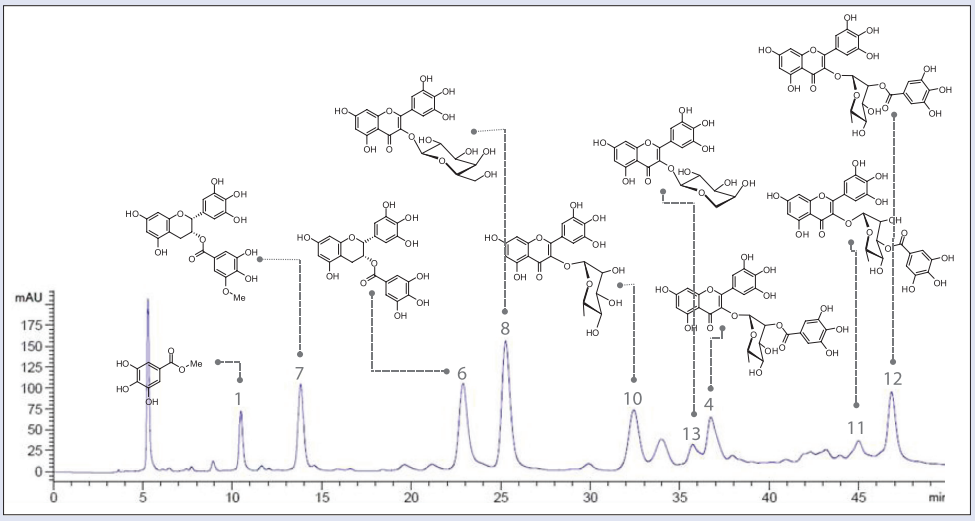

Figure 2: High-performance liquid chromatography of the ethyl acetate fraction of *Limonium tetragonum* extract at the wavelength of 254 nm. 1: gallincin, 4: quercetin-3-O-β-D-galactopyranoside, 6: (–)-epigallocatechin-3-gallate, 7: (–)-epigallocatechin-3-(3′′-O-methyl) gallate, 8: myricetin-3-O-β-D-galactopyranoside, 10: myricetin-3-O-α-L-rhamnopyranoside, 11: myricetin-3-O-(2′′-O-galloyl)-α-L-rhamnopyranoside, 12: myricetin-3-O-(3′′-O-galloyl)-α-L-rhamnopyranoside, 13: myricetin-3-O-α-L-arabinopyranoside

Table 3: Inhibitory effects of compounds 1-13 on the activated HSC-T6 cells

Control	Compounds	Percentage of control	
		25 μM	50 μM
		100.0±0.9	100.0±0.7
1	Gallincin	60.4±1.2	45.5±0.8
2	Apigenin-3-O-β-D-galactopyranoside	72.2±0.9	59.9±2.6
3	Quercetin	68.7±1.8	51.1±1.7
4	Quercetin-3-O-β-D-galactopyranoside	70.7±2.0	54.4±3.0
5	(-)-epigallocatechin	98.8±0.9	87.9±1.1
6	(-)-epigallocatechin-3-gallate	72.7±2.2	45.4±1.5
7	(-)-epigallocatechin-3-(3”-O-methyl) gallate	80.1±1.0	52.2±2.4
8	Myricetin-3-O-β-D-galactopyranoside	67.7±1.7	57.7±2.2
9	Myricetin-3-O-(6”-O-galloyl)-β-D-galactopyranoside	55.2±2.9	32.1±1.4
10	Myricetin-3-O-α-L-rhamnopyranoside	68.9±2.7	53.6±2.3
11	Myricetin-3-O-(2”-O-galloyl)-α-L-rhamnopyranoside	56.7±1.0	37.2±1.7
12	Myricetin-3-O-(3”-O-galloyl)-α-L-rhamnopyranoside	57.1±2.0	39.6±2.3
13	Myricetin-3-O-α-L-arabinopyranoside	62.9±3.0	50.6±3.7

HSC-T6 cells were incubated with each compound at the concentrations of 25 and 50 μM for 48 h. Cell viability was measured by the MTT assay. *P<0.01 compared with nontreated control. HSC-T6: Hepatic stellate cell-T6, MTT: 3-(4,5-dimethylthiazol-2-yl)-2.5-diphenyltetrazolium bromide

reduced expression of transforming growth factor-beta1 (TGF-β1) and alpha-smooth muscle actin (α-SMA) in BALB/cN mice. Myricitrin improved the regeneration of hepatic tissue by inducing proliferating cell nuclear antigen expression. In this animal model, myricitrin was demonstrated to provide a better hepatoprotection when compared to silymarin, which is consistent with its higher *in vitro* antioxidant potential. EtOAc fraction of *L. tetragonum* which is enriched with various myricetin glycosides might therefore be useful for the prevention and/or treatment of liver diseases including fibrosis.

For the extensive usage of medicinal plants as new drug leads or functional food resources, the quality control of plant extract should be preceded. Standardization of plant extract guarantees the reproducible bioactivity and phytoequivalence as well as minimizing undesirable effects. For this, the identification of bioactive constituents in the extract and the selection of one or more constituents as marker compounds is necessary. Furthermore, the development of efficient analytical methods is required. In the present study, we could isolate active compounds classified into catechins and flavonoid glycosides from the EtOAc fraction of *L. tetragonum* extract. Using these isolated compounds, we tried to develop a simultaneous analytical method using HPLC. For the simultaneous determination of major constituents of *L. tetragonum*, the chromatographic condition was first investigated. Various mixtures of acetonitrile, water, and methanol in combination with acetic acid or formic acid in concentration range from 0.01% to 1% were tested as a mobile phase. Acid is known to be effective to improve the separation for phenolic compounds by reducing the tailing of the peaks. Under our chromatographic condition, the addition of 0.1% acetic acid in water was found to improve the resolution of the peaks detected, whereas the distortion of peaks was observed when <0.05% of acetic acid was added. The wavelength for detection was set at 254 nm, where the peaks showed high absorption with the improved S/N (signal-to-noise ratio)

measured by DAD. As a result, the optimal mobile phase consisting of acetonitrile–water with 0.1% acetic acid was subsequently employed for the analysis of the EtOAc fraction of *L. tetragonum* extract, which led to a good resolution and satisfactory peak shape at 254 nm [Table 1]. The presence of eight compounds including gallincin (1), quercetin-3 -O-β-D-galactopyranoside (4), (−)-epigallocatechin-3-gallate (6), myr icetin-3-O-β-D-galactopyranoside (8), myricetin-3-O-α-L-rhamnopy ranoside (10), myricetin-3-O-(2″-O-galloyl)-α-L-rhamnopyranoside (11), myricetin-3-O-(3″-O-galloyl)-α-L-rhamnopyranoside (12), myr icetin-3-O-α-L-arabinopyranoside (13) in the extract was verified by comparing each retention time and UV spectrum with those of each standard compound and spiking with authentic standards [Figure 2].

CONCLUSION

Our data demonstrated that flavonoids and flavonoid glycosides (apigenin, quercetin, and myricetin) and catechins derived from *L. tetragonum* attenuated the proliferation of the activated HSC-T6. Furthermore, a rapid and reliable HPLC method for the simultaneous determination of eight active constituents in EtOAc fraction of *L. tetragonum* has been developed. The EtOAc fraction and the isolated compounds may have promise as candidates for the treatment of liver diseases.

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Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Ksouri R, Megdiche W, Debez A, Falleh H, Grignon C, Abdelly C. Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritima*. Plant Physiol Biochem 2007;45:244-9.
2. Ksouri R, Megdiche W, Falleh H, Trabelsi N, Boulaaba M, Smaoui A, *et al.* Influence of biological, environmental and technical factors on phenolic content and antioxidant activities of Tunisian halophytes. C R Biol 2008;331:865-73.
3. Sehrawat A, Sultana S. Evaluation of possible mechanisms of protective role of *Tamarix gallica* against DEN initiated and 2-AAF promoted hepatocarcinogenesis in male Wistar rats. Life Sci 2006;79:1456-65.
4. Hanen F, Riadh K, Samia O, Sylvain G, Christian M, Chedly A. Interspecific variability of antioxidant activities and phenolic composition in *Mesembryanthemum* genus. Food Chem Toxicol 2009;47:2308-13.
5. Ksouri R, Falleh H, Megdiche W, Trabelsi N, Mhamdi B, Chaieb K, *et al.* Antioxidant and antimicrobial activities of the edible medicinal halophyte *Tamarix gallica* L. and related polyphenolic constituents. Food Chem Toxicol 2009;47:2083-91.
6. Oueslati S, Ksouri R, Falleh H, Pichette A, Abdelly C, Legault J. Phenolic content, antioxidant, anti-inflammatory and anticancer activities of the edible halophyte *Suaeda fruticosa* Forssk. Food Chem 2012;132:943-47.
7. Ksouri WM, Medini F, Mkadmini K, Legault J, Magné C, Abdelly C, *et al.* LC-ESI-TOF-MS identification of bioactive secondary metabolites involved in the antioxidant, anti-inflammatory and anticancer activities of the edible halophyte *Zygophyllum album* Desf. Food Chem 2013;139:1073-80.
8. Yang MH, Kim NH, Heo JD, Sung SH, Jeong EJ. Hepatoprotective effects of *Limonium tetragonum*, edible medicinal halophyte growing near seashores. Pharmacogn Mag 2014;10 Suppl 3:S563-8.
9. Kim NH, Sung SH, Heo JD, Jeong EJ. The extract of *Limonium tetragonum* protected liver against acute alcohol toxicity by enhancing ethanol metabolism and antioxidant enzyme activities. Nat Prod Sci 2015;21:1-5.
10. Kim NH, Heo JD, Kim TB, Rho JR, Yang MH, Jeong EJ. Protective effects of ethyl acetate soluble fraction of *Limonium tetragonum* on diethylnitrosamine-induced liver fibrosis in rats. Biol Pharm Bull 2016;39:1022-8.
11. Lee JI, Kong CS, Jung ME, Hong JW, Noh I, Seo Y. Peroxynitrite-scavenging activity of the halophyte *Limonium tetragonum*. Ocean Polar Res 2011;33:185-91.
12. Bataller R, Brenner DA. Liver fibrosis. J Clin Invest 2005;115:209-18.
13. Vogel S, Piantedosi R, Frank J, Lalazar A, Rockey DC, Friedman SL, *et al.* An immortalized rat liver stellate cell line (HSC-T6): A new cell model for the study of retinoid metabolism *in vitro*. J Lipid Res 2000;41:882-93.
14. Wu J, Zern MA. Hepatic stellate cells: A target for the treatment of liver fibrosis. J Gastroenterol 2000;35:665-72.
15. Chen YW, Li DG, Wu JX, Chen YW, Lu HM. Tetrandrine inhibits activation of rat hepatic stellate cells stimulated by transforming growth factor-beta *in vitro* via up-regulation of Smad 7. J Ethnopharmacol 2005;100:299-305.
16. Nakamuta M, Higashi N, Kohjima M, Fukushima M, Ohta S, Kotoh K, *et al.* Epigallocatechin-3-gallate, a polyphenol component of green tea, suppresses both collagen production and collagenase activity in hepatic stellate cells. Int J Mol Med 2005;16:677-81.
17. Matic S, Stanic S, Bogojevic D, Vidakovic M, Grdovic N, Dinic S, *et al.* Methanol extract from the stem of *Cotinus coggygria* Scop. and its major bioactive phytochemical constituent myricetin modulate pyrogallol-induced DNA damage and liver injury. Mutat Res 2013;755:81-9.
18. Khan RA, Khan MR, Ahmed M, Sahreen S, Shah NA, Shah MS, *et al.* Hepatoprotection with a chloroform extract of *Launaea procumbens* against CCl4-induced injuries in rats. BMC Complement Altern Med 2012;12:114.
19. Domitrovic R, Rashed K, Cvijanovic O, Vladimir-Kneževic S, Škoda M, Višnic A. Myricitrin exhibits antioxidant, anti-inflammatory and antifibrotic activity in carbon tetrachloride-intoxicated mice. Chem Biol Interact 2015;230:21-9.