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ORIGINAL ARTICLE

Ultra High Performance Liquid Chromatography-Electrospray Ionization-Mass Spectroscopy Quantification, Xanthine Oxidase Inhibitory, and Antioxidant Activity Profile of some Medicinal Plants from Albaha Region

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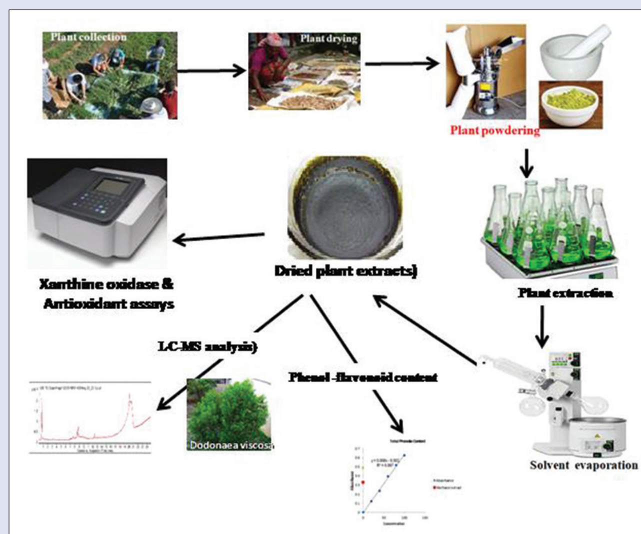
ABSTRACT

Background: There is an urgent need to find new xanthine oxidase (XO) inhibitors with few adverse effects and potent activity, not only for treating gout but also to fight diseases associated with XO activity such as cardiovascular diseases, cancer, diabetes, and obesity. **Objective:** Screening of Saudi medicinal plants for XO inhibitory activity, to quantify the polyphenol–flavonoid content and to study ultra high performance liquid chromatography-electrospray ionization-mass spectroscopy (UHPLC-ESI-MS) profile of compound with best promising XO inhibitory activity among screened extracts. **Materials and Methods:** Sixteen methanol extracts used traditionally for treating gout and/or rheumatism were screened for total polyphenol and flavonoid contents, XO inhibitory, and antiradical activity via 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The degree of XO inhibition was determined by measuring the increase in absorbance at 295 nm associated with uric acid formation. The dose-dependent inhibition profiles of the most active plants were further evaluated by estimating the IC_{50} values of their corresponding extracts. The most promising XO inhibitory extract, i.e., *Dodonaea viscosa* extract was subjected to secondary metabolites profiling using UHPLC-ESI-MS in negative ionization mode and LC-MS analysis. **Results:** Among screened plants, *D. viscosa* leaves, *Punica granatum* flowers, *Ruta chalepensis* leaves, and *Solanum incanum* fruits exhibited the highest activity with an inhibition of 94.4%, 83.4%, 76.2%, and 65.7%, respectively. Extracts of *R. nervosus* leaves, *P. granatum* flowers, and *D. viscosa* leaves showed the highest antiradical activity in the DPPH assay with IC_{50} values of 25.8, 27.4, and 71.2 μg/ml, respectively. The LC/MS spectrum of *D. viscosa* revealed the presence of 13 known compounds along with unknown compounds which belong to flavonoid, terpene, and fatty acid derivatives class. **Conclusion:** The findings obtained from this study revealed that the methanolic extract of *D. viscosa leaf* showed the highest xanthine oxidase inhibition activity and therefore is promising species for isolating active compound between the polyphenol and flavonoid content and the XO inhibitory and radical activity.

Key words: 2,2-diphenyl-1-picrylhydrazyl, albaha, *Dodonaea viscosa,* flavonoids, xanthine oxidase

SUMMARY

- This study was carried out to assess mainly the xanthine oxidase (XO) inhibitory and antiradical activities of sixteen methanol plant extracts used in folkloric medicine in Albaha region, Kingdom of Saudi Arabia. Total polyphenol and flavonoid content of the extracts were determined. The most promising XO inhibitory extract, i.e., *Dodonaea viscosa* extract was subjected to secondary metabolites profiling using ultra high performance liquid chromatography (LC)-electrospray ionization-mass spectroscopy (MS) in negative ionization mode and LC-MS analysis. This study proves scientifically the traditional uses of some plants and shows the relationships.



Abbreviations used: UHPLC-ESI-MS: Ultra high performance liquid chromatography-electrospray ionization-mass spectroscopy; DPPH: 2,2-diphenyl-1-picrylhydrazyl; IC_{50}: Inhibitory concentration; nm: Nano meter; KSA: Kingdom of Saudi Arabia; MeOH: Methanol; NANO2: Sodium nitrite; $ALCL_3$: Aluminum chloride; NAOH: Sodium hydroxide.

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INTRODUCTION

The worldwide incidence and prevalence of hyperuricemia and gout appear to be increasing not only in developed but also in developing countries such as Saudi Arabia where the lifestyle of Saudi Arabians has shifted recently toward to western type due to increasing affluence; this has led to increased incidences of hyperuricemia and gout. It was reported that hyperuricemia is present in a good proportion among Saudi people, but without an accompanying rise in the rate of gout.[1] In addition, there is mounting evidence that uric acid increase may be an independent risk factor for cardiovascular diseases, cancer, diabetes, and obesity;[2-4] therefore, the control of uric acid level may contribute in the prevention and treatment of these diseases that have been widespread among Saudi Arabians.

Urate-lowering agents are represented by xanthine oxidase (XO) inhibitors,[5] uricosuric agents,[6] and uricase agents.[7] XO stimulates the oxidation of hypoxanthine to xanthine and of xanthine to uric acid. Thus, XO inhibitors prevent the synthesis of uric acid. Among the many known XO inhibitors, oxypurinol, allopurinol, and febuxostat have been used widely for the treatment of gout and hyperuricemia, but these drugs can cause severe adverse effects, such as allergic reactions, nephropathy, and hepatitis.[8] Therefore, there is an imperious demand to find new XO inhibitors with few adverse effects and potent activity.

Given the WHO report that medicines derived from plants serve the health needs of approximately 80% of people globally,[9] scientists have turned their attention to explore the potent XO inhibitors from a wide variety of medicinal plants used traditionally for preventing and treating gout.[10-13] The putative therapeutic activity of these natural remedies may be linked to the presence of flavonoids, alkaloids, essential oils, phenolic compounds, tannins, iridoid glucosides, and coumarins that show the potential of antigout actions by their xanthine oxidase inhibition (XOI) activities.[10,14]

XO inhibitory activity of Saudi medicinal plants has not been reported so far, and the flora of Saudi Arabia is extraordinarily rich and diverse. There are many plants that the Saudi people use either in rural or urban areas for the treatment of different ailments in particular rheumatism.[15] This study represents the first report on XO inhibitory evaluation of 16 CMEs from 13 plant species growing in Albaha region, Kingdom of Saudi Arabia (KSA) and used traditionally for the treatment of rheumatoid arthritis and similar ailments.

MATERIALS AND METHODS

Plant materials

The plant material was collected between March and April from different locations in Albaha town and its outskirts [Table 1]. The plants were taxonomically identified at the Faculty of Clinical Pharmacy, Department of Pharmacognosy, Albaha University, KSA. Voucher specimens of the plant material are deposited at the Pharmacognosy Department, Faculty of Clinical Pharmacy, Albaha University, Saudi Arabia.

Preparation of extracts

Ten grams of air-dried, powdered plant material were extracted by shaking at room temperature with MeOH (4 times by 100 mL). The obtained extracts were filtered, evaporated to dryness in vacuo at 40°C, and the yield of each dried extract was calculated in percent. The resulting dried crude extracts were stored at 4°C.

Total flavonoid content

The total flavonoid content (TFC) was measured colorimetrically according to Jothy *et al.*'s method,[16] with minor modification. For each plant extract, 500 µl of the stock (1 mg/ml) was added to 2 ml of distilled water. Then, 0.15 ml of $NaNO_2$ (5% w/v) was added and left for 6 min; then, 0.15 ml of $AlCl_3$ (10%) was added which was left for another 6 min. Finally, 2 ml of NaOH (4% w/v) and distilled water (0.2 ml) were added, left for 15 min at room temperature, and the absorbance was measured at 510 nm. Distilled water was used to blank all samples. Catechin is a flavan-3-ol and used as a standard in determination of total flavonoid content. Different concentrations of catechin were used as standard for plotting the calibration curve ($Y = 0.0041X + 0.0032$, $R^2 = 0.9995$). The TFC was estimated as mg of catechin/100 mg of dried extract.

Total polyphenolic content (TPPC)

The total polyphenolic content (TPPC) in the plant extract was measured by Folin–Ciocalteu reagent based on procedure described by Chen *et al.*[17] with little modifications. 0.3 mL of plant extract (1 mg/ml) was mixed with 1.5 mL of Folin–Ciocalteu reagent (1:10 dilution) and allowed to stand in darkness for 6 min. Then, 1.2 mL of 7.5% sodium carbonate was added, and the mixture was left in dark at 40°C for 90 min. The absorbance of the blue color, that developed, was measured

Table 1: Selected plants studied, ethnobotanical information, and characteristics

Species	Plant family (voucher number)	Part tested (yield in percentage)	Local name	Traditional uses
Achillea biebersteinii Afan	Asteraceae (CP-101)	Fl (1.9)	Thafra	Inflammation[b]
Achillea biebersteinii Afan	Asteraceae (CP-101)	L (3.1))	Thafra	Inflammation[b]
Calotropis procera (Aiton) W.T.Aiton	Asclepiadaceae (CP-091)	L (4.2)	Al Ashur	Rheumatism[a]
Chenopodium murale L	Amaranthaceae (CP-081)	F (8.2)	Jkheara	Inflammation[a]
Dodonaea viscosa Jacq	Sapindaceae (CP-061)	L (2.9)	Shath	Rheumatism[21]
Euphorbia helioscopia L	Euphorbiaceae (CP-051)	AP (4.5)	Al-dehin	Antiseptic
Lavandula dentata L	Lamiaceae (CP-041)	AP (3.3)	Al-shiah	Rheumatism
Pulicaria crispa SCH.BIP	Asteraceae (CP-102)	AP (3.1)	Arararabi	Anti-inflammatory[b]
Punica granatum L	Punicaceae (CP-011)	Fl (2.2)	Al-roman	Rheumatism Shukla *et al.* 2008
Rumex nervosus Vahl (*leaves*)		L (3.7)		
Ruta chalepensis (L)	Rutaceae (CP-121)	L (4.8)	Al-shathab	Inflammatory diseases[b]
Solanum incanum L	Solanaceae (CP-131)	F r (8.1)	Al-hadak	Antiseptic[b]
Solanum incanum L	Solanaceae (CP-131)	L (3.5)	Al-hadak	Leaves as dressing for healing wounds, paste of fruits for treating leishmaniasis[b]
Verbesina encelioides (Cav.) Benth and Hook. f. ex A. Gray	Asteraceae (CP-021)	L (3.2)	Safeara	Wounds, skin diseases[b]
Withania somnifera (L.) Dunal	Solanaceae (CP-011)	Fr (5.2)	Alobeb	Chronic dermatitis[b]
Withania somnifera	Solanaceae (CP 011)	L (4.2)	Alobeb	

[a]Most information obtained from reference,[12] [b]Interviewing with local people. AP: Aerial parts; F: Fruits; L: Leaves; Re: Resins; Fl: Flowers

at 765 nm. Plant extracts that produced absorbances higher than 1.5, a concentration of 100 μg/ml was repeated. The experiments were carried out in triplicates. Gallic acid was used for constructing the standard curve (10–100 μL) (Y = 0.0102X+0.0222; R^2 = 0.9979); and the total phenolic compounds' concentration in each extract was expressed as milligrams of gallic acid equivalent per 100 mg of dried extract (mgGAE/100 mg).

Xanthine oxidase inhibition assay

Using xanthine as substrate, the XO activity was assayed by spectrophotometric method according to Apaya and Hernandez 2011.[18] A mixture containing 1 ml of 100 μg/ml of plant extract or allopurinol, 1.9 ml of 50 mM potassium phosphate buffer, and 1 ml of xanthine substrate (0.6 mM) was preincubated for 10 min at 25°C, and the reaction was started by addition of 0.1 ml of XO enzyme (0.1U/ml in phosphate buffer). The reaction was incubated at 25°C for 30 min, stopped by 1 ml of 1M HCl and the absorbance was measured against phosphate buffer as blank at 295 nm using quartz cuvettes. Allopurinol, the standard XO inhibitor, was used as a positive control. The % XOI was calculated accordingly using the following equation:

$$\% \text{ XOI} = 100 - (A_1 - B) \times 100/(A_o - B)$$

Where A_1 is the activity of the enzyme in the presence of plant extractor standard inhibitor, B is the absorbance in the absence of the enzyme, and A_o is the absorbance in the absence of the plant extractor inhibitor.

Radical scavenging (2,2-diphenyl-1-picrylhydrazyl) assay

The radical scavenging ability of plant extract was quantitatively carried out by the method described by Al-badani *et al.*[19] with minor modification. In a light-protected bottle, 500 μl of plant extract at concentrations ranging from 10 μg/ml to 3 mg/ml was added to 5 ml of 0.004% w/v solution of DDPH in 80% methanol. Ascorbic acid was used as standards and 80% methanol as blank whereas the 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution in the absence of plant extract was used as negative control. The reaction mixture was incubated for 30 min in dark at 37°C, and the absorbance was measured at 517 nm. All assays were measured in triplicates, and DPPH scavenging effect was calculated according to the following equation:

$$\% \text{ DPPH scavenging effect} = (A_o - A_1) \times 100/A_o$$

Where A_o is the absorbance of the negative control, A_1 is the absorbance of DPPH in the presence of plant extract-the absorbance of plant extract blank in 80% methanol. IC_{50} (Inhibitory Concentration) values were calculated from the dose inhibition curve, and results were recorded as average ± standard deviation (SD).

Secondary metabolite profiling

Secondary metabolites were evaluated by UHPLC Accurate-Mass Q-TOF (Agilent 1290 Infinity LC system coupled to Agilent 6520) mass spectrometer with dual ESI source. XDB-C_{18} Agilent Zorbax Eclipse, narrow-bore 2.1 mm × 150 mm, and 3.5 micron (P/N: 930990-902) column was used. The temperature of column was maintained at 25°C, while autosampler temperature was 4°C. The following two mobile phases used: A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) at flow rate of 0.5 mL/min. Injection volume was 1.0 μL. Run time was 25 min and postrun time was 5 min. Mass spectroscopy (MS) analysis full scan was carried out over a range of m/z 100–1000 employing electrospray ion source in the negative ionization mode. Flow rate for nitrogen as nebulizing and drying gas was 25 and 600 L/h, respectively, with drying gas temperature of 350°C. The fragmentation voltage was optimized to 125 V. Capillary voltage for analysis was 3500 V. Data processing was done using Agilent MassHunter Qualitative Analysis B.05.00 (Method: Metabolomics-2017-00004.m). Identification of compounds was done from Search Database: METLIN_AM_PCDL-N-170502.cdb, with parameters as: match tolerance: 5 ppm, positive ions: H^+, Na^+, $[NH_4]^+$, and negative ions: H^-.

Statistical analysis

All results are presented as mean ± SD, and the experiments were performed in triplicate. The % inhibition and IC_{50}s of the CME were calculated using GraphPad Prism version 6.0. (GraphPad Software Inc., California, USA). Pearson correlation coefficient was calculated between TFC, TPPC, and XOI activity of all plant CMEs. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Sixteen CMEs from 13 species of Saudi medicinal plants belonging to nine plant families were screened for XOI activity and antiradical activity using DPPH assay, at concentrations ranging from 25 to 100 μg/ml. The plant species tested in this study were selected on the basis of the folkloric uses as antirheumatic and anti-inflammatory plants [Table 1].

The total phenolic content in the examined plant extracts, which were determined by the Folin–Ciocalteu method, is given in Table 2 as gallic acid equivalent by reference to standard curve (Y = 0.0102X + 0.0222; R^2 = 0.9979). The 16 plant extracts were found to have widely varying phenolic concentration ranging from 1.70 to 22.11 g GAE/100 g plant extract. Extracts of *R. nervosus, Punica granatum* flower, *Solanum incanum* fruits, and *Dodonaea viscosa* leaf exhibited the highest content of phenolics with 24.34, 22.11, 10.69, and 10.67, respectively. The TFC of the tested plants varied from 1.73 to 15.72 mg of catechin equivalent per gram of sample, with a descending order of *R. nervosus* leaves (15.7), *S. incanum* fruits (9.1), *L. dentate* aerial part (4.6), *P. granatum* flowers (4.4), and *D. viscosa* leaves (3.83) [Table 2].

All extracts demonstrated XOI activity at 100 μg/ml, among which namely ten plant extracts showed an inhibition >50%. Among these plants, *D. viscosa* leaves, *P. granatum* flowers, *Ruta chalepensis* leaves, and *S. incanum* fruits exhibited the highest activity with an inhibition of 94.4%, 83.4%, 76.2%, and 65.7% and with IC_{50} values of 54.8, 70.9, 71.5, and 77.2 μg/ml, respectively. Extracts of *R. nervosus* leaves, *P. granatum* flowers, and *D. viscosa leaves* showed the highest antiradical activity in the DPPH assay with IC_{50} values of 25.8, 27.4, and 71.2 μg/ml, respectively.

D. viscose has a wide range of folkloric uses. The stems are used as fumigants to treat rheumatism. The plant extracts were found to have anti-inflammatory, antipyretic, analgesic, and antioxidant properties.[20] Getie *et al.* isolated relatively large amounts of quercetin and kaempferol from *D. viscosa* crude leaf extract that may be partially responsible for XOI activity.[21] In contrast to our potent XOI findings, the hydroalcoholic leaf and branches of extract of *D. viscosa* growing in Australia exhibited weak XOI activity (28.6%).[22]

This variation in the XOI activity could be explained in the light of the presence and/or quantities of bioactive compounds in plants that are influenced by several factors including the plant part used, plant age, environment, seasons, climate, and intraspecies variations. In agreement partially with our results, Mothana *et al.* reported a high effective free-radical scavenging in the DPPH assay with an IC_{50} value of 50 μg/ml for methanolic extract of *D. viscosa* collected from Yemen[23] [Table 3].

The methanol extract of *D. viscose* was subjected to secondary metabolites profiling using ultra high performance liquid chromatography (LC)-electrospray ionization-MS in negative ionization mode. Total ion chromatograms of the extract are shown in Figure 1. The LC/MS spectrum revealed the presence of 13 known compounds along

Table 2: Xanthine oxidase inhibitory, DPPH scavenging activity, and total polyphenol and flavonoid contents of plant extracts

Plant	Total flavonoid content mg catechin/100 mg dried extract	Total phenolic content mg gallic acid/100 mg dry extr act	DPPH scavenging activity IC_{50} (μg/ml)	Percentage XO inhibition (100 μg/ml)	XO IC_{50}
Achillea biebersteinii (Fl)	3.028±0.31	6.194±0.10	188.2	54.4	84.6±5.4
Achillea biebersteinii (L)	4.336±0.57	5.054±0.37	261.2	58.9	97.5±3.4
Calotropis procera (L)	2.759±0.50	3.076±0.24	1756.8	41.8	-
Chenopodium murale (AP)	2.450±0.25	1.700±0.17	1037.5	38.6	-
Dodonaea viscosa (L)	3.832±0.35	10.671±0.52	71.1	94.3	54.8±0.9
Euphorbia helioscopia L (AP)	3.572±0.65	7.171±0.52	103.1	56.2	99.2±26.4
Lavandula dentata (AP)	4.572±0.31	3.377±0.48	309	73.5	90.3±6.9
Punica granatum (Fl)	4.353±0.52	22.109±0.24	27.4	83.1	70.9±3.4
Pulicaria crispa (AP)	3.74±0.26	3.995±0.25	282.1	56.0	101.3±2.1
Rumex nervosus (leaves)	15.718±0.92	24.338±1.23	25.8	35.8	-
Ruta chalepensis (L)	3.141±0.16	4.080±0.38	367.7	76.2	71.5±0.1
Solanum incanum (fruits)	9.060±0.73	10.678±0.55	98.7	65.7	77.2±9.5
Solanum incanum (L)	3.670±0.10	3.995±0.14	293.2	48.7	-
Withania somnifera (fruits)	1.727±0.18	3.792±0.054	379.7	69.5	85.5±0.3
Withania somnifera (L)	3.589±0.18	3.155±0.04	682.7	31.9	-
Verbesina encelioides	2.190±0.14	2.341±0.20	1411.7	34.1	-
Ascorbic acid			19.1	-	-
Allopurinol			-	96.6	0.34±0.1

OX: Xanthine oxidase; IC_{50}: Inhibitory concentration; DPPH: 2,2-diphenyl-1-picrylhydrazyl

Table 3: Pearson correlation coefficient between xanthine oxidase inhibition and different groups and its significance (represented by *P*)

Group	Pearson correlation coefficient (*R*)	Significance (*P*<0.05)
Flavonoid content with XOI	0.291	0.415
Flavonoid content with polyphenolic content	0.417	0.231
Polyphenol content with XOI	0.664	0.037

XOI: Xanthine oxidase inhibition

with unknown compounds. Most of these compounds were found to be flavonoid, terpenes, and fatty acid derivatives. The flavonoids present were mangiferin, guibourtinidol-(4alpha->6)-catechin, and scutellarein 4'-methyl ether 7-(2",6"-diacetylalloside). 6-paradol as a terpene was identified. Fatty acid derivatives found included 14-hydroxy stearic acid, (2S)-2-hydroxyphytanic acid, and docosanedioic acid [Table 4]. From the above-mentioned LC-MS results, it is confirmed that the methanol extract of *D. viscosa* is rich in polyphenolic and flavonoid compounds.

Other interesting source of antigout activity is *P. granatum* flower (IC_{50} 71 μg/mL). Phytochemical and biological investigations of *P. granatum* were recorded;[24-26] but to the best of our knowledge, there is no report available concerning the XOI activity of *P. granatum* flowers; however, methanol extract of *P. granatum* seeds was reported to exhibit inhibition to XO.[27] *P. granatum* has been used by traditional healers to treat diseases such as arthritis,[28] and *P. granatum* flower was found to have antioxidant and anti-inflammatory activity and is rich in flavonoids such as quercetin and phytosterols, for example, β-sitosterol,[25,26] that may be responsible for the XOI activity.[27,29] In addition, the results indicated pomegranate flower extract to exert a more potent antiradical activity in DPPH assay with IC_{50} of 27.4 μg/ml in comparison to what was recorded previously.[30]

R. graveolens is a medicinal plant, which is used widely in Saudi traditional medicine,[15] and is rich in flavonoids as rutin and quercetin that are responsible for its XOI activity. Compared to reported findings about the XOI activity of the plant extract from Iran (IC_{50}: 110 μg/ml),[31] our results showed relatively stronger XOI activity with an IC_{50} value of 71.5 μg/ml. Literature review shows that *S. incanum* is rich in phytochemicals such as carpesterol, solasodine, β-sitosterol, stigmasterol, and khasianine.[32] Lin *et al*. reported the presence of quercetin, kaempferol that may take part in the XOI activity of the plant extract.[33] Besides, members of the *Solanum* genus were known to possess XO inhibitory activity such as *S. melongena*, likely due to the presence of β-sitosterol, stigmasterol present in them.[29] In addition, *S. tornum* is traditionally used to treat anti-inflammatory conditions such as gout and rheumatism in the Philippines, and its methanolic extract exhibited 38.45% XOI activity.[18]

Phenolic compounds of plants are categorized into several categories; the major among these are the flavonoids, which have potent biological activities. Studies on flavonoid derivatives were reported to have a wide range of antibacterial, anti-inflammatory, antiviral, anticancer, hepatoprotective, and antioxidant activities.[34] Flavonoids were explored to inhibit XO activity. The observed activity of the most plant extracts tested could be attributed to the presence of flavonoids. For *P. granatum* flower extract, a significant positive correlation (R_2 it was made for all plants and not one plant) was found between total phenolic and flavonoid content and percent inhibition of XO, while also another weak positive correlation was found for *S. incanum* (fruits), *D. viscosa,* between TFC and % XOI activity. However, a non-significant correlation was found in case of fruit extract of *W. somnifera* between TFC and XOI.

For XO inhibitory activity (% XOI), a significant ($P < 0.05$) but marginal correlation ($R > 0.664$) was obtained for % XOI and TPPC, but nonsignificant correlation ($R > 0.231$ and $R > 0.415$) of TFC with both polyphenolic content and % XOI, respectively. Hence, the XOI activity could be related to the polyphenolic content or the quality of flavonoid in that plant.

CONCLUSION

To the best of our awareness, this is the first report of the evaluation of the XOI activity of the Saudi medicinal plant extracts. The findings obtained from this study revealed that the three methanolic extracts of *D. viscose* leaves, *P. granatum* flowers, and *L. Ruta chalepensis* leaves showed remarkable XOI activity and therefore are promising species for isolating active compounds through a bioassay-guided fractionation that could be used to treat or protect from the chronic gout. In addition, this study confirms scientifically the traditional uses of these plants for preventing or treating gout.

Table 4: Liquid chromatography-mass spectrometry spectral analysis of methanol extract of *Dodonaea viscosa*

Right (min)	Base peak (m/z)	Peak height	Volume (%)	Proposed compounds	Compound class	Mole formula	Mole mass
0.604	225.061	72298	0.51	Glucoheptonic acid	Acid	$C_7H_{14}O_8$	226.068
0.657	421.079	28341	0.39	Mangiferin	Flavonoid	$C_{19}H_{18}O_{11}$	422.086
0.727	133.015	61403	1.34	3,3-Dimethyl-1,2-dithiolane	Aliphatic	$C_5H_{10}S_2$	134.022
1.803	315.070	27133	1.03	2-(4-Chlorophenyl)-3-phenyl-3-(2-pyridinyl) acrylonitrile;	Stilbene nitrile	$C_{20}H_{13}ClN_2$	316.077
6.848	443.191	35282	1.05	Cynaroside A	Glycoside	$C_{21}H_{32}O_{10}$	444.199
8.474	545.143	86252	1.24	Guibourtinidol-(4alpha->6)-catechin	Flavonoid	$C_{30}H_{26}O_{10}$	546.151
8.529	545.128	96409	4.03	Scutellarein 4'-methyl ether 7-(2",6"-diacetylalloside)	Flavonoid	$C_{26}H_{26}O_{13}$	546.136
12.323	221.117	166276	5.48	(6S)-dehydrovomifoliol	Aliphatic	$C_{13}H_{18}O_3$	222.125
14.818	277.180	24678	0.9	6-Paradol	Terpene	$C_{17}H_{26}O_3$	278.187
17.015	299.259	34183	1.26	14-hydroxy stearic acid	Fatty acid	$C_{18}H_{36}O_3$	300.266
18.585	327.291	25762	1.27	(2S)-2-hydroxyphytanic acid	Fatty acid	$C_{20}H_{40}O_3$	328.298
19.208	369.302	39643	1.51	Docosanedioic acid	Fatty acid	$C_{22}H_{42}O_4$	370.309

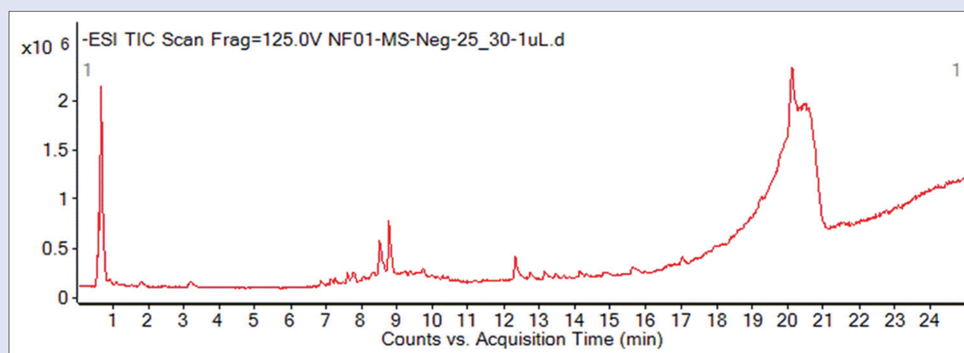

Figure 1: Liquid chromatography-electrospray ionization-mass spectroscopy total ion chromatogram of the methanol extract of *Dodonaea viscosa*

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Conflicts of interest

There are no conflicts of interest.

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