

COMPARATIVE PHYTOCHEMICAL STUDY ON THREE *TETRAENA SPECIES* (ZYGOPHYLLACEAE) GROWING AT DIFFERENT SALINITY LEVELS

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Abstract

Plants grow under harsh conditions produce various phytochemicals which enable them to withstand stress conditions and are involved in resident adaptation. Three species, *Tetraena coccinea*, *Tetraena alba* and *Tetraena simplex* were collected from 3 localities, with different salinity levels, along south Jeddah coast, Saudi Arabia to analyze their phytochemical constituents. Ethanolic extracts of roots, leaves and flowers samples were screened by GC-MS analysis to identify the most abundant phytochemicals. From this study, 50 phytochemical compounds were identified in each extract. These compounds predominantly classified as alkaloids, terpenoids, flavonoids, phenols, steroids, alkanes, alcohols, fatty acids, esters and organic acids. Phytochemicals levels in each species positively correlated with the salinity level of the surrounding habitats. *Tetraena coccinea* that grows in the highest saline conditions possess the highest levels of many active phytochemicals, mainly those belongs to alkaloids and terpenoids, compared to *T. alba* and *T. simplex*. Numerous of the identified compounds are bioactive phytochemicals and proved to hold a broad range of activities, which may help in the defense against incurable illnesses. Hence, from this study it can be confirmed that these species could be used as a new potential source for new drugs and pharmaceutical agents formulations.

Key words: *Tetraena coccinea*, *T. alba*, *T. simplex*, Phytochemicals, Chemical classes, GC-MS analysis.

Introduction

The Zygophyllaceae family of shrubs, herbs and trees is found in tropical and sub-tropical regions of the world in semi-arid and arid areas (Shawky *et al.*, 2019). There are 285 known species of Zygophyllaceae with 27 genera that are categorised into five sub-families (Beier *et al.*, 2003). The largest sub-family is Zygophylloideae which comprises six genera. Its genera are a collection of drought-tolerant succulents, some of which are salt tolerant. These are found in regions with climates that are exceptionally dry (Saleh & El-Hadidi, 1977). It is likely that their tolerance to environmental extremes coupled with their unpalatability explains why they are so abundance of species (Amini-Chermahini *et al.*, 2014). The distribution of the general Zygophylloideae is governed by the chemical composition of the soil in their habitats (Sheahan & Cutler, 1993).

There are eight genera of Zygophyllaceae in the Kingdom of Saudi Arabia: *Balanites* Del., *Fagonia* L., *Nitraria* L., *Peganum* L., *Seetzenia*, *Tetraena* Maxim., *Tribulus* L. and *Zygophyllum* L. (Chaudhary, 2001). The morphological features of *Tetraena* and *Zygophyllum* are broadly alike in terms of the shapes of their fruits, the features of their leaves, their growth habit and their flower traits. Beier *et al.*, (2003). made the most recent taxonomic proposal of *Tetraena* Maxim and *Zygophyllum* and noted that the majority of the Saudi *Zygophyllum* taxa have been switched to *Tetraena*. The *Tetraena* plants in Saudi Arabia have been categorized into six groups by Alzahrani & Albokhari (2018) as follows: *T. alba* (L.) Beier & Thulin, *T. coccinea* (L.) Beier & Thulin, *T. simplex* (L.) Beier & Thulin, *T. propinqua* (Decne.) Ghazanfar & Osborne, *T. hamiensis* (Schweinf.) Beier & Thulin, and *T. decumbens* (Delile) Beier & Thulin.

Of all the *Tetraena* species that are native to Saudi Arabia, the most prevalent is *Tetraena coccinea* (L.) Beier & Thulin (syn. *Z. coccineum* L.). It is found in the south-west and north-west of Saudi Arabia as well as in Kuwait, Palestine, Yemen and north and east Africa. Its ability to tolerate a wide variety of soil types means that it is found in a wide range of habitats. It is a perennial herb with white flowers and fleshy leaves, common in sandy and saline regions close to the coast (Chaudhary, 2001). *Tetraena alba* (L.) Beier & Thulin (syn. *Z. album* L.) is native to the Arabian Peninsula, north and north-east Africa, west Asia, Mauritania, Crete and Spain. It can also be found in saline soils, sand dunes, salt marshes and saline depressions in Egypt, Jordan, Palestine and Tunisia along the Red Sea coast (Beier *et al.*, 2003; Alzahrani & Albokhari, 2018). *Tetraena simplex* (L.) Beier & Thulin (syn. *Z. simplex* L.) is common in Saudi Arabia where it is typically found in sandy soils. It also grows in Arabian Peninsula, Oman, Yemen, the United Arab Emirates, Palestine, Jordan, Iran, India and tropical regions of Africa (Ghazanfar & Patzelt, 2007).

Tetraena species are particularly useful because of their adaptability, helping to bind sand and retain moisture in the soil even in arid and saline deserts as well as preventing soil erosion (Yang & Furukawa, 2006; El-Sheikh *et al.*, 2021). In terms of economic applications, they can be used as animal fodder. They have been utilized also in traditional medicine for various ailments, such as treatment of rheumatism, gout, diabetes, asthma, hypertension, dysmenorrhea, as well as fungal infection (Guenzet *et al.*, 2014; El-Shora *et al.*, 2016; Kchaou *et al.*, 2016). Such medical activities were contributed to their phytochemical constituents. Various classes of compounds including terpenes, flavonoids, saponins, sterols, phenolic, essential oils and esters have been isolated from different

Tetraena species (Abdel-Hamid *et al.*, 2016; Ganbaatar *et al.*, 2016; Abdelhameed *et al.*, 2022; Eltamany *et al.*, 2023). To date, the available literature does not report about chemical composition of *Tetraena* species found mainly in underpopulated area of south Jeddah coast. It is the first attempt to study different phytochemicals including alkaloids, terpenoids, flavonoids, phenols, steroids, alkanes, alcohols, esters, fatty and organic acids obtained from plant roots, leaves and flowers of *T. coccinea*, *T. alba* and *T. simplex*. The second goal of the study is to investigate if there is a relation between the species phytochemicals accumulation ability and the salinity level in its habitat. Therefore, this study constitutes a valuable addition to the scientific literature concerning the studied species.

Material and Methods

Study area: The present study was carried out during March, 2019 on the vegetation of two different coastal sites in Jeddah city which is located in the South Province of the Kingdom Saudi Arabia. The two selected sites are: Al-Sief beach (21°10'31"N 39°10'42"E) and Almojermh aibah (20°30'28"N 39°44'17"E) (Fig. 1).

Samples collection: Three soil samples around each collected species, down to 30 cm depth were collected from each locality, pooled together to form one composite sample, spread over sheets of paper, air dried, passed through 2 mm sieve, and packed in plastic bags ready for

analysis. Some chemical parameters of such soil samples were analyzed. Plant species (*T. coccinea*, *T. alba* and *T. simplex*) were collected, handily cleaned, washed several times with distilled water to remove dust and other residues and separated into roots, leaves and flowers. Then they were dried at room temperature in shaded place for several days till complete dryness and ground in an electric grinder to give a fine powder, and preserved in well stopped bottles for GC-Mass spectrometry.

Soil analysis: The soil EC was determined according to Richards (1954). 10 grams soil put in 250 ml glass beaker with 50 ml distilled water, left overnight, then filtered through filter paper. EC meter was used to evaluate the electrical conductance for soil extracts using decimins/m as a concentration for soil anions according to Conklin (2005). Chloride and sodium ions in the same soil extracts were determined by the atomic absorption spectrometer in the Analytical Chemistry Unit (ACAL), RICI MAAZ Chemical & Environmental Testing Laboratory, Dammam, Saudi Arabia.

Plant extraction: In order to obtain plant extracts, 20 g of each plant powder was mixed with 50 ml ethanol. The mixture was left for 24 h on an orbital shaker with a shaking speed 140 rpm. The extracts were sieved through a fine mesh cloth, centrifuged at 4000 g for 20 min, and evaporated and dried at 45°C under vacuum in a rotary evaporator. The dry crude extracts were stored at 4°C (Tiwari *et al.*, 2011).

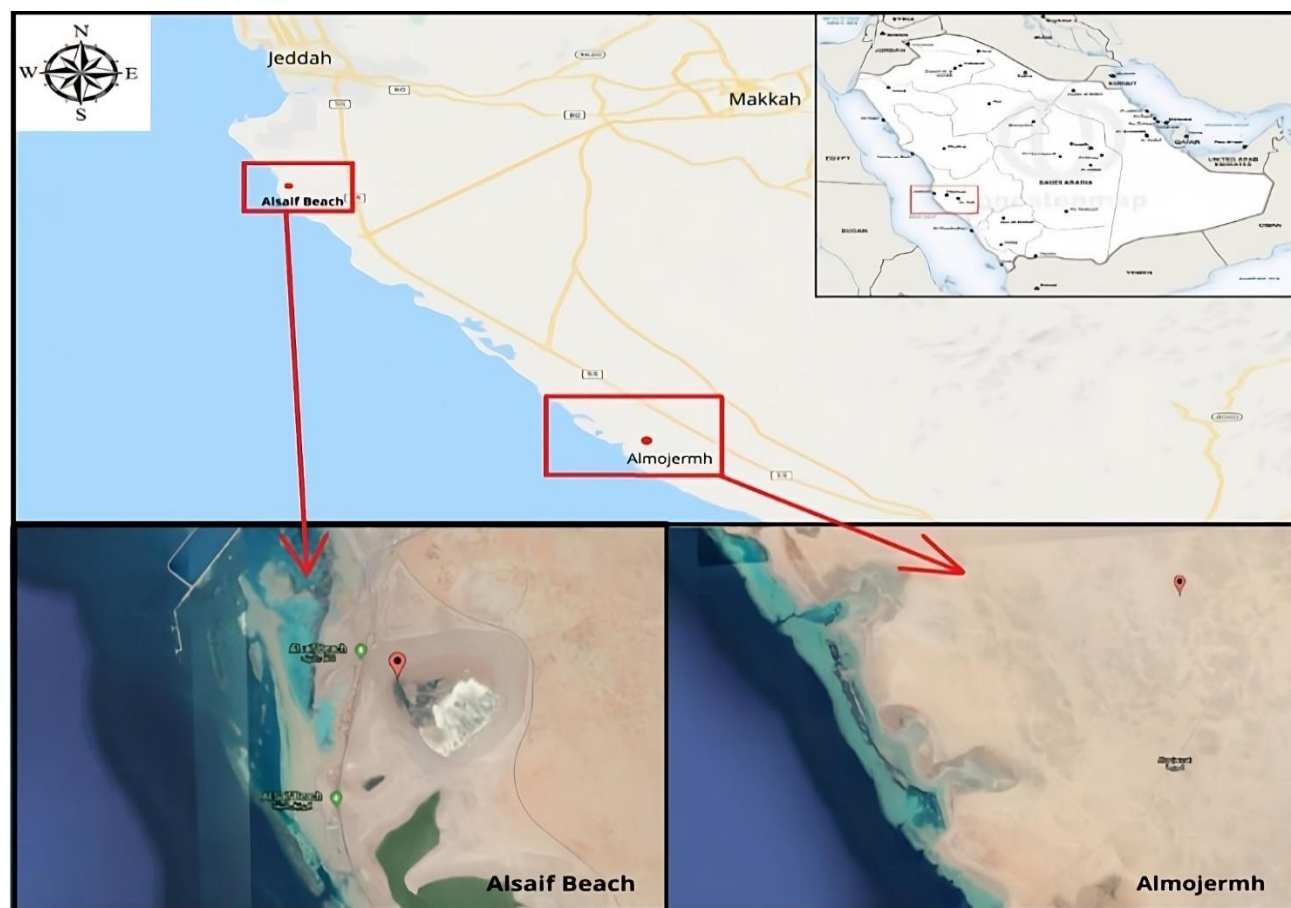


Fig. 1. Location map of Al-Sief beach and Almojermh on south Jeddah coast.

GC-MS analysis: The GC-MS analysis was conducted at the National Research Centre in Egypt. The identification of GC analytes was accomplished at a voltage of 70eV (m/z 50–550; source at 230°C and quadruple at 150°C) using a HP model 6890 GC interfaced to a HP 5791A mass selective detector. In order to facilitate GC, a 30m x 0.25mm i.d., 0.25 μ m film thickness HP-5ms capillary column (J&W Scientific, USA) was employed. The carrier gas was helium which was used at a constant flow rate of 1.0ml/min. The temperature of the injector and MS transfer line was 300°C, while the oven was set to a temperature of 150°C to be used for a period of 2 min, rising at 4°C/min to 300°C and then held at 300°C for 20 min. At a split ratio of 50:1, a volume of 1 μ l was injected for each individual analysis. Interpretation of mass spectrum of GC-MS was done using the database of Wiley and Mainlib. The spectrum of the known component was compared with the spectrum of the known components stored in the inbuilt library. The molecular weight, name, chemical structure and molecular formula of the components of the studied extracts were ascertained.

Statistical analysis

All data collected for the soil analysis were subjected to a one-way variance analysis (ANOVA) test using SPSS statistical package. Multiple range test by Duncan ($p < 0.05$) was used to compare the means.

Results and Discussion

Soil salinity indexes: Electric conductivity signals the salinity levels of the soil. The results of the current study shown that the three species under investigation grow in three different salinity levels. As represented in Fig. 2, EC, Na and Cl concentrations (as salinity indexes) varies considerably between soil samples around each species. EC in soil samples around *T. coccinea* roots was 37.96% higher than that around *T. alba* roots (Fig. 2I). Similarly, Na and Cl ions concentrations around *T. coccinea* roots was about 7 and 2 folds respectively compared to that around *T. alba* roots (Fig. 2II & III). *T. simplex* soil showed the least salinity indexes. Comparable values for EC, Cl and Na ions in the soil inhabited by *Tetraena* sp. in Egypt recorded by El-Amier *et al.*, (2016).

Phytochemicals distribution: For economic and safety reasons, the identification of new sources of natural medicinal and nutraceutical compounds is an encouraging alternative for their usage in the food industry and in defensive medicine to substitute artificial compounds (Tadhani *et al.*, 2007). Falleh *et al.*, (2011) and Ziane *et al.*, (2021) demonstrated that, halophytes, as naturally salt-tolerant plants, have therapeutic and nutritional characters and can be potentially valuable as new sources of bioactive compounds. Therefore, there is an accumulative attention to detect among halophyte species those with high phytochemicals content to be used in the suitable industries.

The current phytochemical investigation of the roots, leaves and flowers extracts of the studied *Tetraena* species has resulted in isolation and structural elucidation of fifty compounds in each extract belonging to different chemical classes. Relative amounts (% peak area) of each one of the identified compounds are presented in Tables 1, 2 and 3 for *T. coccinea*, *T. alba* and *T. simplex* extracts respectively according to their elution order.

As shown in (Table 1), *T. coccinea* roots, leaves and flowers extracts considerably vary in their phytochemicals constituents. The 50 compounds identified in *T. coccinea* extracts includes 10 alkanes, 8 alkaloids, 7 terpenoids, 4 alcohols, 4 esters, 3 phenols, 3 fatty acids, 3 organic acids, 2 flavonoids and 2 steroids. Out of the identified alkanes in *T. coccinea*, the most abundant one was docosane which recorded in leaves (9.23%), roots (11.93%) and flowers (15.76%); followed by Nonacosane in area about 4.93%, 0.58% and 13.36% in *T. coccinea* roots, leaves and flowers respectively; 17-pentatriacontene and octacosane were abundant alkanes also in all *T. coccinea* extracts but recorded their highest values in leaves extract (11.63%) and (15.05%) respectively; dichloromethylethylsulfone and tricosane identified also in all *T. coccinea* extracts in smaller values; anthracene, 2-ethyl and dotriacontane identified in *T. coccinea* roots and flowers; pentacosane identified in leaves and flowers however pentadecylbenzene identified in roots extract only.

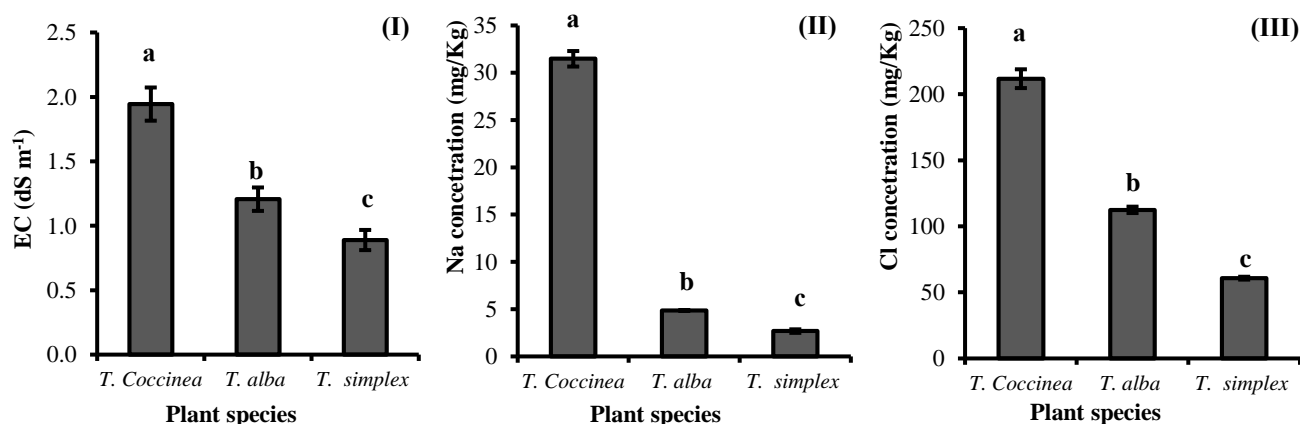


Fig. 2. Soil EC (I), Na concentration (II) and Cl concentration (III). Each histogram is a mean value of three replicates and the vertical bars indicate \pm SE. Bars caring different letters are significantly different at $p < 0.05$.

Table 1. Phytochemicals identified in *Tetraena coccinea* roots, leaves and flowers extracts.

No.	Compound	Peak area (%)			Chemical class
		Roots	Leaves	Flowers	
1.	Dichloroacetoneitrile	0.19	1.29	0.29	Others
2.	5-(Aminomethyl)-2-pyrrolidinone		0.11		Alkaloid
3.	6-Methylheptanoic acid	0.16	0.28		Fatty acid
4.	dichloromethylethylsulfone	1.23	2.42	1.68	Alkane
5.	Cadinene	2.21		1.71	Terpenoid
6.	Anthracene, 2-ethyl	0.30		0.74	Alkane
7.	Cyclo(glycyl-1-tyrosyl)	0.39			Organic acid
8.	α -Eudesmol	8.22	0.91		Terpenoid
9.	3-Cyclohexyl-1-(2-chloroethyl)-1-nitrosoarea(15N1,15N=O)	0.73	0.52		Others
10.	1-Hexadecanol		0.23	0.93	Alcohol
11.	N-Acetyl-DL-tryptophan	0.23		2.62	Organic acid
12.	Ambrosin	0.57			Terpenoid
13.	5-Ethoxy-7-methoxy-2,2-dimethyl-3H-chromen-4-one	1.43			Phenol
14.	tert-Hexadecanethiol	0.36		6.50	Alcohol
15.	Dasycarpidan-1-one	0.32			Alkaloid
16.	Dasycarpidol	0.36		1.90	Alkaloid
17.	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	2.01			Ester
18.	9-Eicosyne	0.93	5.21	7.27	Others
19.	Chromone, 5-hydroxy-6,7,8-trimethoxy-2,3-dimethyl	0.15		1.76	Phenol
20.	Pentadecylbenzene	0.17			Alkane
21.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol		21.41	5.56	Terpenoid
22.	Corynan17ol	2.07	0.12		Alkaloid
23.	1H-Imidazo[1,2-c]oxazol-5-one, tetrahydro-7,7-adihydro-7(4,8-dimethyl-3,7-nonadienyl)	0.84	1.73	1.64	Others
24.	Docosane	9.23	11.93	15.76	Alkane
25.	Tricosane	0.32	0.64	4.20	Alkane
26.	Dasycarpidan-1-methanol, acetate		0.20		Alkaloid
27.	1,5,9,13-Tetrathia-3,11-cyclohexadecaediol	1.14	1.66	1.63	Alcohol
28.	1,2-Benzenedicarboxylic acid, butyl octyl ester	0.39	0.31	1.61	Ester
29.	Erucic acid	2.08	0.24	0.40	Fatty acid
30.	Quercetin-7,3',4'-trimethoxy	1.30			Flavonoid
31.	Quebrachidine	39.34	0.42	1.38	Alkaloid
32.	Pentacosane		6.52	1.61	Alkane
33.	22-Tricosenoic acid	5.88	0.95	11.26	Fatty acid
34.	Prednisolone	0.61	0.44	0.89	Steroid
35.	Phthalic acid, butyl undecyl ester		2.10		Ester
36.	Octacosane	0.23	15.05	0.25	Alkane
37.	Colchifoleine			0.61	Alkaloid
38.	Nonacosane	4.93	0.58	13.36	Alkane
39.	Squalene	0.26	5.84		Terpenoid
40.	24,25-Dihydroxycholecalciferol	2.90	0.15		Terpenoid
41.	D-Mannitol, hexaacetate	0.24	0.89	0.23	Alcohol
42.	Ethyl iso-allocholate	0.78	0.70	0.26	Steroid
43.	Trans-2-phenyl-1,3-dioxolane-4-methyloctadec-9,12,15-trienoate	0.36	0.91		Phenol
44.	Folic Acid	0.47	2.12	0.35	Organic acid
45.	Glucobrassicin		0.26	0.46	Alkaloid
46.	Dotriacontane	1.6		0.43	Alkane
47.	17-Pentatriacontene	1.12	11.63	4.74	Alkane
48.	Phytofluene	0.39		0.41	Terpenoid
49.	Hexadecanoic acid 2-hydroxy-1,3-propanediyl ester	0.69	0.29	6.66	Ester
50.	Lucenin 2	2.67	1.22	0.71	Flavonoid
Total		99.80	99.23	99.81	

There are many alkaloids identified in *T. coccinea* extracts including: quebrachidine which was the most abundant alkaloid in *T. coccinea* extracts and concentrated in its roots (39.34 %) while show less abundance in leaves (0.42%) and flowers (1.38%); corynan17ol that identified in roots (2.07%) and leaves extracts (0.12%), glucobrassicin identified in leaves (0.26%) and flowers (0.46%) extracts; dasycarpidol identified in roots (0.36%) and flowers (1.90%) extracts; dasycarpidan-1-one identified in roots (0.32%) extract only, while dasycarpidan-1-methanol, acetate and 5-(aminomethyl)-2-pyrrolidinone in area of (0.2%) and (11%) respectively recorded in leaves extract only; colchifoleine

recorded in flower (0.61%) extract only. The most abundant terpenoid was 3,7,11,15-tetramethyl-2-hexadecen-1-ol which identified in leaves (21.41%) and flowers (5.56%) extracts; α -eudesmol, squalene and 24,25-dihydroxycholecalciferol were other terpenoids recorded in roots and leaves extracts in area of (8.22%, 0.91%), (0.26%, 5.84%) and (2.90%, 0.15%) respectively; cadinene and phytofluene recorded in leaves and flowers extracts in area of (2.21%, 1.71%) and (0.39%, 0.41%) respectively, While, ambrosin recorded only in roots (0.57%) extract (Table 1).

The most abundant alcohols include tert-hexadecanethiol which represents 6.50% of flowers and

0.36% of roots but did not record in leaves; 1, 5, 9, 13-Tetrathia-3, 11-cyclohexadecaediol which identified in comparable values in all *T. coccinea* extracts, it was 1.14%, 1.66% and 1.63% in roots, leaves and flowers extracts respectively; D-mannitol, hexaacetate that was more abundant in leaves (0.89%) higher than roots and flowers where it was around 0.23% in both of them; the fourth identified alcohol was 1-hexadecanol which recorded in leaves (0.23%) and flowers (0.93%) only. The most abundant esters include: hexadecanoic acid 2-hydroxy-1,3-propanediyl ester where it represents 6.66 % of flowers extracts and 0.69% and 0.29% in roots and leaves extracts respectively; 1,2-benzenedicarboxylic acid, butyl octyl ester that identified in roots (0.39%), leaves (31%) and flowers (1.61%) extracts; 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester which recorded only in roots (2.01%) while phthalic acid and butyl undecyl ester identified only in leaves (2.10%) (Table 1).

Only two flavonoids in identified in *T. coccinea* extracts which are Lucenin 2 that recorded in roots (2.67%), leaves (1.22%) and flowers (0.71%) and quercetin-7,3',4'-trimethoxy that recorded only in roots (1.30%) extract. The most abundant phenol was chromone, 5-hydroxy-6, 7, 8-trimethoxy-2, 3-dimethyl and it recorded mainly in flowers (1.76%) and very small amount in roots (0.15%) while did not appear in leaves extract; trans-2-phenyl-1,3-dioxolane-4-methyloctadec-9,12,15-trienoate is another phenol identified in roots (0.36%) and leaves (0.91%), while 5-ethoxy-7-methoxy-2,2-dimethyl-3H-chromen-4-one identified only in roots (1.43%) extract (Table 1).

22-tricosenoic acid, erucic acid and 6-methylheptanoic acid were the identified fatty acids in *T. coccinea*. 22-Tricosenoic acid and erucic acid recorded in the three extracts in areas of (5.88%, 2.08%), (0.95%, 0.24%) and (11.26%, 0.40%) in roots, leaves and flowers respectively, however 6-methylheptanoic acid recorded in roots (0.16%) and leaves (0.28%) extracts only. The organic acids identified in *T. coccinea* were: folic Acid in roots (0.47%), leaves (2.12%) and flowers (0.35%); N-Acetyl-DL-tryptophan in roots (0.23%) and flowers (2.62%), cyclo(glycyl-1-tyrosyl) which recorded only in roots (0.39%) extract. Only two steroids were identified which are Prednisolone and ethyl iso-allochololate and they are recorded in roots (0.61%, 0.78%), leaves (0.44%, 0.70%) and flowers (0.89% and 0.26%) (Table 1).

According to the results shown in Table (2), the phytochemicals identified in *T. alba* extracts contained 13 terpenoids, 9 alkanes, 8 alkaloids, 5 alcohols, 3 esters, 3 phenols, 3 fatty acids, 2 steroids, 1 organic acid and 1 flavonoid. The most abundant terpenoids in *T. alba* extracts include: squalene which recorded in roots (5.36%), leaves (1.22%) and flowers (0.26%) extracts; furoscrobiculin B, dihydroxanthin and R1-barrigenol that identified only in roots (3.41%, 0.63% and 0.84%) and leaves (0.82%, 0.18% and 1.31%) extracts; neophytadiene that recorded in leaves (6.78%) and flowers (1.57%) only. Roots extract of *T. alba* characterized by the abundance of 5 extra terpenoids which are α -pinene (1.98%), sabinene (2.84%), α myrcene (2.17%), D-carvone (4.22%), b-Selinene (1.82%), while 3,7,11,15-tetramethyl-2-hexadecen-1-ol (3.87%), 4,25-secoobscurinervan, 21-deoxy-16-methoxy-22-methyl (0.71%) and ambrosiol (0.35%) were 3 more terpenoids identified in leaves extract only.

There are many alkanes identified in *T. alba* extracts including: docosane which recorded in relatively high values in both roots (19.36%), leaves (5.26%) and flowers (25.33%) extracts; flowed by octadecane, 17-pentatriacontene, nonacosane and heptacosane which recorded also in roots (4.28%, 0.71%, 0.97% and 1.12 %), leaves (0.41%, 0.23%, 5.20% and 10.98%) and flowers (11.69%, 7.97%, 12.83% and 10.68%) respectively; Cyclohexane, 1, 3, 5-trimethyl-2-octadecyl and pentatriacontane that identified only in leaves (0.27% and 41.08%) and flowers (1.31% and 0.81%) respectively, while nonadecane recorded only in roots (2.59%) and flowers (5.54%) extracts; hexatriacontane (4.88%) identified only in *T. alba* leaves. The most abundant alkaloids in *T. alba* extracts were: 1-phenyl-4-(2-imidazoliny)-5-(p-chlorophenyl)-1, 2, 3-triazole and 2-acetyl-3-(2-cinnamido)ethyl-7-methoxyindole which identified in roots (1.30 and 0.36%), leaves (0.11% and 1.24%) and flowers (0.57% and 0.40%) respectively; 3,7,8-trimethylpyrido [2, 3-d]pyrimidine-2 ,4 (3H, 8H)-dione, 6-bromo-peganol and ceanothine C that recorded in roots (1.54%, 0.36% and 1.63%) and leaves (0.57%, 0.46% and 0.54%) extracts respectively; N-(3'-Methylbut-2'-enoyl)-2-pyrrolidone and 5, 6-Dimethyl-1H-benzo[d]imidazol-2(3H)-one that recorded in roots (2.38% and 12.40%) and flowers (0.27% and 1.07%) extracts respectively; colchifoleine (1.90%) identified only in *T. alba* roots (Table 2).

The most abundant alcohols *T. alba* extracts are: 1-Tetradecanol and tert-hexadecanethiol which recorded in roots (1.42% and 0.59%), leaves (0.61% and 0.58%) and flowers (4.64% and 0.97%) respectively; 1,2-nonadecanediol (0.22%) and Z, Z-3, 15-octadecadien-1-ol acetate (0.51%) recorded in leaves only; 1-docosanol (3.91%) identified only in roots extract. The most abundant esters in *T. alba* extracts are: trichloroacetic acid, hexadecyl ester which was more concentrated in roots (3.12%) and identified in leaves and roots in comparable values (0.9%); phthalic acid, isobutyloctyl ester identified only in roots (1.18%) and leaves (2.18%); benzeneacetic acid, cyclohexyl ester recorded only in roots (1.21%) extract (Table 2).

There are 3 phenols identified in *T. alba* extracts, however their abundance was very low including: 3',8,8'-trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone that identified in roots (0.27%) and leaves (1.64%) extracts; bis (1, 1dimethylethyl) (0.84%) and trans-2-phenyl-1, 3-dioxolane-4-methyloctadec-9, 12, 15-trienoate (0.71%) that identified in roots extract only. No phenols identified in *T. alba* flowers. The most abundant fatty acids are: 5,8,11,14-eicosatetraenoic acid and Ethyl 6,9,12,15-octadecatetraenoate and identified in roots (4.59 and 3.77%), leaves (0.34% and 1.54%) and flowers (2.44% and 0.53%) respectively; Methyl 13,16-docosadienoate (0.22%) that recorded in leaves only. Two steroids identified in *T. alba* which are marsectohexol and ethyl iso-allochololate in roots (0.32% and 0.24%) and leaves (0.16 and 0.32%) respectively. No steroids recorded in flowers extract. Lucenin 2 is the sole identified flavonoid in *T. alba* extracts in values of (0.24%, 3.92% and 0.64%) in roots., leaves and flowers respectively. N, N'-ethylenebis (2-[2-hydroxyphenyl]glycine) (0.30%) is the only identified organic acid in *T. alba* leaves, while no organic acids recorded in its roots or flowers (Table 2).

Table 2. Phytochemicals identified in *Tetraena alba* roots, leaves and flowers extracts.

No.	Compound	Peak area (%)			Chemical class
		Roots	Leaves	Flowers	
1.	à-Pinene	1.98			Terpenoid
2.	Sabinene	2.84			Terpenoid
3.	áMyrcene	2.17			Terpenoid
4.	D-Carvone	4.22			Terpenoid
5.	N-(3'-Methylbut-2'-enoyl)-2-pyrrolidone	2.38		0.27	Alkaloid
6.	5,6-Dimethyl-1H-benzo[d]imidazol-2(3H)-one	12.40		1.07	Alkaloid
7.	b-Selinene	1.82			Terpenoid
8.	3,7,8-Trimethylpyrido[2,3-d]pyrimidine-2,4(3H,8H)-dione	1.54	0.57		Alkaloid
9.	Phenol, bis(1,1dimethylethyl)	0.84			Phenol
10.	1-(2,4-dimethylphenyl)-2-phenylethne	5.56	1.26		Others
11.	1-Tetradecanol	1.42	0.61	4.64	Alcohol
12.	Benzeneacetic acid, cyclohexyl ester	1.21			Ester
13.	Furoscrobiculin B	3.41	0.82		Terpenoid
14.	Octadecane	4.28	0.41	11.69	Alkan
15.	tert-Hexadecanethiol	0.59	0.58	0.97	Alcohol
16.	6-Bromo-Peganol	0.36	0.46		Alkaloid
17.	Ambrosiol		0.35		Terpenoid
18.	Neophytadiene		6.78	1.57	Terpenoid
19.	5,8,11,14-Eicosatetraynoic acid	4.59	0.34	2.44	Fatty acid
20.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol		3.87		Terpenoid
21.	Nonadecane	2.59		5.54	Alkane
22.	1,2-Nonadecanediol		0.22		Alcohol
23.	Ethyl 6,9,12,15-octadecatetraenoate	3.77	1.54	0.53	Fatty acid
24.	Dihydroxanthin	0.63	0.18		Terpenoid
25.	Z,Z-3,15-Octadecadien-1-ol acetate		0.51		Alcohol
26.	Docosane	19.36	5.26	25.33	Alkane
27.	1-Phenyl-4-(2-imidazoliny)-5-(p-chlorophenyl)-1,2,3-triazole	1.30	0.11	0.57	Alkaloid
28.	1-Docosanol			3.91	Alcohol
29.	Phthalic acid, isobutyloctyl ester	1.18		2.18	Ester
30.	Methyl 13,16-docosadienoate		0.22		Fatty acid
31.	N,N'-Ethylenebis(2-[2-hydroxyphenyl]glycine)		0.30		Organic acid
32.	2-Acetyl-3-(2-cinnamido)ethyl-7-methoxyindole	0.36	1.24	0.40	Alkaloid
33.	4,25-Secoobscurinervan,21-deoxy-16-methoxy-22-methyl		0.71		Terpenoid
34.	Cyclohexane,1,3,5-trimethyl-2-octadecyl-		0.27	1.31	Alkane
35.	Heptacosane	1.12	10.98	10.68	Alkane
36.	Marsectohexol	0.32	0.16		Steroid
37.	Trichloroaceticacid,hexadecyl ester	3.12	0.94	0.92	Ester
38.	Bis[(1S,2S,3S,5R)-(+)-isopinocampyl] Phosphorochloridate	3.35	0.56	1.53	Others
39.	Colchifoleine			1.90	Alkaloid
40.	Nonacosane	0.97	5.2	12.83	Alkane
41.	Squalene	5.36	1.22	0.26	Terpenoid
42.	Ethyl iso-allochololate	0.24	0.32		Steroid
43.	Trans-2-phenyl-1,3-dioxolane-4-methyloctadec-9,12,15-trienoate	0.71			Phenol
44.	Ceanothine C	1.63	0.54		Alkaloid
45.	3',8',8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4',4'-tetrone	0.27	1.64		Phenol
46.	17-Pentatriacontene	0.71	0.23	7.97	Alkane
47.	Pentatriacontane		41.08	0.81	Alkane
48.	R1-Barrigenol	0.84	1.31		Terpenoid
49.	Hexatriacontane		4.88		Alkane
50.	Lucenin 2	0.24	3.92	0.64	Flavonoid
Total		99.68	99.59	99.96	

According to the results represented in Table (3), the phytochemicals identified in *T. simplex* extracts contained 10 alkaloids, 9 terpenoids, 7 alkanes, 7 alcohols, 4 esters, 3 flavonoids, 3 fatty acids, 2 phenols, 2 steroids and 1 organic acid. The most abundant alkaloids in *T. simplex* extracts are: pyrrolidine, 1-(1-oxo-7, 10-octadecadienyl), buphanamin and streptovitacin A which recorded in the prepared three extracts in area of (2.36%, 0.78% and 1.19%) in roots, (3.13%, 0.34% and 0.48%) in leaves and (0.91%, 0.43% and 1.45%) in flowers respectively; oxazole, colchifoleine and ceanothine C that recorded only in roots (0.55%, 3.16% and 0.89%) and leaves (0.71%, 4.93% and 2.92%) extracts

respectively, in the same context, 4-(1-phenylethylamino)-1,3-oxazolidin-2-one and isoquinoline are two alkaloids identified in leaves (2.13% and 0.62%) and flowers (0.32% and 0.34%) extracts respectively; tropacocaine (0.49%) recorded in leaves only; while 6-(3-pyridyl) 1-hexyne recorded in flowers (0.35%) only.

The most abundant terpenoids in *T. simplex* extracts are: 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol and tefluthrine which identified in roots (0.82%, 0.43%), leaves (3.88%, 0.89%) and flowers (2.05%, 0.44%) respectively; dehydrobrusatol, phytofluene and 1-acetoxy-p-menth-3-one which identified in leaves (0.47%, 0.62%, 0.40%) and

flowers (0.35%, 0.29%, 0.27%) respectively; R1-barrigenol that identified only in roots (1.64 %) and leaves (1.22 %); lycopene 7 that recorded in roots (0.87%) and flowers (0.37%) extracts; stigmast-5-en-3-ol (2.04) and 1,5-heptadien-4-one,-3,3,6-trimethyl (0.71) which identified in roots extract only. The most abundant alkanes in *T. simplex* extracts are: docosane, dotriacontane, hexadecane and heptadecane, 9-hexyl which identified in all extracts reaching values of (26.28%, 11.39%, 3.91% and 0.52%) in roots, (30.49%, 1.37%, 4.31% and 0.34%) in leaves and (19.30%, 12.74%, 2.85% and 6.88%) in flowers respectively; octadecane identified only in roots (4.32%) and leaves (2.08%) extracts; nonacosane identified only in roots (4.12%) and flowers (2.58%) extracts and finally hexane,3-ethyl-2-methyl which identified only in leaves (4.18%) and flowers (1.36%) extracts (Table 3).

The identified alcohols in *T. simplex* extracts include: 1-hexadecanol, 2-methylhexadecan-1-ol and 1-docosanol which identified in the three extracts reaching total area of (1.03%, 2.45% and 0.65) in roots, (0.40%, 0.53% and 0.30%) in leaves and (1.50%, 0.60% and 0.27%) in flowers respectively; 1-cyclobutylcyclopropan-1-ol that identified only in leaves (0.65%) and flowers (0.28%); tridecanol (0.35%) and 2-hexyl-1-octanol (1.13%) which identified only in flowers; tert-hexadecanethiol (0.95%) recorded only in roots extract. The most abundant esters in *T. simplex* extracts are: hexanedioic acid, dioctyl ester which identified in roots (15.39%), leaves (0.89%) and flowers (16.55%), flowed by 3-isoxazolecarboxylic acid, 4,5-dihydro-5-phenyl, 1,1-dimethylethyl ester which recorded in leaves (5.19%) and flowers (0.27%) and oleic acid, eicosyl ester which identified in roots (0.45%) and leaves (0.96%), while Z 8 -octadecen-1-ol acetate (1.08%) identified in flowers extract only (Table 3).

The most abundant flavonoids are: lucenin 2 that identified in roots (2.10%), leaves (5.15%) and flowers (1.48%) extracts; digitoxin (1.25%) and arenobufagin (30%) which identified in roots and flowers respectively. The three fatty acids identified in *T. simplex* are identified in the three extracts in comparable area % ranging from 1.73% to 0.33% including agaric acid, 9-Octadecenoic acid and methyl 7,10,13-hexadecatrienoate. Only two phenols identified in *T. simplex* extracts: phenol, bis(1,1dimethylethyl) recorded in roots (0.32 %), leaves (1.66%) and flowers (1.52%); carbofuran phenoldinitrophenyl ether (0.80%) recorded only in leaves extract. The identified steroids were ethyl iso-allocholate which recorded in roots (1.13%), leaves (1.33%) and flowers (0.79%) and androst-5, 7-dien-3-ol-17 one, acetate (49%) which recorded in roots extract only. 4-O-methylconhypoprotocetraric acid was the only identified organic acid in *T. simplex* leaves (1.49%) and flowers (3.82%) extracts only (Table 3).

Biological studies on *Tetraena* species have indicated significant antioxidant, antidiabetic, antitumor, antimicrobial and anti-inflammatory activities (Sharma & Ramawat, 2014; Barzegar *et al.*, 2018). Many compounds with medical importance have been identified in this study which vary between the studied species. The most important identified terpenoids include squalene which identified in *T. coccinea* roots and leaves and in all *T. alba* fractions while absent in *T. simplex* on the other hand, many other terpenoids including

lycopene 7, phytofluene, dehydrobrusatol and stigmast-5-en-3-ol identified only in *T. simplex* extracts. These terpenoids are primarily known as natural antioxidants, unique oxygen generators, power immune stimulators, anti-histamine and anti-allergic and anticancer (Kelly, 1999; Engelmann *et al.*, 2011). Also, R1-barrigenol is an important terpenoid that identified in *T. alba* and *T. simplex* extracts known for its antimicrobial activities (Oh *et al.*, 2014). In addition, D-carvone and sabinene are two important terpenoids that identified only in *T. alba* leaves. Many studies showed that D-carvone used as food additive (de Carvalho & da Fonseca, 2006), while sabinene has antioxidant and anti-inflammatory activities (Valente *et al.*, 2013).

Many flavonoids with critical medical importance have been identified in this study including lucenin 2 that known to has anti-inflammatory properties (Kim *et al.*, 2016), identified in all fractions of the three studied *Tetraena* species, and digitoxin that identified in *T. simplex* roots only and is known to be used for chronic cardiac insufficiency (Routledge & Hutchings, 2013). Many important alkaloids have been identified including colchifoleine which recorded in the extracts of the three *Tetraena* species and known to be effective in inflammatory and heart diseases (Hemkens *et al.*, 2016) and Glucobrassicin which recorded only in *T. coccinea* only and known as anticancer (Park *et al.*, 2013). On the other hand, several important steroids have been recorded in this study including ethyl iso-allocholate that identified in the three studied species and prednisolone that identified only in *T. coccinea* extracts have been found to be useful in treating male infertility factor, constipation, inflammations, eye pains and skin infections and explaining its cardiovascular bioactivity (Ogunlesi *et al.*, 2010). 11,2-benzenedicarboxylic acid, butyl octyl ester that identified in all *T. coccinea* extracts is known to possess antimicrobial and antifouling activity (Ingole, 2016).

Although some phytochemical data have been reported on *T. coccinea* (Amin *et al.*, 2011; Mohammed *et al.*, 2021), *T. alba* (Feriani *et al.*, 2020; Abdelhameed *et al.*, 2022; Eltamany *et al.*, 2023) and *T. simplex* (Abdallah & Esmat, 2017; Baky *et al.*, 2020), the number of identified compounds in the literatures is limited. Therefore, the chemical composition of these species has not been fully elucidated. The current study identified 50 chemical constituents in each species distributed between plant roots, leaves and flowers. Although the medicinal and nutritional values of many of the identified compounds in this study are well known, others need additional investigations.

Chemical classes: Table (4) shows the total area (%) for the most abundant chemical classes of the phytochemicals identified in the studied extracts. The overall phytochemicals composition of roots, leaves and flowers of *T. coccinea*, *T. alba* and *T. simplex* showed both qualitative and quantitative differences. It is worth to mention that, in roots extracts, alkaloids represented about 42% out of all identified phytochemicals in *T. coccinea* compared to 19.97 and 8.9% in *T. alba* and *T. simplex* respectively. Alkanes was the second abundant chemical class in roots extracts and it was the highest in *T. simplex* (50.54%) roots followed by *T. alba* (29%) and *T. coccinea* (19.13%). Terpenoids was more abundant in *T. alba* roots (23.27%) followed by *T. coccinea*

(14.55%) and *T. simplex* (3.76%). Flavonoids and steroids were more or less the same values in case of *T. coccinea* and *T. simplex* roots and decreased markedly in *T. alba* roots. Phenoles and fatty acids represented in comparable values in case of *T. coccinea* and *T. alba* roots and decreased markedly in *T. simplex* roots. High amount of esters recorded in *T. simplex* (15.48%) roots followed by *T. alba* (5.51%) and *T. coccinea* (3.09%). The least abundant chemical class in roots extracts was organic acids which represented only by about (1.09%) in *T. coccinea*, while did not recorded at all for *T. alba* and *T. simplex* roots extract.

In leaves extracts, alkanes were the most abundant chemical class in the three studied species. The highest

total alkanes area recorded in *T. alba* (68.31%), followed by *T. coccinea* (48.77%) and *T. simplex* (68.31%). The second abundant chemical group in leaves extract is terpenoids which represent about 28.31%, 15.24% and 10.23% in case of *T. coccinea*, *T. alba* and *T. simplex* respectively. Alkaloids was more abundant in *T. simplex* leaves (15.75%), compared to 2.92% in case of *T. alba* and 1.11% in *T. coccinea* leaves. Flavonoids were more abundant in leaves of *T. simplex* (5.15%), followed by *T. alba* (3.92%) and *T. coccinea* (1.22%). Steroids was the least abundant chemical class in leaves extracts recorded values of 1.14%, 0.48% and 1.33% in case of *T. coccinea*, *T. alba* and *T. simplex* respectively (Table 4).

Table 3. Phytochemicals identified in *Tetraena simplex* roots, leaves and flowers extracts.

No.	Compound	Peak area (%)			Chemical class
		Roots	Leaves	Flowers	
1.	Oxazole	0.55	0.71		Alkaloid
2.	1-Cyclobutylcyclopropan-1-ol		0.65	0.28	Alcohol
3.	Hexane,3-ethyl-2-methyl		4.18	1.36	Alkane
4.	1,5-Heptadien-4-one,-3,3,6-trimethyl		0.71		Terpenoid
5.	6-(3-pyridyl) 1-hexyne			0.35	Alkaloid
6.	Tridecanol			0.35	Alcohol
7.	Phenol, bis(1,1 dimethylethyl)	0.32	1.66	1.52	Phenol
8.	4-(1-phenylethylamino)-1,3-oxazolidin-2-one		2.13	0.32	Alkaloid
9.	1-Acetoxy-p-menth-3-one		0.40	0.27	Terpenoid
10.	2-Hexyl-1-octanol			1.13	Alcohol
11.	Hexadecane	3.91	4.31	2.85	Alkane
12.	1-Hexadecanol	1.03	0.40	1.50	Alcohol
13.	Tropacocaine		0.49		Alkaloid
14.	2-methylhexadecan-1-ol	2.45	0.53	0.60	Alcohol
15.	Tert-Hexadecanethiol	0.95			Alcohol
16.	3-Isoxazolecarboperoxoic acid, 4,5-dihydro-5-phenyl, 1,1-dimethylethyl ester		5.19	0.27	Ester
17.	Methyl 7,10,13-hexadecatrienoate	0.40	0.92	0.41	Fatty acid
18.	Isoquinoline		0.62	0.34	Alkaloid
19.	9-Octadecenoic acid	0.33	0.49	1.73	Fatty acid
20.	α -D-xylofuranose,cyclic 1,2:3,5-bis(butylboronate)	6.40	3.42	10.99	Others
21.	Octadecane	4.32	2.08		Alkane
22.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.82	3.88	2.05	Terpenoid
23.	Streptovitacin A	1.19	0.48	1.45	Alkaloid
24.	Buphanamin	0.78	0.34	0.43	Alkaloid
25.	Docosane	26.28	30.49	19.30	Alkane
26.	Z 8 -Octadecen-1-ol acetate			1.08	Ester
27.	Heptadecane, 9- hexyl	0.52	0.34	6.88	Alkane
28.	1-Docosan	0.65	0.30	0.27	Alcohol
29.	Androst-5,7-dien-3-ol-17 one, acetate	0.49			Steroid
30.	Carbofuran phenoldinitrophenyl ether		0.80		Phenol
31.	Pyrrolidine, 1-(1-oxo-7,10-octadecadienyl)	2.36	3.13	0.91	Alkaloid
32.	4,5-Methylenedioxy-2-bromoN[1-methyl-4-diethylaminobutyl]aniline	2.92	6.66	2.85	Others
33.	Hexanedioic acid,dioctyl ester	15.39	0.89	16.55	Ester
34.	4-O-Methylconhypoprotocetraric acid		1.49	3.82	Organic acid
35.	Colchifoleine	3.16	4.93		Alkaloid
36.	Nonacosane	4.12		2.58	Alkane
37.	Stigmast-5-en-3-ol		2.04		Terpenoid
38.	Arenobufagin			0.30	Flavonoid
39.	Agaricic acid	0.42	0.37	0.55	Fatty acid
40.	Tefluthrine	0.43	0.89	0.44	Terpenoid
41.	Ethyl iso-allocholate	1.13	1.33	0.79	Steroid
42.	Dotriacontane	11.39	1.37	12.74	Alkane
43.	Ceanothine C	0.89	2.92		Alkaloid
44.	R1-Barrigenol	1.64	1.22		Terpenoid
45.	Dehydrobrusatol		0.47	0.35	Terpenoid
46.	Lycopene 7	0.87		0.37	Terpenoid
47.	Phytofluene		0.62	0.29	Terpenoid
48.	Oleic acid, eicosyl ester	0.45	0.96		Ester
49.	Lucenin 2	2.10	5.15	1.48	Flavonoid
50.	Digitoxin	1.25			Flavonoid
Total		99.91	99.96	99.75	

Table 4. The total area (%) of most abundant phytochemical classes identified in the studied *Tetraena* species.

Chemical Class	Roots			Leaves			Flowers		
	<i>T. coccinea</i>	<i>T. alba</i>	<i>T. simplex</i>	<i>T. coccinea</i>	<i>T. alba</i>	<i>T. simplex</i>	<i>T. coccinea</i>	<i>T. alba</i>	<i>T. simplex</i>
Alkaloids	42.09	19.97	8.93	1.11	2.92	15.75	4.35	4.21	3.8
Terpenoids	14.55	23.27	3.76	28.31	15.24	10.23	7.68	1.83	3.77
Flavonoids	3.97	0.24	3.35	1.22	3.92	5.15	0.71	0.64	1.78
Phenols	1.94	1.82	0.32	0.91	1.64	2.46	1.76	-	1.52
Steroids	1.39	0.56	1.62	1.14	0.48	1.33	1.15	-	0.79
Alkanes	19.13	29.03	50.54	48.77	68.31	42.77	42.77	76.16	45.71
Alcohols	1.74	2.01	5.08	2.78	1.92	1.88	9.29	9.52	4.13
Fatty acids	8.12	8.36	1.15	1.47	2.10	1.78	11.66	2.97	2.69
Esters	3.09	5.51	15.84	2.7	0.94	7.04	8.27	3.10	17.90
Organic acids	1.09	-	-	2.12	0.30	1.49	2.97	-	3.82
Others	2.69	8.91	9.32	8.75	1.82	10.08	9.2	1.53	13.84
Total	99.80	99.68	99.91	99.23	99.59	99.96	99.81	99.96	99.75

In flowers extracts, the most abundant chemical class was alkanes where it represents about 76.16%, 42.77% and 45.71% of the total peak area in case of *T. alba*, *T. coccinea* and *T. simplex* respectively. Alkaloids recorded in comparable values in the flower extracts of the three studied species. Terpenoids were more dominant in *T. coccinea* (7.68%), followed by *T. simplex* (3.77%) and *T. alba* (1.83%). Alcohols showed comparable values in *T. coccinea* (9.29%) and *T. alba* (9.52%) flowers and reduced markedly in *T. simplex* (4.13%). Fatty acids represented about 11.66% of the total peak area in *T. coccinea* flowers extract, while reduced to 2.97% and 2.69 % in *T. alba* and *T. simplex* flowers respectively. Esters was more abundant in *T. simplex* (17.9%) flowers, followed by *T. coccinea* (8.27%) and *T. alba* (3.10%). Steroids was the least abundant chemical group in *T. coccinea* and *T. simplex*. Phenols, steroids and organic acids did not record between the most abundant phytochemicals in *T. alba* in flowers (Table 4).

The variation in phytochemical classes between the studied species (Table 4) could attributed to the salinity level in the surrounded habitat. As shown from the results of salinity indexes (Fig. 2), *T. coccinea* grows in significantly higher saline conditions more than *T. alba* and *T. simplex* which could account for its higher concentrations in secondary metabolites content mainly in case of alkaloids and terpenoids. Based on several morphological and anatomical characters as well as eco-physiological values of ionic status, Abd El-Twab & Abd El-Hafeez (2015) demonstrated that, *T. simplex* showed the lowest values of effective salinity, *T. alba* and *T. coccinea* showed alternatively the highest values. They also showed that *T. alba* was less affected by the environmental conditions than *T. coccinea* and *T. simplex*, because its succulence was higher.

Generally, phytochemicals biosynthesis and accumulation significantly depends upon environmental stress variables. As described by Ferrandino & Lovisolo (2014) and Verma & Shukla (2015), many factors including, soil salinity, soil water status, light and temperature can critically affect plant growth and its capability to synthesize secondary metabolites, ultimately leading to the alteration of whole phytochemical profiles which lead to accumulation of bioactive constituents. Large number of plant phytochemicals including flavonoids, alkaloids, terpenoids, steroids and phenolics have been reported to be produced in

many plant species in response to salinity stress and they were shown to be involved in activating plant resistance function (Bourgaud *et al.*, 2001; Sytar *et al.*, 2018).

Conclusion

Overall, from the current study 50 chemical constituents have been identified in each species belonging to different chemical classes. The variation in phytochemical classes between the studied species could attributed to the salinity level in the surrounded habitat. *T. coccinea* that grows in the highest saline conditions possess higher levels of many active phytochemicals mainly those belong to alkaloids and terpenoids. The medicinal and nutritional values of many of the identified compounds is well known, while others need additional investigations. Further studies of the biological importance of the phytochemicals existing in these plants can maximize their therapeutic significance in future and can be an effective and valuable drug source in cheaper rate as it is easily available.

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