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Systematic study of the genus *Nasturtium* R.Br (Brassicaceae) in Iraq

Sura Saleem Albermani, Abdulkarim Albermani, Huda Jasim Altameme*
Department of Biology, College of Science for Women, Babylon University, Iraq
***Corresponding author: E-Mail: huda_jasim@yahoo.com**

ABSTRACT

The current research deals with taxonomic study of the genus *Nasturtium* (Brassicaceae). The study included morphological, anatomical and palynological characteristics and found that these taxonomic traits have importance value to isolate the genus from the others genera in Brassicaceae family. Additionally the chemical compounds from leaves by using Chromatography Gas -Mass Spectrometry was studied.

KEY WORDS: *Nasturtium*, Morphology, Anatomy, pollen grain, seeds, GC-MS, Brassicaceae.

1. INTRODUCTION

Nasturatium R.Br is a genus of Brassicaceae (Syn: Cruciferae or Mustard family) and belongs to Tribe: Arabideae (Koch, 2001; Franzke, 2011). Al-Shehbaz was pointed in 1988 that this genus named firstly by Brow in 1812. There are multiple opinions about the location of *Nasturatium* which has raised controversy and debate about the survival of an independent as a genus or incorporated into the genus *Roripa*, Some researchers favor the survival of *Nasturitum* separately as a genus like (Czerepanov, 1995; Mabberley, 1997) and others from the separation *Nasturtium* about genus *Roripa* on the basis of sequence Chloroplast DNA (*rbc*L) and explained that *Nasturitum* unrelated document *Cardamine* L. likes of Les (1994) and some of them have a different opinion on that integrates this genus with *Roripa* (Jonsell, 1988; Rich, 1991; Rollins, 1993).

There are five species in this genus like *N.officinale* R.B, *N.microphyllum* Boenn.ex Reich, *N.gambellii*, O.E.Schulz, *N.africanum* Braun-Blanq and *N.floridanum* (AL-Shehbaz and Rollins, 1988). In Iraq, there is only one species, a *Nasturtium officinale* (Ridda, and Daood, 1982; Al-Shehbaz and Rollins, 1988) although AL-Rawi (1964), reported the presence of two species, the first was described previously under named *Nasturtium officinale* and the second is *N.amphibium* (L.) R.Br. Synonym of *Roripa amphilia* (L.) Bess. and it spreads within the Sulaimaniya province. The name of *Nasturtium officinale* derives from the Latin language 'nasus tortus' which mean twisted nose relative to the form of fruits. As the common names are Water cress (Goncalvesa, 2009; Bettega, 2016), watercress, KUZELE, KUZAKUZ, KOBANI, HURF AL-MA'I and RASHAD AL-MA'I (Hedge and Lamond, 1980). Sheridan (2001), stated that synonyms names for this genus are *Rorippa nasturtium-aquaticum* (L.) Hayek; *Nasturtium nasturtium-aquaticum* (L.) H. Karst and *Sisybrium nasturtium-aquaticum* L.

Often *Nasturtium officinale* was diagnosed mistake with the Western bittercress (*Cardamine occidentalis*), which grows in moist soil instead of water as it has a longer bloom stalk, a larger and wavy end leaflet, and the largest fruits (WDOE, 2009). *Nasturtium officinale* can also be confused with *Amoracia lacustris* and *Ropipa* species including *Nasturtium microphyllum* which has just a row of seeds in the pods (WIDNR, 2009).

Nasturtium officinale is native to Western Asia, India, Europe, and Africa (WIDNR, 2009), however it is now distributed almost globally. It is considered as being introduced to North and South America, Southern Africa, Australia, and New Zealand (Howard and Lyon, 1952). It is found in shallow, cold, gently moving, fresh water in lakes, reservoirs, streams, rivers, and on damp soil (WIDNR, 2009). It is often found in gently flowing streams, or areas of running water adjacent to springs and riverbanks or on wet soil (Cruz, 2008). This is a plant of suitable plants to eat it is used in salads and cooking due to having hot and sour taste.

Another side *Nasturtium officinale* it contains mustard oil has many medical uses, including anti-bacterial (Bahramikia and Yazdanparast, 2008), and has a protective efficacy against Oral Anti-cancer which is the second disease in terms of deployment of the causes of smoking, and is used in the treatment of many cases diuretic, anemia, eczema, kidney, liver, tuberculosis, boils and warts and tumors, diabetes, bronchitis and scurvy (Halberstein, 2005; Ozen, 2009) and an anti-inflammatory (Molaei, 2014).

And in view of the lack of any morphological or anatomical or chemical information for the unique species in Iraq so it was a goal of the research is an attempt to give a description of this genus in order to distinguish it from the rest of the brassicaceae genera.

2. MATERIALS AND METHODS

Morphological study: The material of the current study are relied on mainly dried specimens were kept in Babylon, Baghdad, Basra and Salahaddin university herbarium. Morphological characters for all plant part were studied in the laboratory under dissecting microscope (Meiji). The dimensions of vegetative parts of the species under study (leaves, petiole, stems, roots and inflorescence, Calyx and corolla) and parts of the reproductive (stamens and pistils) were measured and put it in the tables and documented by some photographs. All information including habitat, identification, localities flowering period were obtain from the label of the specimens and based on the terms set out in (Lawrence, 1951; Radford, 1974).

Anatomical study:

Epidermal preparation: Depending on the method by boiling a dry samples for a period of 1-3min to recover the freshness. The epidermal was collected on the middle part of the lamina was made by peeling and Stripping off method were used to prepare adaxial and abaxial surfaces view of leaf epidermis by using Forceps and Needle, then transferred the epidermis into clean slide contain safranin (1%) prepared in ethyl alcohol (70%) for a period of 2-5 minutes and then wash the epidermis in ethyl alcohol (70%) and a few times to diminish of excess dye then placed under a drop of glycerin and covered with a cover slide and kept in the refrigerator until the examination. After that species samples were examined by a compound microscope and measurements of stomata and epidermal cells using the ocular micrometer and photographed with the Camera installed on the microscope.

Clearing leaves: Followed the method mentioned by Al-Mayah (1983) to study the nature of venation in leaves by placing in a Petri dish containing sodium hydroxide 2-5%, with replace the solution from time to time, then leaves washed with running water several times then added to drops of safranin and left for a period of time until the pigmentation cells sympathetically, then washed with water to get rid of the amount of the excess dye, leaves brushed on the slide glass containing drops of glycerin and put them slide cover to get ready for examination and imaging on the microscope and depending on the terms found in (Hickey, 1973).

Preparation of transverse sections: Leaf, stem, petiole and Peduncle transverse section were prepared by hand cutting according to (Al-Tameme, 2016). The species samples were fixed in alcohol-glycerin (60:40), then Foliar cross sections were prepared from the central leaf. Transverse sections were stained by Safranin. The observations were carried out by light microscope and photographed with camera and recorded measurements profiled by using the ocular micrometer.

Indumentum: Different shapes of trichome in the vegetative and reproductive parts are taken, then it was studied and recorded the quality of trichome and the number of cells per hair and its surface then filmed some of the hairs by the camera installed on the compound microscope.

Palynological Study: According to the method of AL-Dobaissi (2008) Flowering buds of dry samples were collected and boiled for a period ranging between 2-3min and placed on a glass slide under dissecting Microscope type, Meji then removed anther from the rest of the floral parts, added the drops of dye safranin – glycerin to it, then open anther to remove pollen by needle then put the lid gently slide to be examined and photographed the optical microscope compound under oil Immersion lens.

Chemical study: According to Jasim (2015) Methanol extract of *Nasturtium officinale* leaves was analyzed with the help of GC-MS analyzer (Perkin Elmer Gas Chromatography-Mass Spectrum). On Elite-1 column the date was generated. Carrier gas Helium was at a continual flow of 1 ml /min at 280ºC in split mode (10:1). 0.1µ of extract was injected to column at 350^0C injector temperature. The mass Spectrum of compounds present in samples was obtained by electron ionization at 70eV and detector operates in scan mode 45 to 450Da atomic units. A 0.5 seconds of scan interval and fragments from 45 to 450Da was maintained. Total running was 36 minutes. Identification of bioactive component was based on the molecular structure, molecular mass and calculated fragments. Interpretation on mass spectrum GC MS was conducted using the database of National Institute Standard and Technology (NIST).

3. RESULTS AND DISCUSSION

Morphological Descriptions: Perennial Herbs grow in an environment of aquatic or semi-aquatic, height ranges between 13-36 cm at a rate of 22.4 cm. Root system found as Tap root and adventitious root varies between 10 to 16.3cm in length and 0.2-0.5cm in diameter. Stem glabrous, polygonal, hollow, procumbent below then ascending at the end inflorescence 5-(17.6)-41cm in length and 0.3-(0.4)-0.5cm in diameter. Leaves alternate, odd pinnate, petiolate, terminal leaflet larger than lateral, the edges of the leaflets shallow crenate to entire, leaflets ovate opposite or nearly opposite , sessile, The base of leaflet oblique, Apex the leaflet acute, blade length ranges 0.6-(3.62)-6cm, blade width 1-(2.4)-4.4cm. Petiole winged 0.2-(2.8)-5.5cm. Inflorescences short, flowers is terminal raceme in final bunches, pedicel unbranched 0.15×3.7 mm. Calyx green, glabrous, spathulate, dimensions 1×4.2mm. Petals white, rounded at the tip dimensions of 1.8×4.7mm while the dimensions in the claw 1×1.3 mm. Androecium tetradynamus (4 long outside and 2 short inside), filaments purple 1.5×1.6 mm, another yellow, 0.34×0.7, ratio of the length of anther to width is 0.51. Ovary cylindrical elongated tapering terete Purple greenish, Style short or missing, 0.2-0.4mm, the stigma is lobes ovarian 0.32×1.62 mm. Fruits white siliqua may be straight or curved, the dimensions of fruits 0.3×1.34 cm, Fruiting pedicels may be ascending or curved deflexed, 0.54 cm in length. Seeds dark brown biseriate with polygonal depressions on each testa surface, 0.8×1.2mm, ratio length/width is 1.5 (Figure 1).

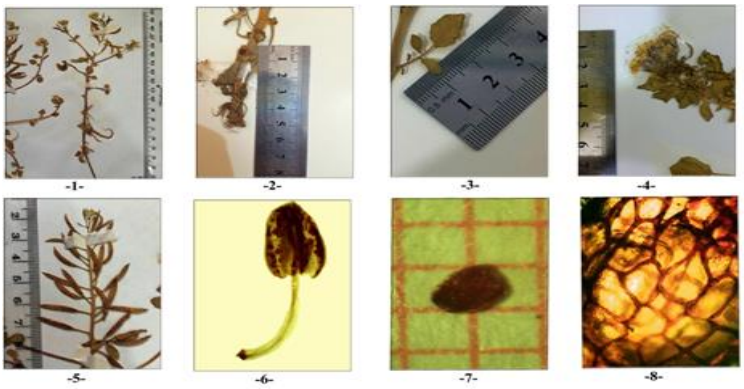


Figure.1. Morphological characters in *Nasturtium officinale*.(1-Whole plant; 2- Root; 3- leaf; 4-inflorescence system; 5-fruits ; 6-stamen; 7- seed; 8- Ornamentation in seed surface)

Nasturtium officinale a glabrous species or has a small hairs, unbranched unicellular, smooth sharp apex. All the results of morphological study agreement with previous studies like (Post, 1933; Jafari and Hassand, 2012) but disagreement with Abdel Khalik (2005) when he pointed that the species under study was glabrous while in this study demonstrated a little unicellular unbranched hairs in surface of petiole, this result was similarity with (Cruz, 2008; Franzke, 2011). Also the study Cumbus and Robinson (1977) confirmed that species *Nasturtium officinale* has two types of roots depending on environmental conditions. When there is a large amount of phosphorus in the water, the roots are adventurous, while the lack of phosphorus in the water, the roots are mainly Tap root. Formation of roots in watercress helped it to widely spread in addition to the direct growth of the seed.

Anatomical Descriptions: The present results reveal that the anticlinal walls of epidermal cells in Cauline leaves a little difference between the adaxial and abaxial surfaces which had cells with undulate sinuous walls and ornamented cuticle. The length of epidermal cells in the adaxial surface of leaves with average 55.2 × 88.5µm and in the abaxial surface with average 53× 72.5µm.

Stomata are rounded or elliptic shaped present on either sides (Amphistomatic leaves) Measurements of stomata are approximately (21 ×27)µm in the adaxial surface of leaves and (20 ×24)µm in abaxial surface of leaves. Guard cells are kidney shaped and Anisocytic type that the stomatal apparatus surrounded by three different cell in size also found Anomocytic type which lack the subsidiary cells. Metcalfe and Chalk (1950) supported this truth when they pointed that stomata in Cruciferae are usually cruciferous. These result confirmed the characters which have proven to be of systematic value are: cuticular characters, epidermis, stomata, and trichomes.

The venation pattern in *Nasturtium officinale* was pinnate, brochidodromous Areolation shape is Polygonal, Complete imperfect or perfect, simple or branched veins according to (Hickey, 1973).

Anatomical characters in transverse sections of stems are represented in Outline as the polygonal and hollow afloat or creepy in shallow marshes. Epidermis biserriate of circular compact cells, (10.0-20.0)µm in thickness. Followed by the cortex consists of 5-9 rows from parenchyma tissue, (50.0-80.0)µm in thickness. Vascular tissue represented by many vascular bundles arranged regularly in the form of broken ring. The vascular bundles are conjoint, collateral, endarch, open, wedge shaped, phloem thickness ranged 0.1-0.3µm and vessels of xylem thickness ranged from 0.2-.0.5µm. Metcalfe and Chalk (1950) indicated to present then endodermis in stem cross section but in study can't differentiated it.

Also, the result reveal a cross section of the inflorescence pedicle was polygonal and has vascular bundles cylindrical in shape as a continuous pericycle in stem. But the petiole appeared in the semicircular Winged through a cross section included the number of package between 9-11 vascular bundle.

Transverse section of the leaf discloses an upper and lower epidermis, a concave of mesophyll and the veins vascular bundles embedded in it. The epidermal cells are rectangular–elongated shaped, compactly arranged in one-layered upper and lower epidermis have a range thickness (20.0-30.0)µm. Mesophyll was composed by 30-50 row of circular-ovate shape and 150-300µm in thickness. Palisade cells which were smaller at the inner layer 50-120µm in thickness. The spongy parenchyma had layers of cells with various shapes loosely arranged 80-100µm thickness. In the mesophyll, the veins vascular system is embedded and represented by vascular bundles similar with those described in the stem, number of bundle approximately 3-6 bundles. However in vascular bundles, xylem is near upper and phloem is near lower surface. The thickness of xylem a ranged between 190-200µm. The central vascular bundle has a broad circular form range between 120-180µm in diameter, the number of vessels in the vascular rows 3-5 row, the number of vessels in each row between 3-5 vessels. The vessel diameter of between 30-60µm. Results were not different to what Ratikanta (2012) brought.

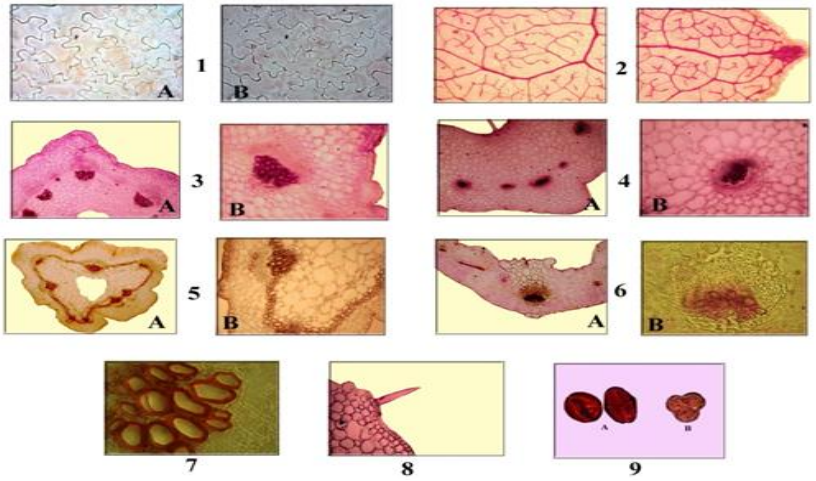
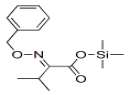
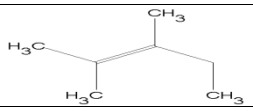
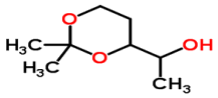
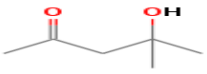
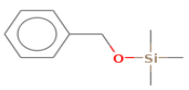
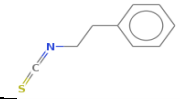
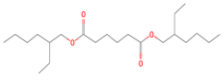


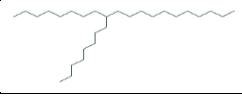
Figure.2. variations in the anatomical characters in *Nasturtium officinale*

1-A-Lower epidermis, B- upper epidermis 40x; 2-Venation 40x; 3- A, B-C.S. of Stem in 10x and 40x; 4-A, B C.S. of Petiole in 10xand 40x; 5- A, B- C.S. of pedicle in 10xand 40x; 6- A,B- C.S. of leaf in 10xand 40x; 7- vessels in vascular bundle 40x; 8- simple hairs; 9- pollen grain in 100x.

Palynological Descriptions: The result showed that, the pollen grains are tricolporate, spineless, length of polar axis is (10.0-16.0) µm , and the length of equatorial axis is (10.0-17.0)µm, prolate-spheroidal (P/E = 1.1) and usually categorized in small grains according to (Erdtman, 1971). The grains in *Nasturtium* was spheroidal or prolate shaped in equatorial outline and triangular-obtuse in polar outline. The pollen has distinct equatorial depressions between the apertures and without distinct lacunae. The exine thickness is 1-2µm. Thus, the result in this study agreed with Conaway (1996) but disagreed with the study Perveen (2004), that described pollen grains at generally as Sub-polate, and others (Lahham, 1987) described as Sub-spheroidal.

Table.1. GC-MS analysis of eleven major phytocomponents identified in the methanolic leaves extract *Nasturtium officinale*

RT	Area%	Biochemical component name	C.F.	Molecular weight	Chemical structure
2.426	27.48	Butanoic acid,2-[(phenylmethoxy)imino], trimethylsilyl ester	$C_{14}H_{21}NO_3Si$	279.40	
3.503	8.05	2-Pentene,2,3-dimethyl-	C_7H_{17}	98.18	
4.136	0.81	1-(2,2-Dimethyl[1,3]dioxin-4-yl)ethanol	$C_8H_{16}O$	128.21	
4.175	56.46	2-Pentanone,4-hydroxy-4-methyl-	$C_6H_{12}O_2$	116.15	
4.898	1.92	Silane, trimethyl (phenylmethoxy)-	$C_{10}H_{16}OSi$	180.31	
17.723	1.00	Cetene	$C_{16}H_{32}$	224.42	
16.148	1.35	Benzene,(2-isothiocyanatoethyl)-	C_9H_9NS	193.23	
20.51	0.60	E-15-Heptadecenal	$C_{17}H_{32}O$	252.43	
22.244	1.22	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	370.56	
27.579	0.57	Hexanedioic acid,bis (2-ethylhexyl)ester	$C_{22}H_{42}O_4$	294.76	

32.507	0.55	Eicosane, 9-octyl-	$C_{28}H_{58}$	394.76	

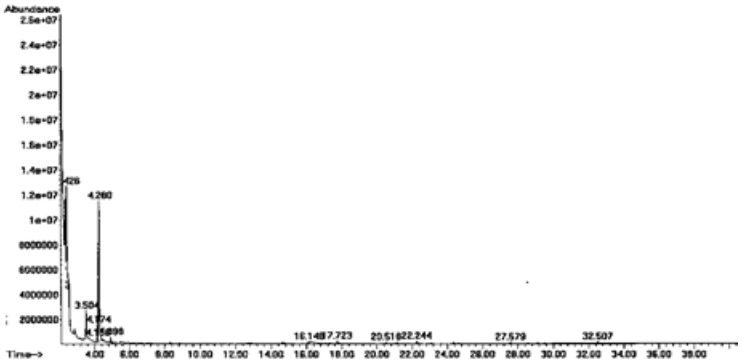


Figure.3. GC-MS Chromatogram of methanolic leaf extract of *Nasturtium officinale*

Chemical Description: GC-MS chromatogram of the methanolic leaf extract of *Nasturtium officinale* (Figure.3) displayed 11 peaks indicating the presence of eleven compounds. The chemical compounds identified in the methanolic extract of leaf presented in Table.1. GC-MS analysis discovered that the presence of The 11 compounds mainly composed of 27.48% of Butanoic acid, 2 - [(phenylmethoxy) imino], trimethylsilyl ester ; 8.05% of 2-Pentene, 2,3, -dimethyl-; 0.81% from 1 - (2,2-Dimethyl [1,3] dioxin-4-yl) ethanol; 56.46% of 2-Pentanone, 4-hydroxy-4-methyl-; 1.92% of Silane, trimethyl (phenylmethoxy) - 1.00% from Cetene; 1.35% of Benzene, (2-isothiocyanatoethyl)-; of 0.60% of the E-15-Heptadecenal and 1.22% of Hexadecanoic acid, methyl ester; 0.57% of Hexanedioic acid, bis (2-ethylhexyl) ester and% 0.55 Eicosane, 9-octyl. Wallig (1998); Bianchet (1999); and Mahjoub (2009) emphasized the watercress was a richer a glcosinate, and Alam (2015) pointed it contained a high percentage of phenolic compounds by using HPLC technique.

4. CONCLUSION

As a conclusion from the above observation, it can be concluded that combination of morphological, anatomical, Palynological and chemical study to play a significant role for delimitation and isolation of taxa.

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