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Green Synthesis of Silver Nanoparticles from Capitula extract of Some *Launaea* (Asteraceae) with Notes on their Taxonomic Significance

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DATA are used to re-assess the relationships between certain species of the genus *Launaea* Cass. belonging to the family Asteraceae. Taxonomic diversity of 10 taxa belonging to 8 species and 2 subspecies of *Launaea* Cass. is provided using morphological criteria concerned with vegetative and reproductive organs in addition to FTIR spectroscopy. Ecofriendly silver nanoparticles were synthesized from *Launaea's* capitula extract and characterized by FTIR spectroscopy. NTSYS-pc software was used in order to analyze the data of FTIR spectroscopy and morphological characters. FTIR technique was used to recognize the functional groups of the active compound according to the peak value in Infrared radiation region. Cluster analysis based on FTIR data divided the ten studied taxa into three major groups; the first group comprises the four species of sect. Microrhynchus (*L. nudicaulis, L. intybacea* and *L. massauensis*) and sect. Launaea (*L. capitata*), the second group comprises the four subspecies of *L. angustifolia* and *L. fragilis*; the third group comprises the two-allied species (*L. mucronata* and *L. cassiniana*). FTIR technique found to be a rapid and accurate method for differentiating between *Launaea* taxa under investigation.

Keywords: *Launaea*, Asteraceae, FTIR, Spectroscopy, Systematic, Silver nanoparticles.

Introduction

Taxonomists always found a problem in the systematic of the genus *Launaea* due to its confusing taxonomic history (Zareh et al., 2016a). *Launaea* Cass. comprises 55 species and it is mainly distributed in the S. Mediterranean, Africa, and SW Asia (Kilian, 1997). Until that time, several species today included in *Launaea* had already been described but placed in such different genera as *Chondrilla, Lactuca, Leontodon, Prenanthes* and *Sonchus*. For the most of the 19th century, the present day *Launaea* species were dispersed and moved chiefly between *Sonchus* and *Lactuca*. According to Zareh et al. (2016a), the genus *Launaea* was represented in the flora of Egypt by twelve species and being the largest genus of the tribe Cichorieae.

There is a worldwide interest in synthesis of silver nanoparticles from environmentally friendly materials such as bacteria, enzymes, fungi and leaf extract (Bhainsa & D'souza, 2006; Jain et al., 2009; Saifuddin et al., 2009 and Willner et al., 2007). Green synthesis of silver nanoparticles (AgNPs) provides numerous benefits due to its environmental friendly effect as well as its low-cost. Recently, several literatures pay attention to the importance of using plant extract to synthesis silver nanoparticles (Dhand et al., 2016; Francis et al., 2017; Gomathi et al., 2017; Kumar et al., 2014; Saravanakumar et al., 2017 and Singh et al., 2010).

Mondal et al. (2011) synthesized ecofriendly silver nanoparticles from $AgNo_3$ by the latex of six different plant taxa belonging to six different families; they proposed to use this modern technique for the taxonomy of angiosperms based on the ability of plants to synthesis silver nanoparticles which are variable in concentration, shape and size.

Chemotaxonomy has been strongly inclined the whole field of biology, which is also very useful for plant taxonomy (Mariswamy et al., 2012). FTIR spectroscopy (Fourier transform infrared) is a non-invasive, fast and a high-resolution analytical technique for recognized different types of chemical bonds by producing the infrared absorption spectrum that is like a molecular fingerprint (Griffiths & de Haseth, 1986 and Smith,

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1996). The application of a combination with numerical methodologies, FTIR is recommended and has many advantages. This technique has been successfully used for classifying of aged and normal soybean seeds (Kusama et al., 1997 and Wang et al., 2002). One of the most important applications of the IR spectroscopic study is the diagnostic value in establishing the occurrence of certain organic constituents in the plants (Velmurugan, 2006).

Recently, FTIR has been introduced as a metabolic fingerprinting tool for the plant sciences (Parveen et al., 2007 and Shen et al., 2008). In such an attempt, either the variation of the intensity or the frequency shift of some characteristic absorption bands can be used. According to Kim et al. (2004), the FTIR approach is strongly recommended to use in chemotaxonomic classification of flowering plants. The coming decades are likely to bring new approaches which will change our understanding of plants architecture and structure what is currently known in model organisms (Heywood et al., 2007).

In this study, ecofriendly silver nanoparticles were synthesized from capitula extract of eight closely similar taxa of *Launaea* (Table 1) and illustrate the relationships among the study taxa based on its chemical characteristics using FTIR approach in conjunction with the morphological characters.

Materials and Methods

Morphological studies were carried out on fresh material and voucher specimens deposited in different Egyptian herbaria: CAI, CAIM and ASTU. The investigated specimens used in morphological and FTIR spectroscopic studies are summarized in Table 1.

For observation the silver nanoparticles, the method followed Eri et al. (2014) with little modification as follows: The extract was made using 1g of capitula; the capitula were cleaned thoroughly using distilled H_2O and cut into small pieces, the capitula sample then added to 100ml of dist. H_2O and then boil for 5 min. The solution was then left to cool to normal temperature (approximately 25°C). Following this step, the extract was then filtered through the filter paper.

The silver nitrate ($AgNO_3$) used in this study was obtained from sigma Aldrich, 40ml of capitula extract was added to 60ml of 10^{-3}µ $AgNO_3$ solution and the reaction was left to take place at room temperature. The formation of silver nanoparticles was indicated by the changing the coloure from colouress to yellow and finally to dark brown. The solution then centrifuges to collect the silver nanoparticles. The nanoparticles were washed two times using distilled H_2O.

The formation of silver nanoparticles by capitula extract were scanned in the 300-900nm wave-length rang using a double beam spectrophotometer (Perkin-Elmer lambda 750 spectrophotometer). The FTIR spectrum was obtained in the mid IR region of 400-4000cm^{-1}. The dried experimental sample was directly placed on the potassium bromide crystals and the spectrum was recorded in transmittance mode.

Data scoring and analysis

The analyses were carried out using NTSYSpc2 software. A cluster analysis was performed using the average taxonomic distance and UPGMA clustering. The correlation coefficient between the tree matrix and the distance matrix was calculated in order to examine how the cluster analysis fits the distance matrix.

For FTIR, each separate character was scored as present (1) or absent (0) for calculating different coefficients such as Simple Matching (SM), Sorensen-Dice (SD) and Jaccard (J). All detectable and clear characters were scored. Two phenograms were constructed, the first was based on morphological criteria and the second was based on combined characters of FTIR (Fig. 1 and 2).

Results

The studied 76 morphological characters concerned with stem, leaves, stipules, inflorescence, flowers, capsules, seeds and embryo were studied, and the morphological criteria used in computer analysis were indicated in (Table 2 and Appendix 1). The cluster analysis based on morphological data (Fig. 1) divided the studied taxa into two major groups; the first group (A) comprises the species of sect. Zollikoferia which characterized by papillose marginal achenes and absence of pappus disk (namely: *L. angustifolia*, *L. fragilis L. mucronata* and *L. cassiniana*); the second group comprises the species of the other two sections (Launaea and Microrhynchus) that characterized by wrinkled or weakly papillose marginal achenes (Zareh et al., 2016b).

TABLE 1. Plants used in FTIR analysis.

NO.	Taxa	Locality
1	*Launaea nudicaulis* (L.) Hook. f.	Suez, Wadi Hagol, 13.4.2010, *Zareh & Aboul-Ela* s.n. (ASTU)
2	*Launaea intybacea* (Jacq.) Beauverd	Gebel Elba, Wadi Laseitit, 7.2.1962, *Täckholm et al.* 1687 (CAI)
3	*Launaea massauensis* (Fresen.) Sch.Bip. ex Kuntze	Marsa Allam, Wadi Abu Ghusun, 7.3.1989, *Zareh & Fargali* s.n. (ASTU)
4	*Launaea capitate* (Spreng.) Dandy	Marsa Matrouh, AL-Omyed, 5.4.2012, *Zareh & Aboul-Ela* s.n. (ASTU)
5	*Launaea angustifolia* (Desf.) Kuntze subsp. angustifolia	Alamein - Alexandria road, 10.4.2010, *Zareh & Aboul-Ela* s.n. (ASTU)
6	*Launaea angustifolia* (Desf.) Kuntze subsp. *arabica* (Boiss.) N. Kilian	The Siwa Oasis, 29.12.1969, *Zahran* s.n. (CAI)
7	*Launaea fragilis* (Asso) Pau subsp. *fragilis*	Al Alamein, Sidi Abdel Rahman, 4.4.2012, *Zareh & Aboul-Ela* s.n. (ASTU)
8	*Launaea fragilis* (Asso) Pau subsp. *tenuiloba* (Boiss.) Zareh & Mohamed	Alexandria-Matrouh, Kilo137, 4.4.2012, *Zareh & Aboul-Ela* s.n. (ASTU)
9	*Launaea mucronata* (Forssk.) Muschl.	Suez, Wadi Hagol, 13.4.2010, *Zareh & Aboul-Ela* s.n. (ASTU)
10	*Launaea cassiniana* (Jaub. & Spach) Kuntze	Mersa Alam, Wadi Abu Ghusun, 8.3.1989, *Zareh & Faraghali* s.n. (ASTU)

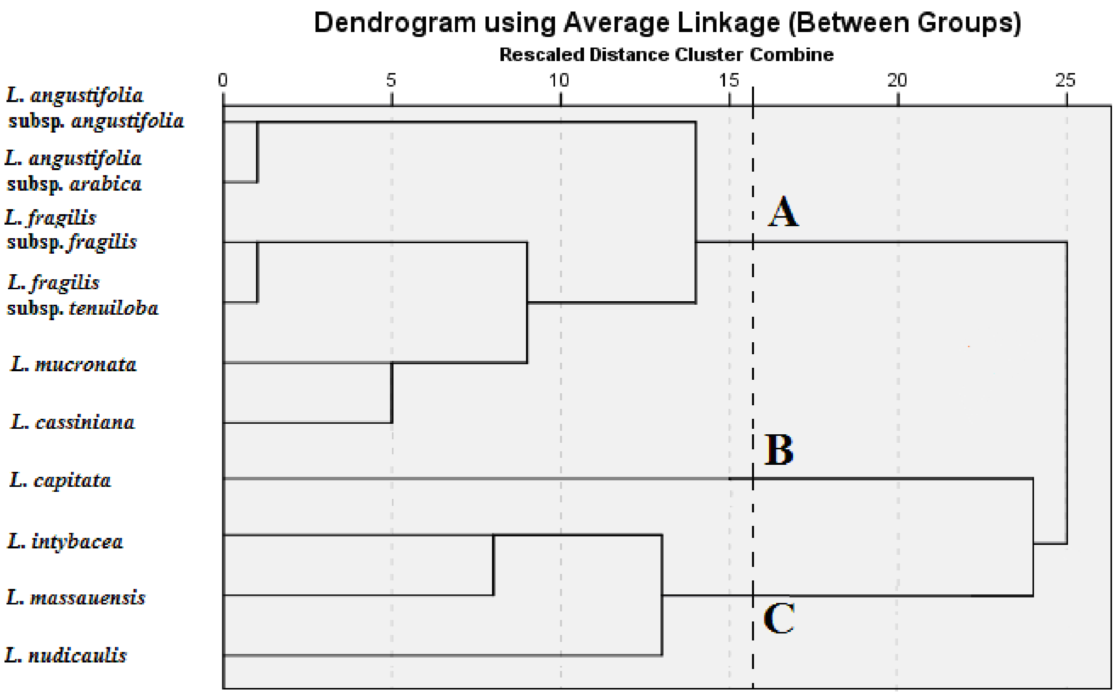

Fig. 1. Dendrogram illustrating the relationships among the 10 studied taxa of *Launaea* based on the morphological characters. A, sect. Zollikoferia; B, sect. Launaea and C, sect. Microrhynchus.

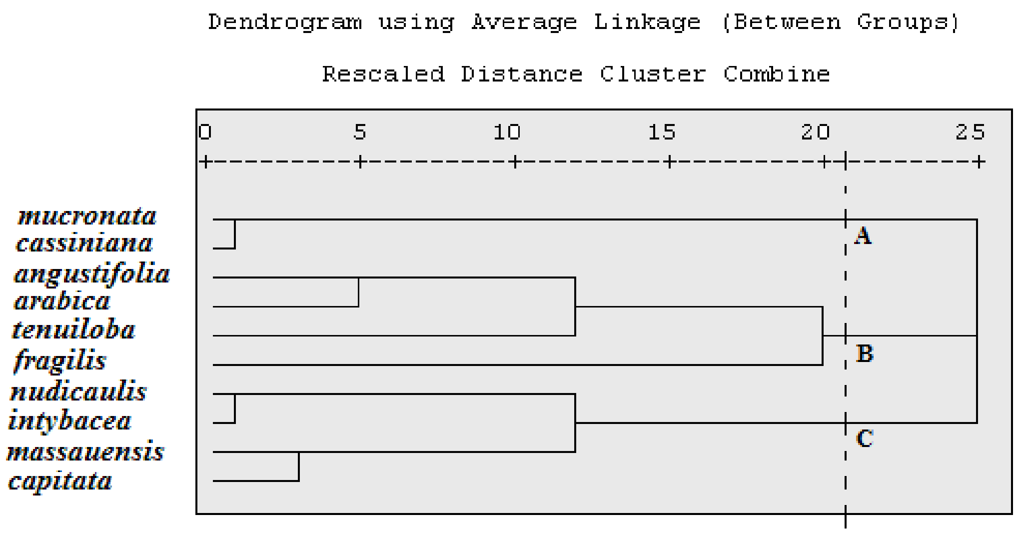

Fig. 2. Dendrogram illustrating the relationships among the 10 studied taxa of *Launaea* based on the FTIR characters.

TABLE 2. Characters and character state used in morphological analysis

	Character & character state		**Character & character state**		**Character & character state**
1	Habit	14	Basal leaves apex	27	Head long at anthesis
	1. Annual		1. Acute		1. 8-9 mm
	2. Perennial		2. Obtuse		2. 10-12 mm
	3. Shrub		3. Acuminate		3. 15-20 mm
2	Plant nature	15	Cauline leaves blade	28	Head long at fruiting
	1. Spinescent		1. Reduced		1. 9.0-10.0 mm
	2. Spineless		2. Ovate		2. 10.5-12.0 mm
3	Growth pattern		3. Auriculate		3. 12.5-14.0 mm
	1. Prostrate	16	Cauline leaves apex		4. 15.0-20.0 mm
	2. Procumbent		1. Reduced	29	Involucral bracts margin
	3. Twining		2. Acute		1. Not scarious
	4. Erect		3. Obtuse		2. narrow scarious
4	Plant form	17	Early leaves margin		3. broadly scarious
	1. Scape-like		1. Sub-entire	30	Outer bracts apex nature
	2. Caulescent		2. Serrate-dentate		1. Fleshy
5	Plant base	18	Longer leaf length		2. Callous-tipped
	1. Woody		1. (7-8 cm)		3. Cartilaginous
	2. Herbaceous		2. (9-10 cm)	31	Outer bracts apex shape
6	Plant height		3. (11-12 cm)		1. Acute
	1. (± 10 cm)		4. (15-16 cm)		2. Obtuse
	2. ((± 25 cm)	19	Synflorescence		3. Acuminate
	3. ((± 30 cm)		1. Heads single	32	Outer bracts broad
	4. ((± 40 cm)		2. Heads glomerate		1. 1.5-2mm
	5. ((± 100 cm)	20	Head broad		2. ± 3mm
7	Branching		1. less than 2.5 mm	33	Outer bracts length
	1. From base		2. more than 4.0 mm		1. 1.5-2.5 mm
	2. Above	21	Peduncles long		2. ±3mm
8	Branch leafness		1. Absent	34	Median bracts shape
	1. Leafy		2. 0.3-0.8 mm		1. oblong-lanceolate
	2. Leafless		3. 1.0-2.5 mm		2. ovate-lanceolate
9	Branch texture		4. 30 - 70 mm	35	Median bracts apex nature
	1. Glabrous	22	Receptacle diameter at fruiting		1. Fleshy
	2. Tomentose		1. 1.5-2.0 mm		2. Cartilaginous
	3. Puberulent		2. 2.5-3.0 mm	36	Median bracts apex shape
	4. Glaucescent		3. 3.5-5.5 mm		1. acute
10	Leaf margin nature		4. 6.0-10 mm		2. obtuse
	1. White cartilaginous	23	Head shape		3. acuminate

TABLE 2 Cont.

	Character & character state		Character & character state		Character & character state
	2. Green fleshy		1. Cylindrical	37	Median bracts shape
11	Basal leaves presence		2. Broadly cylindrical		1. oblong-lanceolate
	1. Rosetted		3. Narrowly cylindrical		2. ovate-lanceolate
	2. Early deciduous		4. Campanulate	38	Median bracts broad
	Basal leaves shape	24	Head color		1. (1.5-2.0 mm)
	1. Spathulate		1. Green		2. (2.5-3.0 mm)
12	2. linear to linear-spathulate		2. Grayish green		3. (3.5-4.0 mm)
	3. Oblong	25	Head broad at anthesis	39	Median bracts length
	4. Lanceolate		1. 2-2.5mm		1. (4.0-5.0 mm)
	5. Oblanceolate-elliptic		2. 4-5mm		2. (5.5-6.0 mm)
13	Basal leaves margin		3. 6-7mm		3. (6.5-7.0 mm)
	1. Entire	26	Head broad at fruiting	40	Inner bracts apex nature
	2. Dentate		1. 2-3mm		1. Fleshy
	3. Sinuate-dentate to pinnatifid		2. 4-5mm		2. Cartilaginous
	4. Sinuate-dentate to pinnatisect		3. 6-7mm		
41	Inner bracts apex shape	52	Achenes wings	64	Inner achenes color
	1. Acute		1. Not winged		1. Whitish
	2. Obtuse		2. 1-winged		2. Brown
42	Inner bracts broad		3. 2-3-winged		3. Yellow
	1. 1-1.5 mm	53	Achenes length	65	Inner achenes shape
	2. 2-2.5 mm		1. 2.0-3.0 mm		1. columnar
43	Inner bracts length		2. 3.5-4.0 mm		2. compressed
	1. 8.0-9.0 mm		3. 4.5-6.0 mm	66	Inner achenes apex
	2. 9.5-10.0 mm		4. 6.5-8.0 mm		1. cuspidate
	3. 10.5-12.0 mm	54	Achenes width		2. not cuspidate
	4. 12.5-15.0 mm		1. 0.5 - 0.6 mm	67	Inner achenes sculpture
44	Flowers per capitulm		2. 0.7-0.9mm		1. smooth
	1. 6-10		3. 1-1.3mm		2. wrinkled
	2. 15-20	55	Achenes base		3. papillose
	3. 25-33		1. obtuse teethed	68	Inner achenes ribs
	4. 35-50		2. not teethed		1. 4 main
	5. ≥55	56	Marginal achenes color		2. 5 main
45	Ligules length		1. whitich		1. 2, distinct
	1. 4.0 – 6.0 mm		2. brown		2. not differentiated
	2. 6.5 – 8.0 mm		3. grey	69	Inner achenes secondary ribs
	3. 8.5-12.0 mm		4. blackish		1. 2, distinct
	4. 12.5-20.0mm		5. yellow		2. not differentiated
46	Ligules width	57	Marginal achenes angles	70	Inner achenes ribs surface
	1. 0.8 - 1.5 mm		1. ±5 angular		1. transversally wrinkled
	2. 1.7 - 2.2 mm		2. ±4 angular		2. glabrous
	3. 2.4 – 2.8 mm		3. not angular		3. squamulose-papillose
	4. 3.0 – 5.5 mm	58	Marginal achenes length	71	Pappus long
47	Flowers tube length		1. shorter than inner		1. 5-6 mm
	1. 3.5 - 6.0 mm		2. longer than inner		2. 7 - 8 mm
	2. 6.5 – 9.0 mm	59	Marginal achenes shape		3. 9-10 mm
48	Anther length		1. columnar	72	Pappus
	1. 1.2 – 2.2 mm		2. compressed		1. persistent
	2. 2.5-3.2 mm	60	Marginal achenes apex		2. deciduous
	3. 3.5-5.5 mm		1. truncate	73	Pappus symmetry
49	Style-arms long		2. cuspidate		1. monomorphic
	1. 0.5-1mm	61	Marginal achenes sculpture		2. dimorphic
	2. 3-3.5mm		1. wrinkled	74	Pappus disk
	3. 3.5-4mm		2. papillose		1. present
50	Style sweeping hairs color	62	Achenes secondary ribs		2. absent
	1. yellow		1. ±2 distinct	75	Inner setaceous
	2. blackish		2. not distinct		1. absent
51	Achenes symmetry	63	Achene ribs surface		2. small number
	1. sub homomorphic		1. hirsute at angles		3. large number
	2. heteromorphic		2. transversally wrinkled	76	Outer downy
			3. squamulose-papillose		1. absent
					2. large number

The later group can be divided into two groups; one (B) comprises the species of sect. *Launaea* (*L. capitata*) which are characterized by prostrate habit and aggregate heads; the second group (C) comprises the species of section Microrhynchus (*L. nudicaulis, L. intybacea* and *L. massauensis*) that can be separated based on pappus characters. This grouping agrees with regards of Kilian (1997) in which he arranged the studied species in three different tribes.

The cluster analysis based on FTIR data (Fig. 2) divided the ten studied taxa into three major groups; the first group comprises the four species of sect. Microrhynchus (*L. nudicaulis, L. intybacea* and *L. massauensis*) and sect. Launaea (*L. capitata*), the second group comprises the four subspecies of *L. angustifolia* and *L. fragilis*; the third group comprises the two allied species (*L. mucronata* and *L. cassiniana*).

Discussion

Launaea cassiniana (Jaub. & Spach) Kuntze was described by both Kilian (1997) and Boulos (2002) as *L. mucronata* subsp. *cassiniana*. According to Täckholm (1974) and Zareh et al. (2016b), *L. mucronata* can easily distinguished from *L. cassiniana* by several morphological characters which summarized in Table 3.

On the other hand, *L. tenuiloba* (Boiss.) Kuntze is treated by Kilian (1997) and Boulos (2002) as a synonym to *L. fragilis*, the plant is differing in being procumbent, branched from base with subentire leaves and wrinkled inner achenes, thus it is treated here as *L. fragilis* subsp. *tenuiloba* (Boiss.) Zareh & Mohamed comb. nov. (Zareh et al., 2016b).

According to Griffiths & De Haseth (2007), FTIR approach allows distinguishing the whole range of infrared spectrum in the measurements of plant specimens. The biosynthesized silver nanoparticles were formed in several different shapes such as hexagonas, spherical, monodispersed and uniformly distributed. Capitulum release reducing agents into the solution which are responsible for the formation of silver nanoparticles. Also, the particles are not aggregate, which might be an indication to the presence of a capping agent.

TABLE 3. Morphological differences between *L. mucronata* and *L. cassiniana*.

Taxa / Character	*Launaea mucronata*	*Launaea cassiniana*
Involucre bracts	Marginal scarious	Non marginal scarious
Achenes length	Shorter than 6mm	Longer than 6mm
Achenes shape	Compressed, 5-angeled	Columnar, not angular
Pappus	Hetero-morphic	Mono-morphic

TABLE 4. General band assignments of the average FTIR spectrum of plants

O-H stretching vibrations of hydroxyl groups	N-H stretching of aliphatic and aromatic amines
H-bonded alcohols, phenols	C=C or C-N stretching of aromatic amines
N-H stretching of 1° and 2° amines and amides	C-O vibration
C-H stretching of CH2	C-Cl bending
(NH)C=O stretching	-C-O-C
NO_2	C=C stretching of alkene
C-N stretching of aliphatic amines or to alcohols or phenols	C-N stretching of aromatic and aliphatic amines
O-SI-O stretching	C–C–N asymmetric stretching vibration
C-H stretching of aldehyde	C-N,C=N stretching
C-N stretching of primary and secondary amines	C-Cl stretching
NH2 Wagging of amino	C-N stretching of amino
Lead	C-H stretching of alkane

The FTIR results adequate with the morphological characters; the species of the first group are characterized by wrinkled achenes and truncate base, the second are characterized by papillose achenes and horned base and the third groups are characterized by papillose achenes and tubular base. Nevertheless, the FTIR analysis showed that the two subspecies of *L. fragilis* (subsp. *fragilis* and subsp. *tenuiloba*) is apparently different in their components, this adequate with the treatment of Täckholm (1974) as two different taxa. The summary of the most general band assignments observed in the studied taxa are presented in Table 4 and Appendix 2, the FTIR spectra also are showed in Fig. 3 and 4.

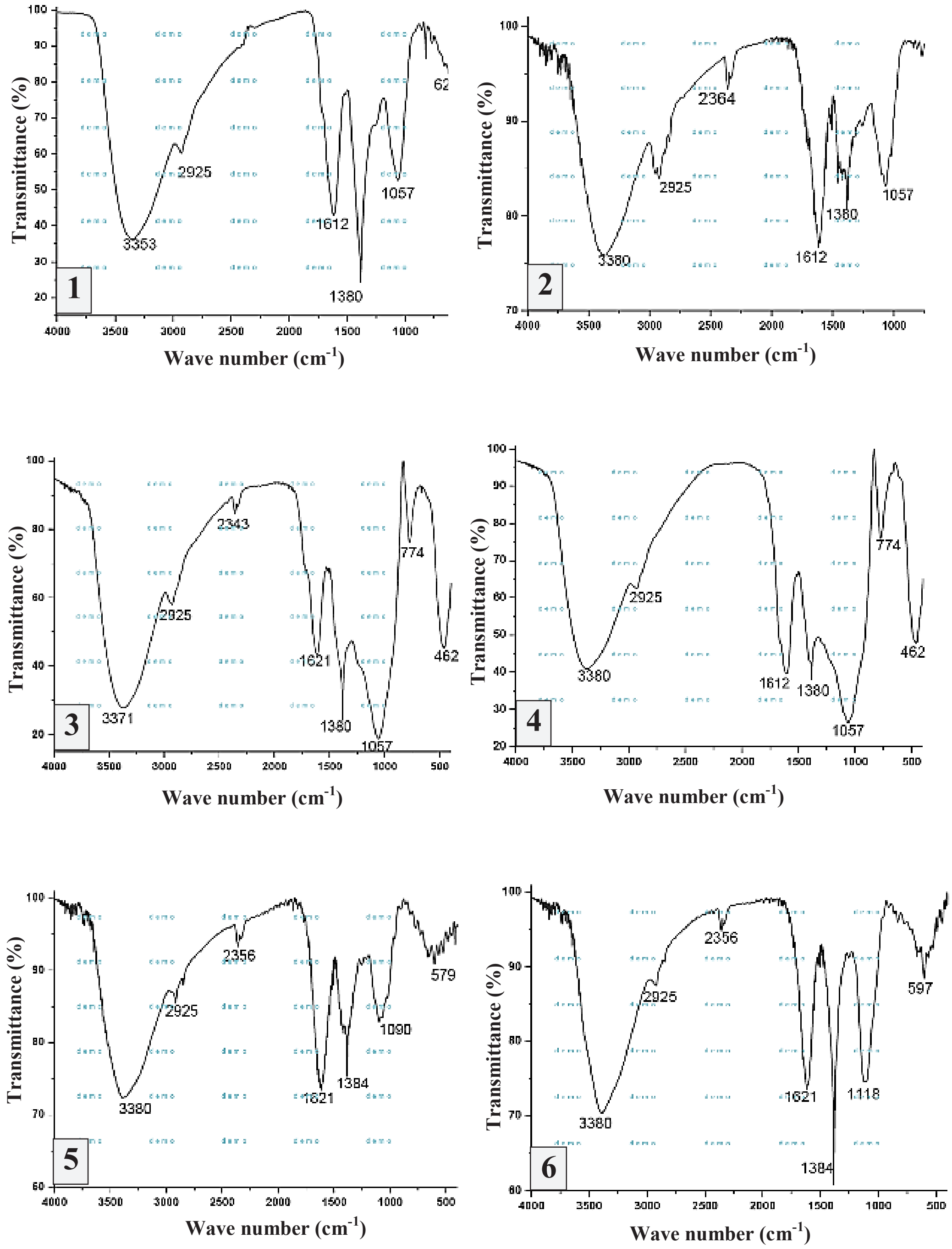

Fig. 3. FTIR spectra of the *Launaea* taxa. 1, *L. nudicaulis*; 2, *L. intybacea*; 3, *L. massauensis*; 4, *L. capitate*; 5, *L. angustifolia* subsp. angustifolia and 6, *L. angustifolia* subsp. arabica.

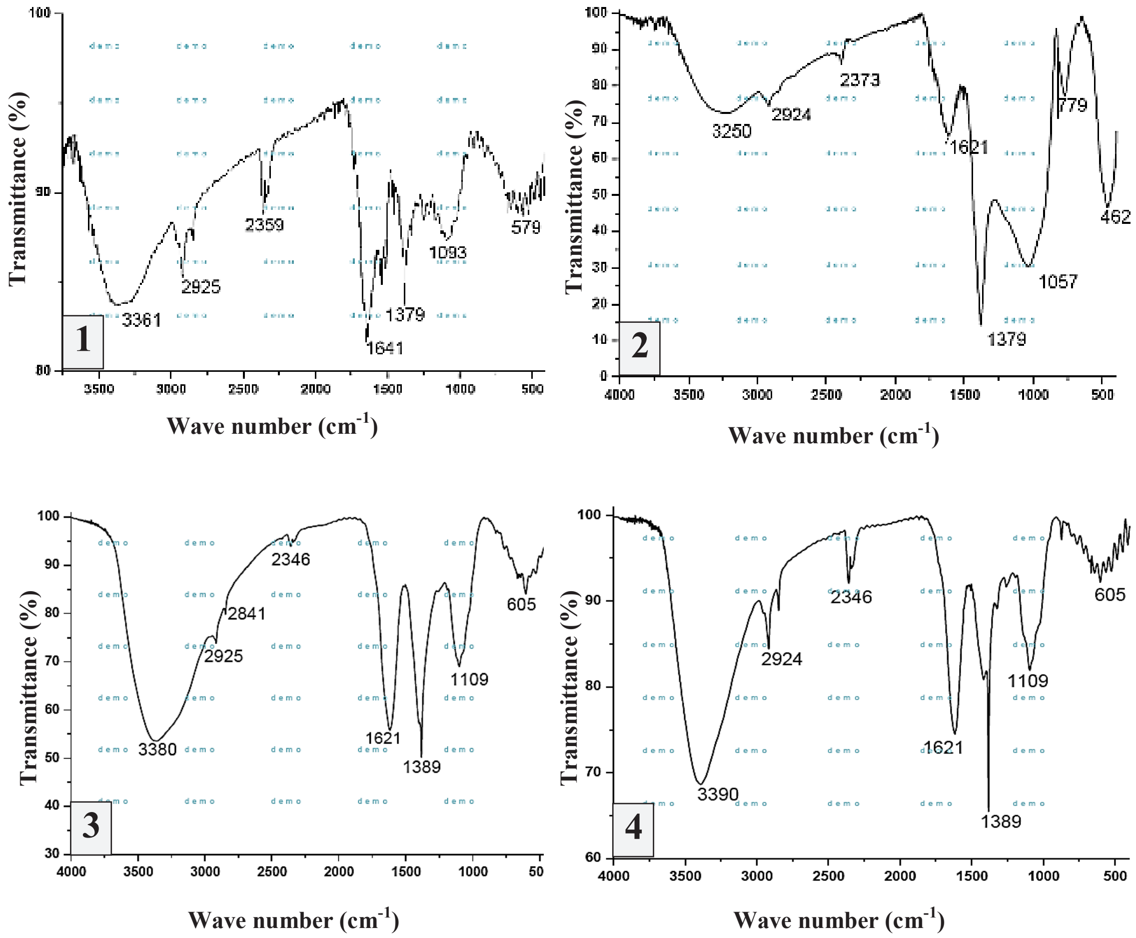

Fig. 4. FTIR spectra of the *Launaea* taxa. 1, *L. fragilis* subsp. fragilis; 2, *L. fragilis* subsp. tenuiloba; 3, *L. mucronate* and 4, *L. cassiniana*.

Conclusion

Morphological and FTIR spectroscopic studies was applied on ten closely related taxa of the genus *Launaea* belonging to family Asteraceae. The functional groups of the active components were identified based on the peak value in Infrared radiation region for each taxon. The results show that the FTIR spectroscopic technique was found to be an accurate and a rapid method to differentiate between closely and confused related taxa and have provided a valid source of taxonomic evidence for addressing the relationships at the species taxonomic levels.

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التخليق الأمن لجسيمات الفضة النانوجزيئية من مستخلص نورات بعض أنواع اللاونيا (الفصيلة المركبة) مع ملاحظات حول دلائلها التصنيفية

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استخدمت النتائج في هذه الدراسة من اجل إعادة تقييم العلاقات بين بعض أنواع جنس اللاونيا التابع للفصيلة المركبة. تم تخليق جسيمات الفضة النانوجزيئية الصديقة للبيئة من مستخلص نورات عدد عشرة وحدات تصنيفية ينتمون لثماني أنواع وتحت نوعين من جنس اللاونيا. تضمن البحث دراسة التنوع التصنيفي للوحدات التصنيفية محل الدراسة باستخدام الصفات المورفولوجية المرتبطة بالأعضاء الخضرية والتكاثرية بالإضافة إلى الدراسات الطيفية باستخدام تقنية FTIR. تم استخدام برنامج NTSYS-pc لتقييم التحليل الإحصائي لجميع النتائج التي تم الحصول عليها. واظهرت النتائج امكانية استخدام تقنية النانوتوكنولوجي كطريقة للتفرقة بين بعض الوحدات والأنواع المتداخلة تصنيفيا، وكمصدر لصفات تصنيفية جديدة يمكن من خلالها معرفة العلاقات بين الوحدات التصنيفية محل الدراسة.