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Original Research Article

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 chromatoraphy-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*

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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 *Centuarea* 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 is a rich source of triterpenoids, steroids and series of five long-chain alkanoic 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 accetate 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 ethanol as eluting solvents. Xanthotoxin/furanocoumarin was obtained as a colourless needle like crystals (0.16 g, m.p. 148 ^{o}C , lit. m.p. 149 ^{o}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 $CHCl_3$:EtOAc (6:4). Salvigenin (0.71g) was obtained as pale yellow crystals (m.p. 188 ^{o}C, lit. m.p. 189 ^{o}C [18]. Further elution of the same column gave cirsimaritin (0.52 g as yellow crystals, m.p. 196 ^{o}C, lit. m.p. 198 ^{o}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 $CHCl_3$:EtOAc (5:5). Santin (0.45 g) was obtained as colourless crystals (m.p. 161 ^{o}C, lit. m.p. 162 ^{o}C) [19, 20]. Ethyl acetate fraction of the methanolic extract of *K. linearis* gave, after solvent evaporation, a colorless amorphous powder which was subjected to column chromatography (120 x 2 cm, with 650 g silica gel) eluting with $CHCl_3$:EtOAc (6:4). Two compounds were isolated, purified and identified after characterization as estradiol (2.40 g as colourless crystals, m.p. 172 ^{o}C, lit. 171 ^{o}C) [21] and β-amyrin (0.92 g as colourless crystals, m.p. 188 ^{o}C, lit. m.p. 189 ^{o}C) [22]. All spectroscopic data (UV, IR, ^{1}H-NMR, ^{13}C-NMR, Mass) of estradiol β-amyrin were compared with literature [14,21,23].

Antimicrobial screening

The hydrodistilled oil and extracts of *C. pseudosinaica and others 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), *Syncephalastrum racemosum* (RCMB 05922), *Geotricum candidum* (RCMB 05097), *Candida albicans* (RCMB 05036) and bacteria such as Gram +ve bacteria (*Streptococcus pneumonia* (RCMB 010010), *Bacillis 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 +ive bacteria and *gentamicin* in DMF (30 µg) for Gram –ive bacteria were used as reference antibiotics. The incubation of plates for fungi was done for 72 hrs at 28 ^{o}C while one day at 37 ^{o}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 ^{o}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 harmone), 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 anthraqinones 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 *amphoterocin* 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 Bacillis 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 amphotericin, 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)

Table 2: Antimicrobial activities of essential oil and extracts

Sample	*C. pseudosinaica*		*T. auriculatum*	*K. linearis*	*Amphotericin B*
	Oil	*Extract*	*Extract*	*Extract*	**(30µg)**
Fungi					
A. fumigatus	16.7 ± 0.36	22.3 ± 0.25	24.3 ± 0.63	11.3 ± 0.34	23.7± 0.1
S. racemosum	11.2 ± 0.58	16.5 ± 0.25	17.6 ± 0.27	12.1 ± 0.25	19.7 ± 0.2
G. candidum	17.6 ± 0.44	25.8 ± 0.58	26.9 ± 0.35	15.3 ± 0.38	28.7 ± 0.2
C. albicans	13.3 ± 0.36	23.5 ± 0.32	24.6 ± 0.34	9.6 ± 0.24	25.4 ± 0.1
Gram +ve bacteria					***Ampicillin (30µg)***
S. pneumoniae	16.4 ± 0.32	19.5 ± 0.44	20.6 ± 0.34	15.0 ± 0.43	23.8 ± 0.2
B. subtilis	25.1 ± 0.45	29.8 ± 0.58	33.7 ± 0.25	17.4 ± 0.53	32.4 ± 0.3
Gram –ve bacteria					***Gentamycin* (30µg)**
P. aeruginosa	5.2 ± 0.52	12.3 ± 0.25	13.1 ± 0.32	10.5 ± 0.22	17.3 ± 0.1
E. coli	5.1 ± 0.58	17.6 ± 0.19	18.3 ± 0.58	11.2 ± 0.33	19.9 ± 0.3

Table 3: MIC of essential oil and extracts

Sample	*C. pseudosinaica*		*T. auriculatum*	*K. linearis*	*Amphotericin* **B (30**
Fungi	*Oil*	*Extract*	*Extract*	*Extract*	**µg)**
A. fumigatus	12.5 ± 0.16	1.95 ± 0.03	0.97 ± 0.02	50.0 ± 0.63	0.97 ± 0.02
S. racemosum	15.0 ± 0.16	12.5 ± 0.14	31.25 ± 0.28	50.0 ± 0.24	7.81 ± 0.06
G. candidum	0.81 ± 0.05	0.48 ± 0.02	0.24 ± 0.01	12.5 ± 0.20	0.03 ± 0.02
C. albicans	2.5 ± 0.16	1.95 ± 0.01	0.97 ± 0.01	-	0.48 ± 0.01
Gram +ve bacteria					***Ampicillin* (30 µg)**
S. pneumoniae	-	7.81 ± 0.02	7.81 ± 0.01	12.5 ± 0.16	0.97 ± 0.02
B. subtilis	-	0.03 ± 0.02	0.03 ± 0.02	31.25 ± 0.20	0.007 ± 0.001
Gram –ve bacteria					***Gentamicin* (30 µg)**
P. aeruginosa	-	50.0 ± 0.24	50.0 ± 0.32	-	31.25 ± 0.20
E.coli	-	11.25 ± 0.20	15.26 ± 0.24	50.0 ± 0.23	7.81 ± 0.16

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 ^{o}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* pharmaco-logic effect of plant extracts on hepato-renal 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

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	39.30± 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	-

Table 5: Pharmacologic effects of plant extracts on hepato-renal variation of mice induced with yeast

Treatment	ALT (U/L)	AST (U/L)	Bilirubin (mg/dL)	Protein (g/dL)	Albumin (g/dL)	Urea (mg/dL)	Creatinine (mg/dL)
Controlled (ref)	67.5±3.48	146.3±5.20	1.6±0.11	8.6±0.22	3.7±0.01	37±2.35	0.43±0.22
C. pseudosinaica	67.5±2.78	146.9±5.32	1.7±0.12	8.5±0.31	3.7± 0.27	35± 2.20	0.40±0.21
T. auriculatum	66.6±2.78	147.9±5.34	1.8±0.11	8.5±0.30	3.6± 0.27	36± 2.40	0.39±0.20
K. linearis	64.8±2.88	151.4±6.84	1.6±0.14	8.7±0.36	3.4± 0.21	36± 2.56	0.42±0.26

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 phytomolecules 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 phytomolecules 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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