jaundice [3,4,11]. The enzyme is produced by the intestinal *E. coli*, which has activity in both intestinal tissues and the bacteria. β-glucuronidase is found in animals, plants, and bacteria and causes β-glucuronide hydrolysis [12,13]. Intestines of neonates are free of bacteria and break down the conjugated bilirubin in the intestinal tract in adults and make it easier to remove it [12,13]. On the other hand, the activity of β-glucuronidase is high in the intestinal mucosa of the infants [7]. This results in the conversion of conjugated bilirubin into the unconjugated bilirubin in the intestines, which is reabsorbed through the intestinal epithelial cells and enters the intestinal-hepatic cycle. This action increases the level of unconjugated bilirubin and causes jaundice in newborn infants [4,7,14]. On the other hand, β-glucuronidase increases CPT-11 activity, an anti-cancer drug, and plays an important role in various types of cancers, including colorectal, lung, breast, gastric, pancreatitis, cervix and ovarian cancers [15,16]. CPT-11 is converted to SN-38 in the liver by the carboxylesterase, and SN-38 is converted to SN-38G by the UTP-glucuronyltransferase [16]. SN-38G passes through the digestive system and is considered as a substrate for the intestinal β-glucuronidase [16]. By converting SN-38G to SN-38, which is a topoisomerase, β-glucuronidase increases drug activity through fixing single-strand DNA breaks and cancer cell apoptosis [17].

In some cases, the enzyme activity has been reported to cause diarrhea [16]. Therefore, regulating the enzyme activity by increasing or decreasing activity can be effective in balancing the effectiveness of the drug. The use of plants as medicines has a long history [18-22]. Many studies on the inhibition of β-glucuronidase have been done in Korea [23], Japan [24], and Afghanistan [25].

In this study, we aimed to find hydro-methanolic extracts of 100 different plants to inhibit β-glucuronidase. Beta-glucuronidase inhibitors are well known for controlling various diseases, including hepatitis, hepatic necrosis, arthritis, and even colorectal cancer [8]. D-glucuno-1-4-lactone is currently one of the most important β-glucuronidase inhibitor, which is used in the treatment of several cancers, including ovarian, uterus, and prostate. The use of chemical drugs has always had some complications.β-glucuronidase also has two different properties: in some cases, its inhibition results in the treatment of the disease, while in some other cases increase in its activity is in benefit of the patient. Depending on the type of disease, patients should be advised to use or avoid using active substances. Considering the long history of plants used as medicines as well as biodiversity in different areas, we aimed to investigate the effect of hydro-methanolic extract of 100 plants on β-glucuronidase in order to find cheaper and safer new medicines in treatment and control of various diseases.

2. Materials and Methods

2.1. Plants.

One hundred plants were collected during summer 2016 and or purchased from the medicinal herbal markets in Kerman, and all of them were botanically identified by a botanist. A voucher specimen was deposited at the herbarium of the Herbal Medicines Research Center, Faculty of Pharmacy, Kerman University of Medical Sciences, Iran (Table 1). Those herbs

which were purchased from Kerman medicinal markets; therefore, the codes are not known to the researchers.

Each of the plants was air-dried and grounded into a fine powder. Twenty grams of each plant powder was suspended in 200 mL of methanol (70%), evenly soaked and stirred for one hour using the shaker, then placed at room temperature in dark conditions for 24 hours. The suspension was filtered and air-dried at room temperature. The resulting powders were stored in dark vials at -20°C until use [20,21,26].

2.2. Enzyme assay.

The β-glucuronidase, Phenolphthalein glucuronide, and 4-nitrophenyl β-D-glucuronide (4-NPG) were purchased from Sigma CO., USA. The β-glucuronidase activity was measured for each substrate by the spectrophotometric method [27]. In the method in which Phenolphthalein glucuronide was used as the substrate, 1 and 5 mg/ml of the extract was dissolved in methanol (10%) and 0.1 ml of the solution was added to 0.6 ml of sodium acetate 1 M and 0.2 ml of phenolphthalein glucuronide 0.01 M. Mixture was incubated in 37 °C in a water bath for 5 min. Negative control was prepared without the extract in a similar manner. Similarly, a blank sample was also prepared without the extract. β-glucuronidase (0.1 ml) at a concentration of 2 mg/ml was added to the test tubes, and 0.1 ml of water was added to the blank then incubated for 30 min. To terminate the reaction, after 30 minutes, 1 ml of the inhibitors (a mixture of 0.2 M Glycine and 0.2 M sodium chloride) were added to the tubes. L-aspartic acid was used as a positive control [28].

In another method, in which 4-NPG was used as the second substrate, a solution of 10 mg/ml of 4-NPG was prepared in phosphate buffer (0.05 M), pH 7.20. Sixty µl of the substrate and 20 µl of the extract was added to 50 µl of the enzymatic solution (2 mg/ml in phosphate buffer). The mixture was incubated in a 37 °C water bath for 20 min. Then 200 µl of Na_2Co_3 0.2 M was added as a reaction inhibitor. Finally, the absorbance of the tubes was measured against distilled water at 405 nm. Each test was performed 3 times, and the mean value was used as the inhibitory activity of the plant extract. L-aspartic acid was used as a positive control [27]. The inhibitory activity was calculated using the following formula [27,29]:

$$\text{Inhibition (\%)} = [\text{A Control-A Extract / A Control}] \times 100$$

2.3. Kinetic study.

In order to measure the inhibition mode by hydro-methanolic extract with high activity, β-glucuronidase activity was assayed with: a) increasing concentrations of Phenolphthalein glucuronide the first substrate (0.39,0.775, 1.5, 3.1 and 6.2 mM) in the absence or presence of one of the extracts at two different extract concentrations (2.5 and 5 $mg.mL^{-1}$); b) increasing concentrations of 4-NPG as the second substrate (0.5, 0.793, 1, 1.578, 2 and 3.03 mM) in the absence or presence of one of the extracts at two different extract concentrations (2.5 and 5 $mg.mL^{-1}$). Optimal doses of the extracts were determined based on the results from the inhibitory activity assay, as described above. The inhibition type for these extracts was determined by Lineweaver-Burk plot analysis [30].

3. Results and Discussion

Using Phenolphthalein glucuronide, *Rosa damascena*, and *Ipomoea tricolor* and showed more than 85% inhibitory effect on β-glucuronidase, while other plants extracts

showed less than 85% inhibitory effect or even no inhibitory effect on this enzyme (Table 1). Using 4-nitrophenyl β-D-glucuronide, *Stevia* and *Cerasus avium* showed more than 65% inhibitory effect on β-glucuronidase, while other plants extracts showed less than 65% inhibitory effect or even no inhibitory effect on this enzyme (Table 2).

Inhibition mode of two plants (*Rosa damascena* and *Ipomoea tricolor*) against Phenolphthalein glucuronide was analyzed by Lineweaver–Burk plots. The enzyme kinetics demonstrated a competitive inhibition by *Rosa damascena.* Enhancing the concentration of this extract showed an increase in the $K_{m,}$ and no effect on V_{max} values (Figure 1). While competitive inhibition on β-glucuronidase activity was observed by *Ipomoea tricolor*, enhancing the concentration of this extract showed a decrease in V_{max} and K_m values (Figure. 2). The K_i values for *Rosa damascena* and *Ipomoea tricolor* were 0.276 mg/ml and 0.486 mg/ml, respectively. The inhibition mode of two plants (*Stevia* and *Cerasus avium*) against 4-NPG was analyzed by Lineweaver–Burk plots. The enzyme kinetics demonstrated a non-competitive inhibition by *Stevia* and *Cerasus avium*. Enhancing the concentration of these extracts showed a decrease in the V_{max} and no effect on K_m values (Figures 3 and 4). The K_i values for *Stevia* and *Cerasus avium* were 1.67 mg/ml and 1.8 mg/ml, respectively.

In neonatal jaundice, the amount of non-conjugated bilirubin increases. β-glucuronidase converts conjugated bilirubin to non-conjugated and causing neonatal jaundice [3,4,11]. Severe jaundice can cause deafness, cerebral palsy, kernicterus, and other forms of brain damage [10]. β-glucuronidase also increases the activity of CPT-11, an anti-cancer drug, by converting SN-38G to SN-38 [15]. D-glucono-1-4-lactone is currently one of the most important β-glucuronidase inhibitor, which is used in the treatment of several cancers, including ovarian, uterus, and prostate [8]. However, the use of chemical drugs always has some complications. In this study, the inhibitory effect of hydro-ethanolic extracts of 100 plants on β-glucuronidase activity. Using Phenolphthalein glucuronide, the inhibitory effect of *Rosa damascena* and *Ipomoea tricolor* on β-glucuronidase were 94% and 86%, respectively. *Rosa damascena* showed a competitive inhibition on the β-glucuronidase, but *Ipomoea tricolor* showed a non-competitive inhibition. Using 4-nitrophenyl β-D-glucuronide, the inhibitory effect of *Stevia and Cerasus avium* on β-glucuronidase was 75% and 68%, respectively. Both plants demonstrated a non-competitive inhibition. Hydro-methanolic extract of *Rosa damascena*, *Ipomoea tricolor*, *stevia*, and *Cerasus avium* showed an inhibitory effect on β-glucuronidase. In this study, no plant showed an activating effect on β-glucuronidase. It is worth mentioning that several plants with less than 10% activating effect were found in this study, which is not enough to activate the β-glucuronidase.

β-glucuronidase released from bacteria, related to a higher incidence of Crohn's disease and colon cancer and, therefore, the inhibition of this enzyme released from bacteria living in the gut, is related to improving the health system [12]. Karak et al. (2019 and 2017) reported that plant extract from *Swertia* species and also *Piper betle* have β-glucuronidase inhibitory effects, which confirmed our findings [18,19]. In a study in which β-glucuronidase inhibitory effects of some plants were evaluated, they found that *Mentha piperita* strongly inhibited the enzyme activity (88.9%), in our study we found that the inhibition is dependent on the substrate and *Mentha piperita* inhibited β-glucuronidase activity 63% and 9% when we used phenolphthalein glucuronide and 4-NPG as substrate, respectively. They used phenolphthalein glucuronide as the substrate and found higher inhibition activity by *Mentha piperita* that can be related to the type of extract used by them. In their study, they used the total flavonoid

contents of *Mentha piperita*, but in the current study, we used a crude hydro methanolic extract of *Mentha piperita* [13].

The β-glucuronidase expression was up-regulated in patients with some cancers, including breast, lung, leukemia, and colon. Also, it up-regulated in diseases such as diabetes, HIV, and rheumatoid arthritis. It seems that β-glucuronidase can be considered as biomarkers in those diseases and also may be involved in their pathogenesis. Therefore, β-glucuronidase inhibition reduces those pathological conditions and attenuates their adverse consequences [8].

Table 1. Plants and their inhibitory effect on phenolphthalein glucuronide.

Plants name	Family	Used part	Inhibition (%)
Rosa damascena	Rosaceae	Galls	94
Ipomoea tricolor	Convolvulaceae	Galls	86
Ziziphora tenuir	Lamiaceae	Leaves	84
Nigella sativa L.	Ranunculaceae	Seeds	83
Origanum majorana	Lamiaceae	Whole plant	81
Syzygium aromaticus	Caryophyllaceae	Aerial part	78
Huscoriaria	Anacardiaceae	Seeds	77
Scrophularia striata	Compositae	Aerial part	77
Olea europaea	Oleaceae	Leaves	77
Zataria multiflora	Lamiaceae	Aerial part	76
Lavandula angustifolia	Lamiaceae	Whole plant	75
Rosa	Rosaceae	Galls	75
Avena sativa	Grasses	Seeds and shell	74
Echium amoenum	Boraginaceae	Galls	74
Cerasus avium	Rosaceae	Fruit Tails	73
Glycyrrhiza glabra	Legumes	Aerial part	70
Myrtus communis	Myrtaceae	Leaves	70
Equisetum arvense	Equisetaceae	Whole plant	70
Tanacetum parthenium	Asteraceae	Galls	69
Centaurea depressa	Grasses	Galls	68
Papaver somniferum	Papaveraceae	Seeds	67
Cucumis sativus	Cucurbitaceae	Seeds	67
Otostegia persica	Violaceae	Aerial part	65
Sanguisorba minor	Geraniacea	Aerial part	64
Mentha piperita	Lamiaceae	Leaves	63
Arctium lappa	Asteraceae	Whole plant	62
Cubeb berries	Piperaceae	Fruits	61
Phoenix dactylifera	Arecaceae	Galls andFloret	61
Medicago sativa L.	Legumes	Seeds	61
Matricaria recutita	Composite	Galls	61
Hypericum perforayum	Labiatae	Aerial part	60
Astragalus adsccendens	Psyllidae	Gum	59
Crataegus oxyacanta	Rosaceae	Galls and Fruits	58
Coriandrum sativum	Apiaceae	Aerial part	58
Alhagi maurorum	Fabaceae	Gum	57
Citrus aurantium	Rutaceae	Galls	56
Crataegus oxyacanta	Astraceae	Whole plant	55
Cotoneaster	Rosaceae	Gum	53
Hyssopus officinalis	Lamiaceae	Plant flowers	52
Calendula officinalis L.	Asteraceae	Whole plant	51
Urticadioica	Urticaceae	Aerial part	49
Punica granatum	Punicaceae	Skin	49
Sesamum indicum	Pedaliaceae	Seeds	48
Cinnamomum Zeylanicum	Lauraceae	Roots	48
Stevia	Asteraceae	Leaves	47
Rosmarinus officinalilis	*Lamiaceae*	Aerial part	47
Artemisia absinthium	Compositae	Leafy branches	44
Lippia citriodora	*Verbenaceae*	Leaves	44
Allium sativum L.	Lily	Gland	44
Polygonatum orientale Desf.	Umbelliferae	Roots	41
Camellia sinensis	Theaceae	Leaves	40
Mentha longifolia	Labiatae	Aerial part	39
Achillea wilhelmsii	Compositae	Aerial part	37
Lactuca sativa L.	Compositae	Seeds	36

Plants name	Family	Used part	Inhibition (%)
Anacardium occidentale	Umbelliferea	Seeds	36
Artemisia abrotanum	Compositeae	Aerial part	34
Hypericum perforatum	GuttiferaeHypericaceae	Plant flowers	34
Lavandula stoechasl	Lamiaceae	Aerial part	34
Melissa officinalis	Lamiaceae	Leaves and shoot	33
Salix alba	Salicaceae	Aerial part	29
Heracleum persicum	Apiaceae	Fruits	29
Teucrium polium	*Lamiaceae*	Aerial part	28
Lepidium sativum L.	Cruciferae	Seeds	26
Pimpinella anisum	Cruciferae	Seeds	25
Peganum harmala	Zygophyllaceae	Aerial part	24
Zingiber officinale	Zingiberaceae	Rhizomes	24
Panax ginseng	*Araliaceae*	Roots	23
Cydonia oblonga	Rosaceae	Seeds	22
Valeriana hispida	Valerianaceae	Rhizomes	22
Citrullus colocynthis	Cucurbitaceae	Fruits	20
Ficus carica	Moraceae	Leaves	18
Rosa canina	Rosaceae	Fruits	16
Ocimum basilicum L.	Lamiaceae	Seeds	15
Trigonella foenumgraecum	Leguminosae	Seeds	15
Bunium persicum	Apiaceae	Seeds	14
Papaver orientale	Papaveraceae	Aerial part	14
Cichorium intybus	Compositae	Roots	11
Althae officinallis	Malvaceae	Galls	11
Hibiscus gossypifolius	Labiatae	Galls	10
Ranunculus arvensisl	Ranunculaceae	Aerial part	10
Portulaca oleracea	*Portulacaceae*	Seeds	9
Artemisia dracunculus	Compositae	Leaves	7
Citrus aurantium	Rutaceae	Galls	6
Hibiscus gossypifolius	Malvaceae	Fruits and Calix	5
Coriandrum sativum	Graminaceae	Stem soup	4
Physalis alkekengi	Solanaceae	leaves	4
Foeniculum vulgare	Umbelliferaeapiaceae	Fruits	4
Carthamus oxyacantha	Compositae	Whole plant	3
Ziziphus spinachristi	Rhamnaceae	leaves	0
Alhagi camelorum	Fabaceae	Aerial part	0
Fumaria parviflora	Fumariaceae	Aerial part	0
Rubia tinctorium	Rubiaceae	Roots	0
Terminalia chebulla	Combertaceae	Fruits	0
Cordiamixa	Boraginaceae	Fruits	0
Fraxinus excelsior	Oleaceae	Aerial part	0
Vaccinium arctostaphylus	Ericaceae	Fruits	0
Ocimum basilicum	Lamiaceae	Seeds	0
Eucaliptus galbie	Eucalypteae	Leaves	0
Anacardium occidentale	Apiaceae	leaves	0
Malva sylvestris	Malvaceace	Galls	0

Table 2. Plants and their inhibitory effect on 4-Nitrophenyl β-D-glucuronide.

Plants name	Family	Used part	Inhibition (%)
Stevia	Asteraceae	leaves	75
Cerasus avium	Rosaceae	Fruit Tails	68
Rosa damascena	Rosaceae	Galls	67
Calendula officinalis L.	Asteraceae	Whole plant	67
Ziziphor atenuir	Lamiaceae	leaves	58
Crataegus oxyacanta	Rosaceae	FruitsGalls and	58
Origanum majorana	Lamiaceae	Whole plant	49
Hyssopus officinalis	Lamiaceae	Plant flowers	49
Scrophularia striata	Compositae	Aerial part	45
Otostegia persica	Violaceae	Aerial part	43
Zatari amultiflora	Lamiaceae	Aerial part	35
Melissa officinalis	Lamiaceae	Leaves and Shoot	34
Ipomoea tricolor	Convolvulaceae	Galls	27
Tanacetum parthenium	Asteraceae	Galls	27
Crataegus oxyacanta	Astraceae	Whole plant	27
Papaver somniferum	Papaveraceae	Seeds	25
Cucumis sativus	Cucurbitaceae	Seeds	24

Plants name	Family	Used part	Inhibition (%)
Nigella sativa L.	Ranunculaceae	Seeds	22
Lippia citriodora	Verbenaceae	leaves	19
Sanguisorba minor	Geraniacea	Aerial part	18
Glycyrrhiza glabra	Legumes	Aerial part	16
Artemisia abrotanum	Compositeae	Aerial part	16
Ranunculus arvensisl	Ranunculaceae	Aerial part	16
Rhus coriaria	Anacardiaceae	Seeds	14
Anacardium occidentale	Umbelliferea	Seeds	13
Teucrium polium	*Lamiaceae*	Aerial part	13
Avena sativa	Grasses	Seeds and shell	11
Sizigium aromaticus	Caryophyllaceae	Aerial part	10
Lavandula angustifolia	Lamiaceae	Whole plant	10
Centaurea depressa	Grasses	Galls	10
Citrus aurantium	Rutaceae	Galls	10
Cotoneaster	Rosaceae	Gum	10
Mentha piperita	Lamiaceae	leaves	9
Phoenix dactylifera	Arecaceae	Galls and Floret	9
Hypericum perforatum	Guttiferae Hypericaceae	Plant flowers	9
Sesamum indicum	Pedaliaceae	Seeds	8
Cichorium intybus	Compositae	Roots	8
Olea europaea	Oleaceae	leaves	6
Coriandrum sativum	Apiaceae	Aerial part	6
Panax ginseng	*Araliaceae*	Roots	5
Artemisia absinthium	Compositae	Leafy branches	4
Rosa canina	Rosaceae	Fruits	4
Ocimum basilicum L.	Lamiaceae	Seeds	4
Rosa	Rosaceae	Galls	3
Echium amoenum	Boraginaceae	Galls	3
Astragalus adsccendens	Psyllidae	Gum	3
Achillea wilhelmsii	Compositae	Aerial part	3
Citrullus colocynthis	Cucurbitaceae	Fruits	3
Trigonella foenumgraecum	Leguminosae	Seeds	3
Artemisia dracunculus	Compositae	leaves	3
Coriandrum sativum	Graminaceae	Stem soup	3
Physalis alkekengi	Solanaceae	leaves	3
Myrtus communis	Myrtaceae	leaves	2
Cubeb berries	Piperaceae	Fruits	2
Hypericum perforayum	Labiatae	Aerial part	2
Urtica dioica	Urticaceae	Aerial part	2
Rosmarinus officinalilis	*Lamiaceae*	Aerial part	2
Mentha longifolia	Labiatae	Aerial part	2
Heracleum persicum	Apiaceae	Fruits	2
Peganum harmala	Zygophyllaceae	Aerial part	2
Valeriana hispida	Valerianaceae	Rhizomes	2
Bunium persicum	Apiaceae	Seeds	2
Citrus aurantium	Rutaceae	Galls	2
Hibiscus gossypifolius Mill.	Malvaceae	Fruits and calix	2
Foeniculum vulgare	Umbelliferaeapiaceae	Fruits	2
Carthamus oxyacantha	Compositae	Whole plant	2
Equisetum arvense	Equisetaceae	Whole plant	0
Arctium lappa	Asteraceae	Whole plant	0
Medicago sativa L.	Legumes	Seeds	0
Matricaria recutita	Composite	Galls	0
Alhagi maurorum	Fabaceae	Gum	0
Punica granatum	Punicaceae	skin	0
Cinnamomum zeylanicum	Lauraceae	Roots	0
Lactuca sativa L.	Compositae	Seeds	0
Pimpinella anisum	Cruciferae	Seeds	0
Zingiber officinale	Zingiberaceae	Rhizomes	0
Cydonia oblonga Mill.	Rosaceae	Seeds	0
Ficus carica	Moraceae	leaves	0
Papaver orientale	Papaveraceae	Aerial part	0
Althae officinallis	Malvaceae	Galls	0
Hibiscus gossypifolius	Labiatae	Galls	0
Portula caoleracea	Portulacaceae	Seeds	0
Ziziphus spinachristi	Rhamnaceae	leaves	0

Plants name	Family	Used part	Inhibition (%)
Alhagi camelorum	Fabaceae	Aerial part	0
Terminalia chebulla	Combertaceae	Fruits	0
Cordia mixa	Boraginaceae	Fruits	0
Fraxinus excelsior	Oleaceae	Aerial part	0
Vaccinium arctostaphylus	Ericaceae	Fruits	0
Ocimum basilicum	Lamiaceae	Seeds	0
Eucaliptus galbie	Eucalypteae	leaves	0
Anacardium occidentale	Apiaceae	leaves	0
Malva sylvestris	Malvaceace	Galls	0
Allium sativum L.	Lily	gland	2
Polygonatum Orientale des.	Umbelliferae	Roots	2
Camellia sinensis	Theaceae	leaves	4
Lavandula stoechasl	Lamiaceae	Aerial part	5
Salix alba	Salicaceae	Aerial part	5
Lepidium sativum L.	Cruciferae	Seeds	6
Fumaria parviflora	Fumariaceae	Aerial part	8
Rubi atinctorium	Rubiaceae	Roots	8

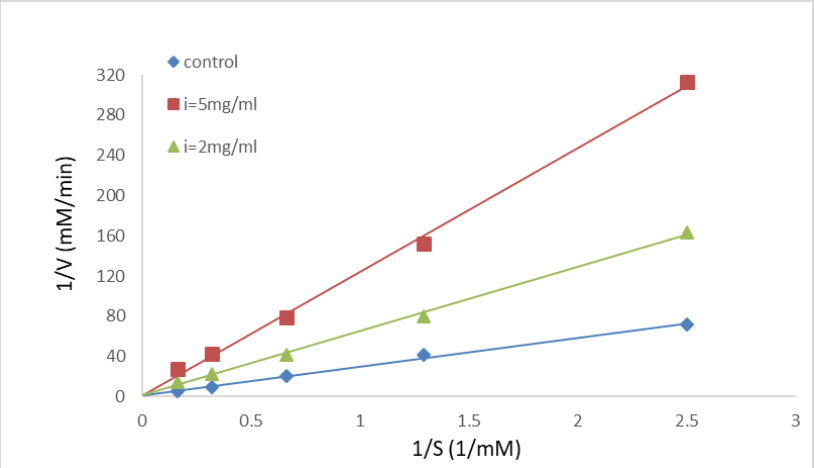

Figure 1. The Lineweaver-Burk plot of kinetic analysis for β-glucuronidase at two different concentrations of *Rosa damascena* (2.5 and 5 mg mL-1) in the presence of 5 different Phenolphthalein glucuronide concentrations.

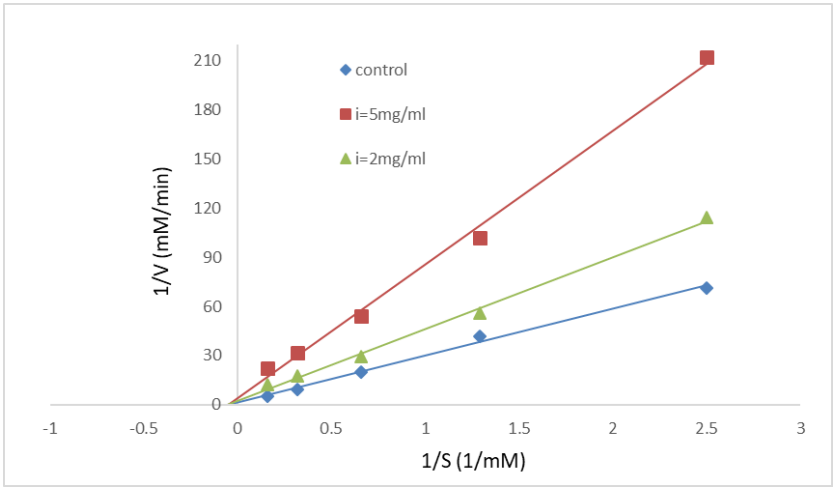

Figure 2. The Lineweaver-Burk plot of kinetic analysis for β-glucuronidase at two different concentrations of *Ipomoea tricolor* (2.5 and 5 mg mL-1) in the presence of 5 different Phenolphthalein glucuronide concentrations.

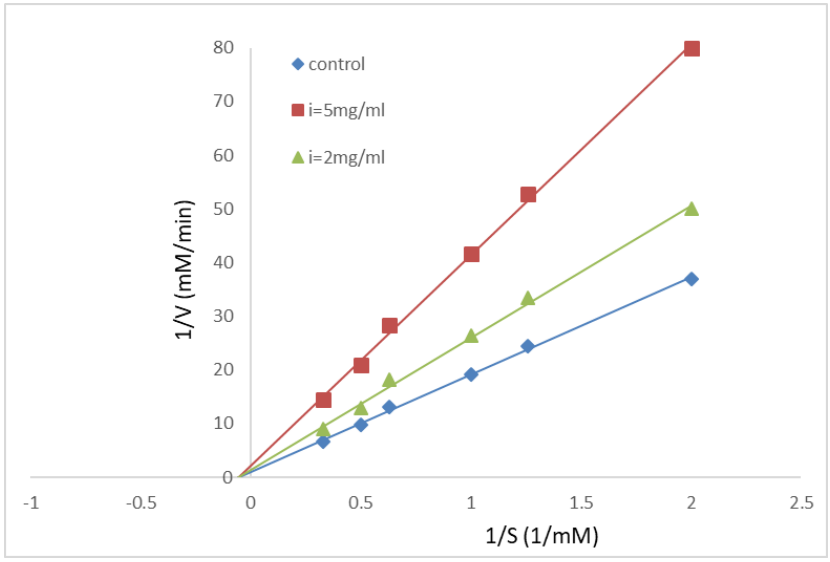

Figure 3. The Lineweaver-Burk plot of kinetic analysis for β-glucuronidase at two different concentrations of *Stevia* (2.5 and 5 mg mL-1) in the presence of 5 different 4-NPG concentrations.

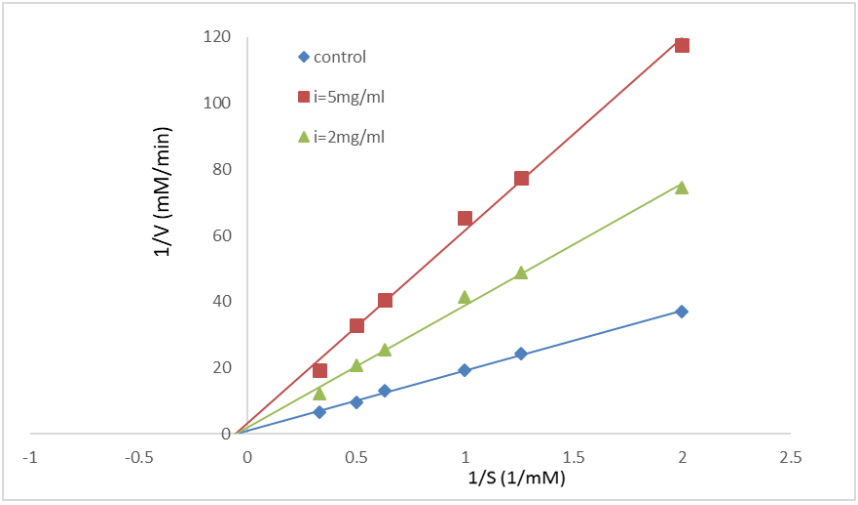

Figure 4. The Lineweaver-Burk plot of kinetic analysis for beta-glucuronidase at two different concentrations of *Cerasus avium* (2.5 and 5 mg mL-1) in the presence of 5 different 4-NPG concentrations.

4. Conclusions

Hydro-methanolic extract of *Rosa damascena*, *Ipomoea tricolor*, *Stevia*, and *Cerasus avium* showed an inhibitory effect on β-glucuronidase. Since β-glucuronidase inhibition is considered as a biomarker of cancer pathology (especially colon cancer) and other disease condition including diabetes, therefore, its inhibitor must be evaluated in such disease for possible beneficial effects. The results provided by the current study scientifically validate the use of these plants in the treatment of the related diseases, and we need to evaluate the hydro-methanolic extract of *Rosa damascena*, *Ipomoea tricolor*, *Stevia* and *Cerasus avium* for their beneficial effects in diseases mentioned above models.

Funding

This research received no external funding.

Acknowledgments

This study was financially supported by the Kerman Medical University Research Council.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Seyedi, R.; Mirghafourvand, M.; Jannat Dost, A.; Mohammad-Alizadeh-Charandabi, S.; Asghari Jafarabadi, M. Relationship between Neonatal Skin Bilirubin Level and Severe Jaundice with Maternal, Childbirth, and Neonatal Characteristics. *Iranian Journal of Neonatology* **2019**, *10*, https://doi.org/10.22038/ijn.2019.33282.1478.
2. Fakhri, M.; Farhadi, R.; Mousavinasab, S.N.; Yosefi, S.S.; Hosseinimehr, S.J.; Azadbakht, M. Effect of Natural Products on Jaundice in Iranian Neonates. *Jundishapur J Nat Pharm Prod* **2019**, *14*, https://doi.org/10.5812/jjnpp.83042.
3. Roche. S.P.; Kobos, R. Jaundice in the adult patient. *Am fam physician* **2004**, *69*, 299-308.
4. Liu, W.; Liu, H.; Wang, T.; Tang, X. Therapeutic effects of probiotics on neonatal jaundice. *Pak J Med Sci* **2015**, *31*, 1172-1175, https://doi.org/10.12669/pjms.315.7921.
5. Gazzin, S.; Masutti, F.; Vitek, L.; Teribelli, C. The molecular basis of jaundice: An old symptom revisited. *Liver Int* **2017**, *37*, 1094-1102, https://doi.org/10.1111/liv.13351.
6. Zahed Pasha, Y.; Alizadeh-Tabari, S.; Zahed Pasha, E.; Zamani, M. Etiology and therapeutic management of neonatal jaundice in Iran: a systematic review and meta-analysis. *World J Pediatr* **2020**, https://doi.org/10.1007/s12519-020-00339-3.
7. Bader, D.; Yanir, Y.; Kugelman, A.; Wilhelm-Kafil, M.; Riskin, A. Induction of early meconium evacuation: is it effective in reducing the level of neonatal hyperbilirubinemia? *Am J Perinatol* **2005**, *22*, 329-333, https://doi.org/10.1055/s-2005-871529.
8. Awolade, P.; Cele, N.; Kerru, N.; Gummidi, L.; Oluwakemi, E.; Singh, P. Therapeutic significance of b-glucuronidase activity and its inhibitors: A review. *Eur J Med Chem* **2020**, *187*, https://doi.org/10.1016/j.ejmech.2019.111921.
9. Whitehead, M.W.; Hainsworth, I.; Kingham, J.G. The causes of obvious jaundice in south west wales: perceptions versus reality. *Gut* **2001**, *48*, 409-413, https://doi.org/10.1136/gut.48.3.409.
10. Saluja, S.; Agarwal, A.; Kler, N.; Amin, S. Auditory neuropathy spectrum disorder in late preterm and term infants with severe jaundice. *Int J Pediatr Otorhinolaryngol* **2010**, *74*, 1292-1297, https://doi.org/10.1016/j.ijporl.2010.08.007.
11. Maisels, M.J.; McDonagh, A.F. Phototherapy for neonatal jaundice. *N Eng J Med* **2008**, *358*, 920-928, https://doi.org/10.1056/NEJMct0708376.
12. Mahran, E.; Keusgen, M.; Morlock, G.E. New planar assay for streamlined detection and quantification of b-glucuronidase inhibitors applied to botanical extracts. *Anal Chim Acta* **2020**, *4*, https://doi.org/10.1016/j.acax.2020.100039.
13. Molan, A.L.; Saleh Mahdy, A. Iraqi medicinal plants: Total flavonoid contents, free-radical scavenging and bacterial beta-glucuronidase inhibition activities. *IOSR-JDMS* **2014**, *13*, 72-77, https://doi.org/10.9790/0853-13527277.
14. Wong, R.J.; Bhutani, V.K. *Pathogenesis and etiology of unconjugated hyperbilirubinemia in the newborn*. *UpToDate* .Waltham, MA: UpToDate, **2020**.
15. Rothenberg, M.L. Irinotecan (CPT-11): recent developments and future directions–colorectal cancer and beyond. *Oncologist* **2001**, *6*, 66-80, Https://doi.org/10.1634/theoncologist.6-1-66.
16. Roberts, A.B.; Wallace, B.D.; Venkatesh, M.K.; Mani, S.; Redinbo, M.R. Molecular insights into microbial β-glucuronidase inhibition to abrogate CPT-11 toxicity. *Mol Pharmacol* **2013**, *84*, 208-217, https://doi.org/10.1124/mol.113.085852.
17. Hsieh, Y.T.; Chen, K.C.; Cheng, C.M.; Cheng, T.L.; Tao, M.H.; Roffler, S.R. Impediments to enhancement of CPT-11 anti-cancer activity by E. coli directed beta-glucuronidase therapy. *PloS One* **2015**, *10*, https://doi.org/10.1371/journal.pone.0118028.
18. Karak, S.; Nag, G.; De, B. Metabolic profile and β-glucuronidase inhibitory property of three species of Swertia. *Rev bras farmacogn* **2017**, *27*, 105-111, http://dx.doi.org/10.1016/j.bjp.2016.07.007.
19. Karak, S.; Das, S.; Biswas, M.; Choudhury, A.; Dutta, M.; Choudhury, K.; De, B. Phytochemical composition, β-glucuronidase inhibition, and antioxidant properties of two fractions of Piper betle leaf aqueous extract. *J of Food Biochemistry* **2019**, https://doi.org/10.1111/jfbc.13048.
20. Perumal, A.; Naidu Krishna, S.B.; Sershen, K.; Pillay, K.; Govender, P. Phytochemical composition and biological investigation of Trichilia emetica Vahl. seed extracts. *Letters in Applied Nano Bio Science* **2020**, *9*, 1111-1116, https://doi.org/10.33263/LIANBS92.11111116.
21. Gandham, R.G.; Raji, P.; Rohan, B.; Divya Kumar, M.; Kripu Sharma, V.; Keerthana, D.; Karishma, S.; Iyappan, P.; Thrumurugan, R.; Samrot, A.V.; Ponnaiah, P.; Pattamadath, S.; Purayil, S.K.; Javad, P.T.M.; Prakash, P. Green synthesis and antibacterial activity of silver nanoparticles from the aqueous extracts of Cassia alata. *Letters in Applied Nano Bio Science* **2020**, *9*, 1037-1041, https://doi.org/10.33263/LIANBS92.10371041.

22. Kumari, R.; Chandra Mishra, R.; Parkash Yadav, J. Antioxidant and cytotoxic studies of Acacia nilotica twig extract and their green synthesized silver nanoparticles. *Letters in Applied Nano Bio Science* **2020**, *9*, 975-980.
23. Shim, S.B.; Kim, N.J.; Kim, D.H. β-Glucuronidase inhibitory activity and hepatoprotective effect of 18β-glycyrrhetinic acid from the rhizomes of Glycyrrhiza uralensis. *Planta Med* **2000**, *66*, 40-43, https://doi.org/10.1055/s-2000-11109.
24. Summart, R.; Chewonarin, T. Purple rice extract supplemented diet reduces DMH-induced aberrant crypt foci in the rat colon by inhibition of bacterial β-glucuronidase. *Asian Pac J Cancer Prev* **2014**, *15*, 749-755, https://doi.org/10.7314/APJCP.2014.15.2.749.
25. Nawaz, H.R.; Malik, A.; Khan, P.M.; Shujaat, S.; Rahman, A. A novel β-glucuronidase inhibiting triterpenoid from Paeonia emodi. *Chem Pharm Bull (Tokyo)* **2000**, *48*, 1771-1773, https://doi.org/10.1248/cpb.48.1771.
26. Joukar, S.; Askarzadeh, M.; Shahouzehi, B.; Najafipour, H.; Fathpour, H. Assessment of safety and therapeutic efficacy of rosa damascena L. and quercus infectoria on cardiovascular performance of normal and hyperlipidemic rabbits: Physiologically based approach. *Journal of Toxicology* **2013**, *2013*, https://doi.org/10.1155/2013/769143.
27. Acharya, J.; De, B. Bioactivity-guided fractionation to identify β-glucuronidase inhibitors in Nymphaea pubescens flower extract. *Cogent Food & Agriculture* **2016**, *2*, https://doi.org/10.1080/23311932.2015.1134379.
28. Meister, A. *Advances in enzymology and related areas of molecularbiology*. Volume 70, John Wiley & Sons, **2009**.
29. Gholamhoseinian, A.; Sharifi–Far, F.; Rahimi–Naiini, M. Screening of methanol extracts of sixty plants from Kerman for their potential xanthine oxidase inhibitory activity. *J Herbmed Pharmacol* **2017**, *6*, 126-129.
30. Kim, Y.M.; Jeong, Y.K.; Wang, M.H.; Lee, W.Y.; Rhee, H.I. Inhibitory effect of pine extract on α-glucosidase activity and postprandial hyperglycemia. *Nutrition* **2005**, *21*, 756-761, https://doi.org/10.1016/j.nut.2004.10.014.