Phytochemical analysis and bioactivity screening of three medicinal plants of Saudi Arabia

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Abstract

Purpose: To investigate the phytochemical analysis and bioactivity screening of some Asteraceae medicinal plants.

Methods: The chemical constituents were isolated by column chromatography and elucidated using chemical and extensive spectroscopic methodologies including gas chromatography-mass spectrometry (GC-MS), Fourier transform infrared spectroscopy (FTIR), as well as 1D and 2D nuclear magnetic resonance (NMR). The plant extracts were obtained by solvent extraction method while hydrodistillation was used to isolate plant essential oils. Furthermore, cup-plate agar diffusion was applied for antimicrobial activity evaluation while minimum inhibitory concentration (MIC) was assessed by microdilution technique.

Results: Centaurea pseudosinaica, Tripleurospermum auriculatum, and Koelpinia linearis afforded previously undescribed three coumarins (xanthotoxin, cirsimaritin, salvigenins) from C. pseudosinaica, one steroid (estradiol) and a pentacyclic triterpene (β-amyrin) from T. auriculatum and a coumarin (santin) from K. linearis in good yields. In addition, the plant extracts and oils exhibited remarkable bioactivities including antifungal, antibacterial and antipyretic etc.

Conclusion: The results reveal the presence of bioactive phytomolecules from Asteraceae plant extracts and volatile oils from three Asteraceae plants.

Keywords: C. pseudosinaica, T. auriculatum, K. linearis, Xanthotoxin, Salvigenin, Cirsimaritin, Santin, Estradiol, β-amyrin, Antimicrobial activity

INTRODUCTION

Asteraceae or sunflower is a big and widespread family of flowering plants with more than 1,911 genera and 32,913 species [1]. The useful chemotaxonomic markers of the genus Centaurea are characterized by the presence of terpenoids, flavonoids, alkaloids, coumarins, and sesquiterpene lactones [2]. Centaurea pseudosinaica is native to Middle East and locally abundant mainly in the northern region of Saudi Arabia. To the best of our knowledge, this is the first phytochemical investigation of C. pseudosinaica and its bioactivities except one.
previous study on the antibacterial activity of this plant against the Gram-+ve bacteria [3].

Nothing appears to have been published before on the chemical constituents of T. auriculatum, although the genus Tripleurospermum has been previously studied and several significant medicinal properties including anti-inflammatory, anti-fungal, anti-bacterial and anti-oxidant were reported [4]. The plant K. linearis, a unique species in the genus Koelpinia is a rich source of triterpenoids, steroids and series of five long-chain alkanolic acid esters of lupeol and lupenone [5]. The purpose of our present study is to determine the phyto-chemical constituents of the plants mentioned above and the evaluation of their bioactivities.

EXPERIMENTAL

Plant collection, identification and extract preparation

The entire plant materials including stems, leaves and flowers of C. pseudosinaica, T. auriculatum and K. linearis were procured from Central Saudi Arabia (Riyadh) in early May 2014. Identification of the plant specimen was performed by a taxonomist (Dr Jacob T. Pandalayil) from Herbarium Division, College of Science, KSU, Riyadh, KSA. Samples of the plants were kept in our laboratory under the specimens numbers HZK-121, HZK-122 and HZK 123, respectively. The plants materials were air-dried in shade, crushed into fine powder and then extracted with methanol. The methanolic extracts were filtered, dried and subjected to column chromatography. The hydro-distillation of C. pseudosinaica (whole plant, 90 gm) in a Clevenger-type apparatus afforded yellow colored oil in the yield of 0.67 % v/w (fresh weight basis).

Isolation and identification of chemical constituents

Fractionation of methanolic crude extract (90 g) of C. pseudosinaica with water and ethyl acetate gave, after evaporation of solvent, 48 g from the ethyl acetate fraction. This fraction was further subjected to column chromatography (120 x 3 cm in size) on a silica (520 g) using various proportion of petroleum ether, chloroform and ether as eluting solvents. Xanthotoxin/furanoocoumarin was obtained as a colourless needle like crystals (0.16 g, m.p. 148 °C), lit. m.p. 149 °C [16,17]. The compound characterization was carried out through NMRs, IR and mass spectroscopy. Similarly, the ethanol extract (56 g) of C. pseudosinaica gave after solvent evaporation, a dark green viscous material which was initially subjected to column chromatography (140 x 2.5 cm, with 870 g silica gel) eluting with CHCl₃:EtOAc (6:4). Salvigenin (0.71g) was obtained as pale yellow crystals (m.p. 188 °C, lit. m.p. 189 °C [18]. Further elution of the same column gave cirsimaritin (0.52 g as yellow crystals, m.p. 196 °C, lit. m.p. 198 °C [18-20]. The butanol extract (20 g) of T. auriculatum gave after solvent evaporation, a greenish material which subject to column chromatography (140 x 2.5 cm, with 870 g silica gel) eluting with CHCl₃:EtOAc (5:5). Santin (0.45 g) was obtained as colourless crystals (m.p. 161 °C, lit. m.p. 162 °C) [19, 20]. Ethyl acetate fraction of the methanolic extract of K. linearis gave, after solvent evaporation, a colourless amorphous powder which was subjected to column chromatography (120 x 2 cm, with 650 g silica gel) eluting with CHCl₃:EtOAc (6:4). Two compounds were isolated, purified and identified after characterization as estradiol (2.40 g as colourless crystals, m.p. 172 °C, lit. 171 °C) [21] and β-amyrin (0.92 g as colourless crystals, m.p. 188 °C, lit. m.p. 189 °C) [22]. All spectroscopic data (UV, IR, 1H-NMR, 13C-NMR, Mass) of estradiol β-amyrin were compared with literature [14,21,23].

Antimicrobial screening

The hydrodistilled oil and extracts of C. pseudosinaica and other plant extracts T. auriculatum, K. linearis were taken for anti-microbial screening by just dissolving 20 mg (oil/extract) in 1 mL dimethylformamide (DMF) and 50 μL was applied (equivalent to 1mg). The cup-plate agar diffusion technique was used for the antimicrobial screening [24]. The selected pathogens were fungi for example Aspergillus fumigates (RCMB 02568), Syncphaelastrum racemosum (RCMB 05922), Geotricum candidum (RCMB 05097), Candida albicans (RCMB 05036) and bacteria such as Gram +ve bacteria (Streptococcus pneumonia (RCMB 010010), Bacillus subtilis (RCMB 010067) and Gram -ve bacteria (Pseudomonas aeruginosa (RCMB 010043), Escherichia coli (RCMB 010052) which were obtained from stock culture at College of Science, KSU, Riyadh. For the positive controls, amphotericin B in DMF (30 μg) for fungi, ampicillin in DMF (30 μg) for Gram +ve bacteria and gentamycin in DMF (30 μg) for Gram -ve bacteria were used as reference antibiotics. The incubation of plates for fungi was done for 72 hrs at 28 °C while one day at 37 °C for bacteria and subsequently inhibition zones were detected and recorded. The minimum inhibitory concentration (MIC) was determined.
with microdilution method [25a] using serially
diluted (2-fold) of plant extract.
Mice (200-250g) used for in-vivo experiment
were taken from the Veterinary Section (animal
house), College of Science, KSU, Riyadh and
weighed form 200 to 250g. Before inoculation,
they were initially tested for negative pyretic
where yeast induced pyrexia (10 ml/kg) was
suspended in 0.9 % saline. The determination of
bilirubin, albumin, ALT (alanine transaminase),
AST (aspartate transaminase), total protein,
urea and creatinine was done through standard
protocols [25b]. The infective dose (sample) was
prepared as an organism loopful (placed in agar
slant), transferred into 10 ml test-tube with
sterilized peptone water which was then
incubated at 36.5 °C for 1 day.

Statistical analysis

The results from the antimicrobial, antipyretic
and MIC studies were demonstrated as mean ±
and the standard deviation of the triplicate
results were calculated and plotted using
software Microsoft Excel 2016. The standard
deviation was found to be well within the
acceptable range as demonstrated in the plot.

RESULTS

Chemical constituents

Bioactive coumarins: xanthotoxin, (a phototoxic
furanocoumarin), salvigenin (7-O-methylated
flavonoid lipid molecule) and cirsimaritin or
skrofulein were isolated from C. pseudosinaica
in good yield after recrystallization from alcohol.
Another bioactive coumarin, santin was isolated
in excellent yield first time from the whole genera
Tripleurospermum. Like-wise, a steroid (estradiol
– a female sex hormone), and β-amyrin (a
pentacyclic triterpene) were obtained from the
genus Koelpinia in high yields. Phytochemical
screening of the three plants studied in our
present study showed the presence of
glycosides, terpenes, steroids, tannins, flavonoids (except in K. linearis), coumarins (except in T. auriculatum), chlorides and oxalates
while alkaloids and anthraquinones were not
present (Table 1). These variations in
phytochemical content of the plant are believed
due to different environmental factors.

Bioactivity screening

The susceptibility of different pathogens to the
inhibitory effect of oils and extracts from C.
pseudosinaica and extracts of T. auriculatum, K.
linearis have been established and found to show
various significant activities for biological
applications. As can be seen in Table 2, significant
anti-fungal activities were demonstrated for both hydrodistilled oils and
alcoholic extracts of all three plants against vital
human pathogens like A. fumigates, S. racemosum, G. candidum and C. albicans. In
addition, comparison with the standard antibiotic
amphterocin B (30 µg) clearly revealed that
alcoholic extracts showed overall excellent
activities against all microorganisms especially
against C. albicans where the activities increased
significantly. Similar control was observed for
anti-bacterial activities for plant species that
showed remarkable results when compared with
standard broad spectrum antibiotics (ampicillin
and gentamycin) against Gram +ve bacteria such
as Streptococcus pneumoniae and Bacillus
subtilis and Gram –ve bacteria like Pseudomonas
aeruginosa and Escherichia coli) whereas, K.
linearis exhibited prominent activities as
compared to T. auriculatum against all
pathogens (fungi and bacteria).

In order to explore the pathogens sensitivity, the
in vitro minimum inhibitory concentration (MIC)
analyses was examined to evaluate plant oils
and extracts effectiveness. As depicted from
(Table 3) compared to the controlled reference
antibiotics such as amplifiercin, ampicillin and
gentamycin which showed an encouraging
counter effect against all pathogens whereas C.
albicans and P. aeruginosa were completely
resistant to K. linearis extract. Similarly, Gram
+ve and Gram –ve bacteria were also found
resistant to C. pseudosinaica oils.

Table 1: Phytochemical profile of plant extract

| Plant Species | Glycosides | Terpenes | Steroids | Tannins | Flavonoids | Coumarins | Chlorides | Oxalates | Saponins |
|---------------|------------|----------|----------|---------|------------|-----------|-----------|----------|----------|
| C. Pseudosinaica | +++ | +++ | * | + | +++ | ++ | ++ | + | |
| T. auriculatum | +++ | +++ | +++ | ++ | + | - | +++ | +++ | - |
| K. linearis | +++ | +++ | +++ | - | - | ++ | +++ | + | |

Key: +++ (high), ++ (low), + (very low)
Antipyretic effect of plant extracts

The in-vivo antipyretic effect of plant extracts has revealed that the intake of infective dose of these extracts into mice with yeast induced pyrexia is safe and does not affect the functionality of the kidney and liver. From Table 4, it is shown that the plant extracts’ efficiency in lowering the temperature of mice with respect to the standard drugs i.e. paracetamol and aspirin, is fully sensitive and quite effective. The rectal temperature of mice was fluctuating from 1 to 2 °C after regular time intervals but surprisingly it remained constant and control after 16h of treatment as compared to Paracetamol. Similarly, as compared to aspirin, plant extracts have also shown the controlled antipyretic effect when they used the average standard doses of 400 mg/kg.

In another experiment, the in-vivo pharmacologic effect of plant extracts on hepatorenal variation of mice induced with yeast, where concentration of C. pseudosinaica, T. auriculatum and K. linearis extracts have shown a balanced effect as compared to the controlled treatment (Table 5). The average values for each of ALT, AST, bilirubin, total phosphorous, Urea, and creatinine were found to be within normal range, indicating no harmful effect on hepatorenal functions.

Table 4: Antipyretic effect of plant extracts in yeast-induced pyrexia in mice

| Treatment       | Amount (mg) | Mice body Temp. after 16 hours | Temperature after treatment |
|-----------------|-------------|--------------------------------|-----------------------------|
|                 |             | 1 h                            | 2 h                         | 3 h | 4 h |
| Controlled (ref)| 00          | 39.28±0.29                     | 39.63±0.29                  | 39.50±0.33 | 39.80±0.29 | 39.20±0.22 |
| Paracetamol     | 150         | 39.86±0.32                     | 37.91±0.18                  | 37.76±0.18 | 38.01±0.33 | 38.70±0.29 |
| C. pseudosinaica| 400         | 39.76±0.30                     | 39.83±0.31                  | 39.84±0.33 | 39.84±0.32 | 39.84±0.31 |
| T. auriculatum  | 400         | 39.45±0.38                     | 39.11±0.42                  | 38.21±0.38 | 38.30±0.48 | 39.13±0.22 |
| K. linearis     | 400         | 39.85±0.36                     | 39.11±0.38                  | 38.21±0.38 | 39.15±0.43 | 39.06±0.36 |
| Controlled (ref)| 00          | -                              | 10.52±0.50                 | 10.52±0.50 | 10.52±0.50 | - |
| Aspirin         | 200         | -                              | 16.78±0.51                 | 17.17±0.28 | 17.36±0.48 | - |
| C. pseudosinaica| 400         | -                              | 10.54±0.33                 | 11.12±0.22 | 11.03±0.32 | - |
| T. auriculatum  | 400         | -                              | 9.93±0.23                  | 9.57±0.43  | 9.83±0.38  | - |
| K. linearis     | 400         | -                              | 13.67±0.41                 | 14.23±0.61 | 14.05±0.63 | - |
protein, albumin, urea and creatinine during 35 days dose at 400 mg/kg were considered for the study. These findings further demonstrated that the functionality of kidney and liver were remained basically unchanged in mice after the intake of these plant extracts.

**DISCUSSION**

As an ongoing research on the medicinal plants of Saudi Arabia [6-8], a first time detailed phytochemical study on some Asteraceae plants growing in Saudi Arabia has been described in this report. The chemical constituents explored in this study and their characterization were compared with the literature.

To the best of our knowledge, *C. pseudosinaica* has not been previously evaluated for its phytochemical constituents. All three coumarins were isolated in good yield and have been reported to exhibit anti-microbial and anti-proliferative activities [9,10]. Likewise, Santin from *T. auriculatum*, a steroids (estradiol) and a triterpene (β-amyrin) from *K. linearis* were isolated, characterized and exhibited anti-oxidant, neuroprotection, anti-ulcer and anti-microbial activities [13,14].

The findings of the results in the current investigation revealed that the variations in bacterial response to the respective plants (oil and extracts) might be due to the structural difference of bacteria and the constituents mode of action against these bacterial species. The notable antimicrobial (antifungal and antibacterial) bio-activity of the studied plants is attributed to the phytochemicals due to unfavourable desert’s environment. These results revealed the occurrence of some bioactive volatile/non-volatile compounds in the plant essential oils and extracts could be used to develop anti-microbial agents.

**CONCLUSION**

In this study, various phytochemicals such as xanthotoxin, cirsimaritin, salvigenins, estradiol, β-amyrin and santin were isolated from three Asteraceae plants of Saudi Arabia. Biological activity evaluation of extracts and essential oils from these plants revealed significant antimicrobial activities and hence, these plants might be used as a suitable candidate for the treatment of microbial diseases.

**DECLARATIONS**

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**Conflict of interest**

No conflict of interest is associated with this work.

**Contribution of authors**

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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**REFERENCES**

1. Skaltsa H, Lazari D, Panagouleas C, Georgiadou E, Garcia B, Skovic M. Sesquiterpene lactones from...
Centaurea thessala and Centaurea attica. Antifungal activity. Phytochemistry 2000; 55: 903-908.

2. Formisano C, Rigano D, Senatore F, Bancheva S, Maggio A, Rosselli S, Bruno M. Flavonoids in Subtribe Centaureinae (Cass.) Dumort. (Tribe Cardueae, Asteraceae): Distribution and 13C-NMR Spectral Data. Chem. Biodivers 2012; 9: 2096–2158.

3. Ramzi AA, Mothana SAA, Abdo SH, Faisal MNA, Sama AZA, Ulrike L. Antimicrobial, Antioxidant and Cytotoxic Activities and Phytochemical Screening of Some Yemeni Medicinal Plants. Evid Based Complement and Alternat Med 2010; 7(3):;323-330.

4. Hosseini M, Parvin S, Bakhtiarian A. Antiinflammatory, analgesic activity of Tripleurospermum disciforme extract in rats. Toxicol Lett 2007; 1725.

5. Koul S, Razan TK, Andotra CS, Kalla AK, Koul S, Taneja SC, Dhar KL. Koelpinin-A, B and C-three triterpenoids from Koelpinia linearis. Phytochemistry 2000;., 53,: 305-309.

6. Alwahaibi LHN, Mahmood A, Khan M, Alkhathlan HZ. Comparative study on the essential oils of Artemisia judaica and A. herba-alba from Saudi Arabia. Arabian Journal of Chemistry 2018; https://doi.org/10.1016/j.arabjc.2018.03.004

7. Khan M, Khan ST, Khan NA, Mahmood A, Al-Kedhairy A, Alkhathlan HZ. The composition of the essential oil and aqueous distillate of Origanum vulgare L. growing in Saudi Arabia and evaluation of their antibacterial activity. Arabian Journal of Chemistry 2018; 11: 1189-1200 https://doi.org/10.1016/j.arabjc.2018.02.008

8. Khan M, Mahmood A, Alkhathlan HZ. Characterization of leaves and flowers volatile constituents of Lantana camara growing in central region of Saudi Arabia. Arabian Journal of Chemistry 2016; 9; 764-774.

9. Walasek M, Grzegorczyk A, Malm A, Skalicka-Woźniak K. Bioactivity-guided isolation of antimicrobial coumarins from Heracleum mantegazzianum Sommier & Levier (Apiaceae) fruits by high-performance counter-current chromatography. Food Chem 2015; 186: 133–144.

10. Sen A, Ozbas TS, Blits L. Bioactivity-guided isolation of anti-proliferative compounds from endemic Centaurea kilaea. Pharm Biol 2017; 55(1): 541-546.

11. Kim HJ, Kim S, Dong Y, Lee IS, Kim JS, Kim J, Woo JT, Cha BY. Melanogenesis-Inducing Effect of Cirsimaritin through Increases in Microphthalmia-Associated Transcription Factor and Tyrosinase Expression. Int. J. Mol. Sci 2015; 16: 8772-8788.

12. Kouamé PBK, Jacques C, Bedi G, Silvestre V, Loquet D, Barillé-Nion S, Robins RJ, Tea I. Phytochemicals Isolated from Leaves of Chromolaena odorata: Impact on Viability and Clonogenicity of Cancer Cell Lines. Phytotherapy Research 2013; 27: 835–840.

13. Prossnitz ER, Barton M. Estrogen biology: new insights into GPER function and clinical opportunities. Mol. Cell. Endocrinol 2014; 389: 71–83.

14. Zheng Y, Huang W, Yoo J, Ebersole JL, Huang CB. Antibacterial compounds from Salsola grosvenorii leaves. Natural Products Research 2011; 25(9): 890-897.

15. Randhawa, M. A, Alenazy, A. K., Alrowaili, M. G., Basha J. An active principle of Nigella sativa L., thymoquinone, showing significant antimicrobial activity against anaerobic bacteria. J Intercult Ethnopharmacol 2016; 6(1): 97-101.

16. Wu CM, Koehler PE. Isolation and identification of xanthotoxin (8-methoxypsoralen) and bergapten (5-methoxypsoralen) from celery infected with Sclerotinia sclerotiorum. Ayres, J. C. Appl Microbiol 1972; 23(5): 852-856.

17. Schonborn A, Sina A. Some New β-Diketones Containing the Trifluoromethyl Group 1a. J. Am. Chem. Soc 1950; 72: 4826-4828.

18. Tešević V, Aljancić I, Milosavljevic’ S, Vaja V, Dordević I, Jadranić M, Menković N, Matevski V. J. of serbian Chem. Soc 2014; 79: 1-13.

19. Bautista E, Calzada F, Ortega A, Yépez-Mulia L. Antiprotozoal Activity of Flavonoids isolated from Mimosa tenuiflora. J. Mex. Chem. Soc 2011; 55(4): 251-253.

20. Sülsen VP, Cazorla SI, Frank FM, Redko FC, Anesini CA, Coussio JD, Malchiodi EL, Martino VS, Muschietti LV. Trypanocidal and Leishmanicidal Activities of Flavonoids from Argentine Medicinal Plants. Am. J. Trop. Med. Hyg 2007; 77(4): 654–659.

21. Abbas FA, Ateya AM, Estradiol, Estrione, Estrone and Novel Flavonoids from Date Palm Pollen. Australian Journal of Basic and Applied Sciences 2011; 5(8): 606-614.

22. Vázquez LH, Palazon J, Navarro-Ocaña A. The Pentacyclic Triterpenes α, β-amyris: A Review of Sources and Biological Activities. Phytochemistry - A Global Perspective of Their Role in Nutrition and Health 2012; 483-502. DOI: 10.5772/27253

23. Jabeen K, Javaid A, Ahmad E, Athar M. Antifungal compounds from Melia azedarach leaves for management of Ascochyta rabiei, the cause of chickpea blight. Natural Products Research 2011; 25(3): 264-276.

24. Woods GL, Washington JA, Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH. (Eds.). Manual of Clinical Microbiology 1995; 6th Ed. ASM Press, Washington, D.C., 1327–1341.

25. Khan, ZA, Siddiqui MF, Park S. Current and Emerging Methods of Antibiotic Susceptibility Testing. Diagnostics 2019; 9(2): 49-66. b) Reitman S, Frankel S. Colourimetric method for the determination of serum transaminases. Am. J. Clin. Pathol 1957; 28: 56-61.