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RESEARCH ARTICLE

Determination of some chemical compounds in *Citrus* species in Iraq*Aseel K. AL-anbari^{1,3}, Talib. A.alkhesraji², Athia al-Mashhadani³, HazimSaadoon Aljewari³.

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Manuscript Info**Abstract****Manuscript History:**

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We indicated the concentration of eight compounds for each volatile oils (EOs) (Limonene, 2,6-Octadien-1-ol, Citral, Methoprene, Geranylmethylether, Mehap, Octenal, Erucylamide) and phenols (Benzoic acid, Cinamic acid, Sinapic acid, acid Coumaric, Furanocoumarin, Naringin, Inetin, Caffeic acid) by extracted and determinates in 16 *Citrus* species from Iraq. the results have implications for the further elucidation of biochemistry methods and also define the species that more useful to extraction specific chemical compound in the future work.

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Introduction

Citrus as importance medicinal plants and implicated in medical treatments because of their high content of active ingredients especially essential oils, phenols (Blumenthal, 2002). Volatile oil that called the essential oils. EOs from citrus plant contain a high percentage of monoterpene hydrocarbons (70-95%) along with smaller amounts of sesquiterpeneoxygenated derivatives and aromatic hydrocarbons (Mohamed *et al.*, 2010, Siddique *et al.*, 2011).

Citrus oil mainly consists of Limonene, α -pinene, β -pinene, β -myrcene and linalool. These oils and antibacterial properties, fungi and viruses, as well as antioxidant properties in cells. It has been found (Blumenthal, 2002) that oils extracted from lemon, have the effectiveness of a powerful anti-oxidant to own mechanics enzymatic and effectively to remove free radicals.

phenolic components and more secondary metabolic compounds that are widespread in the plant kingdom, which normally present in the leaves.

They include a large number of multiple vehicles basis phenol C₆H₅OH and the majority of complex installation contains many of the episodes aromatic Aromatic ring bearing one or more of the site to the occupation of hydroxyl (Al-Sahar, 1992) and that the phenolic compounds tend to melt in water and often are found associated with the sugar in Glycosides that

each plant sophisticated contains a special form or distinctive of phenolic compounds so.

The natural phenols as flavonoids as antioxidants potential effect to the cells by stopping DNA doubled in the stage of S-phase. The aim of this study is define the quantitative chemical compounds in the *Citrus* species that useful for future studies to easier choosing the species that content high concentration of determinate chemical compounds.

Material and Methods

Plant materials:

Citrus leaves of adult trees were collected from different cities in Iraq (table 1).

Samples were divided two types (1) fresh to study EOs, (2) dry to study phenols

Table 1.list if specimens included in the study

Species	Locality	Collector numbers
<i>C. auarantifoliavar.acidica</i> (Christm.)Swingle	Salah aldeen	A. Al anbari 10
<i>C. aurantium</i> L.	Diyala	A. Alanbari 637
<i>C. deliciosa</i> Ten.	Babel	A. Alanbari 331
<i>C. grandis</i> Osbeck	Baghdad	A. Alanbari 21
<i>C. japonica</i> Thunb.	Salah aldeen	A. Alanbari 13
<i>C.latifolia</i> Tanaka	Babel	A. Alanbari 261
<i>C. limetta</i> Risso	Baghdad	A. Al anbari 26
<i>C. limon</i> (L.) Burm.f.	Karbala	A. Alanbari 528
<i>C. medica</i> L.	Diyala	A. Alanbari 12
<i>C. paradise</i> Macfad	Baghdad	A. Alanbari 31
<i>C.reszni</i> Hort. ex Tanaka	Diyala	A. Alanbari 20
<i>C. reticulate</i> var. <i>clementine</i> Blanco	Karbala	A. Alanbari 438
<i>C. sinensis</i> Osbeck	Baghdad	A. Alanbari 51
<i>C. aurantium</i> L. x <i>C. trifoliata</i> (L.) Raf.	Diyala	A. Alanbari 98
<i>C. sinensis</i> var. <i>moro</i> Osbeck	Diyala	A. Alanbari 95
<i>C.volkameriana</i> Pasq.	Babel	A. Alanbari 120

EOs extraction and analysis

Method of extracting oil (fig 1) distillation manner as mentioned(Rovio, 1999)(in terms of soft Leaves passed samples Btoren (the mobile phase and solid phase oil was purified through filter leaf was chapter and diagnosis in a High Performance Liquid Chromatography - HPLC was injected samples using a column of species

Column: (3μ m(

Partical size: (50 x 2 mm I.D(

Solid phase: Silica column (Hexane: Methylene colride 50:50 v \ v(

Mobile phase: content of

Solvent A-15% formic acid in acetonitrile

Solvent B-45% formic acid in acetonitrile

Starting from 15% 45% A B

Deionized water 80:20

Detection UV set at 254 nm.

Temperature: 25 C °

Flow rate: 1 ml / min

Sample Injection volum: 20μl

Modal: FLC-Shimadzu 10AV-LC

Pump modal: LC-10A Shimadzu

Monitored: VV-VIS 10A-SPD

Was calculated concentration of the oil in the sample according to the following equation:

$$\text{Conc. of Sample } \mu\text{g} / \text{mg} = (\text{Area of sample}) / (\text{Area of standard}) \times \text{conc.of standard} \times \text{dilution factor}$$

Phenols extraction and analysis

phenolic compounds in Citrus genus (fig 2)developing in Iraq for extraction of phenolic compounds from parts of the vegetative and the results were in terms of separation and diagnosis manner HPLC High performance Liquid Chromatography. Method is used (Haborne, 1973) as stated in the (Al- Jibouri, 2010) with some modification, Dried leaves of each species studied and grindthe electric

Filtrate were separated by a central chapter quickly and 7500 r / min for 15 minutes.

Method is used the HPLC e analysis (to isolate compounds and diagnosis.(

Column was composed of

Column: Zorbaxclips XDB-C-18, 3M m

particle size (50 x 4.6 mm I.D(

Mobile phase: water: methanol: acetonitrile (50:40:10)
gradient program from 0% B to 100% B for 8 minutes
Detection UV set at 280 nm.

Temperature: ambient

Flow rate: 1 ml / mi

Sample Injection volum: 20µl

Was calculated concentration of phenolic material in the sample according to the following equation:

$$\text{Conc. of Sample Mg / m} = (\text{Area of sample}) / (\text{Area of standard}) \times \text{conc. of standard} \times \text{dilution factor}$$

Results and discussion:

Essential oils

EOs have shown compounds oils and their presence in a wide range of mattresses genus under study may be due to the existence of rules and genetic fixed materials Demonstrated genetic basis for these oils and proved, the chemical has been possible to diagnose and season (Fadiet *al*, 2012) Vehicles belonging to groups oils are different and these oils are:

Limonene The presence of this compound has extensive basic species of genus has recorded the highest value in species *C.latifoliata* that can used in the lower of Cholesterol level, also 2,6-Octsdien-1-ol, Geranylmethylether, and Erucylamide are common oils in the plant spread mattresses genus under study has presence in all species of genus, while Octenal founded in most species except *C.aurantifoliavar.acidica*, but Citral not exist in the *C. aurantium*, *C.volkameriana* as well as Mehap no see in the *C.volkameriana*, *C.limon*, while the Methoprene founded in two species are *C.aurantifoliavar.acidica*, *C.grandis*. Therefore we can extraction the methoprene from leaves to inhibitor for musca Larva as Mohammed and Shefik (2009) reported. We registered vehicles Limonene ,2,6-Octsdien-1-ol, Geranylmethylether, Erucylamide presence "in all mattresses genus under study which contributes to isolate these species according vehicles oily From this, Citral composite presence in the species *C. aurantium*, *C.volkameriana*. This indicates the presence of a genetic relationship of the two species, considering that species *C. volkmeriana* is a hybrid from *C. aurantium*, this results agree with Nicolsi(2000). Results were obtained as in the figures (3)(2)

Phenols

The study showed the components of phenols compounds in species of genus studied. This is the first study of its species in Iraq have included this study, diagnosis of 8 compounds belonging to groups phenolic different based on what is available from a standard vehicle standard compounds species have shown clear differences in the contents of whether the level of Statistics vehicles or complex's Home While certain substances appeared in specific species of articles appeared in other species of this material is

Benzoic acid one of a group of phenolic acids have people in all species of genus under study and record active substance benzoic acid is intended to be used in horticulture (floriculture) for the control of fungi, bacteria, viruses and viroids. It's known as inhibit the bacteria cell multiplication. The Cinamic acid one of the many spread phenolic acids in the plant has been found in all mattresses under study as well as Sinapic acid while Naringin not exist in *C.limetta*,

Furanocoumarin compound was in the most species, indicating the spread except species *C.sinensis*, *C.medica*. Inetin and Coumaric acid not found in *C.paradisialso C. limon*, *C.medica* no inetin, while *C. aurantium* and *C.limetta* no coumaric acid but Caffic acid It compounds has few of the prevalence in the plant. We don't founded in the *C.grandis*, *C. limtta*, *C. reshni* and *C.aurantium x C. trifoliata*

Table (2) standard Essential oils and phenolic compounds used in the study

seq	Essential oils stender .	seq	Phenol comp.
1	Limonene	1	Benzoic acid
2	2,6-Octsdien-1-ol	2	Cinamic acid
3	Citral	3	Sinapic acid
4	Methoprene	4	Coumaric acid
5	Geranylmethylether	5	Furanocoumarin
6	Mehap	6	Naringin
7	Octenal	7	Inetin
8	Erucylamide	8	Caffeic acid

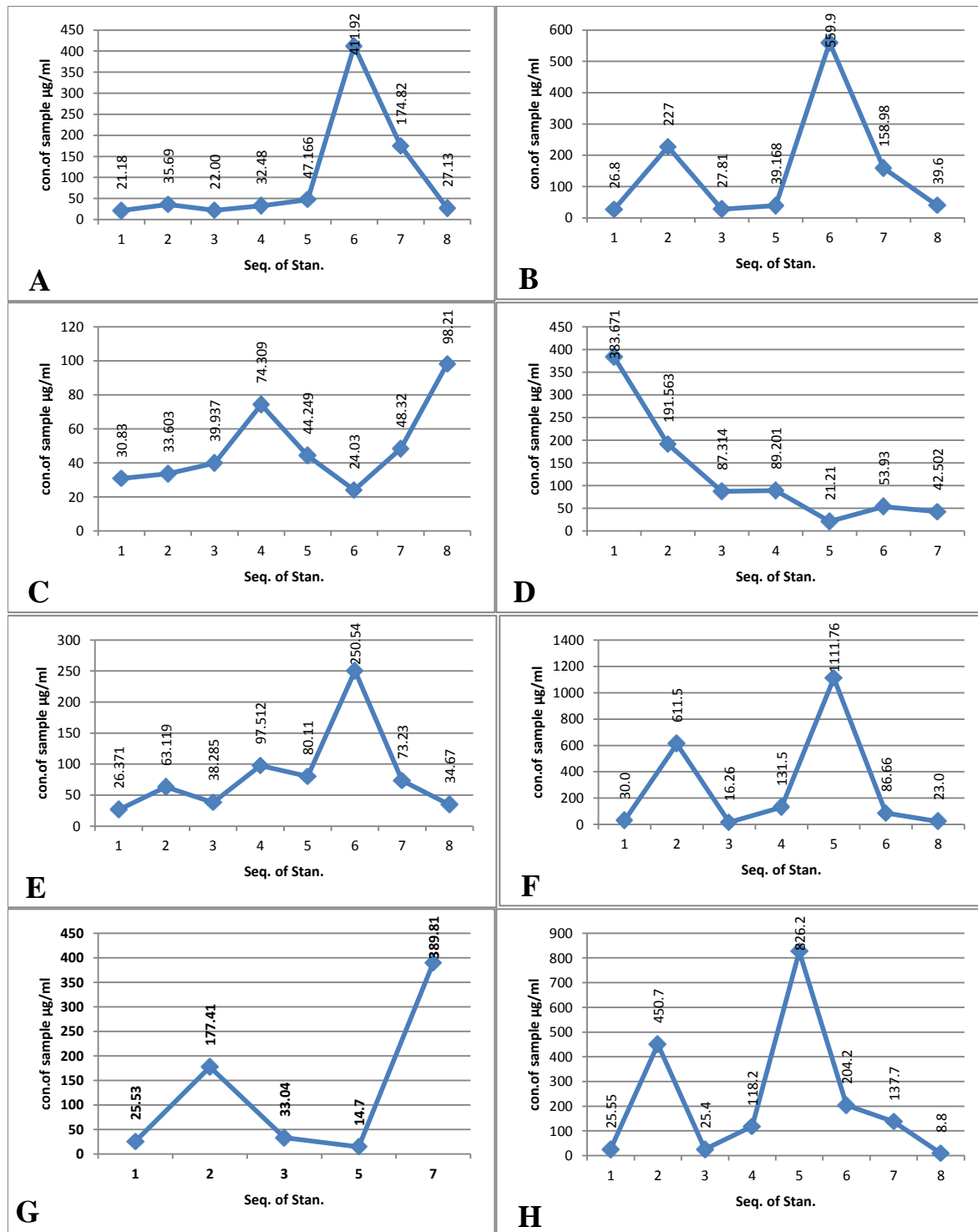


Fig 1. Essential oils in Citrus species
 A= *C. aurantifolia* var. *acidica*, B= *C. aurantium*, C= *C. deliciosa*,
 D= *C. grandis*, E= *C. japonica*, F= *C. latifolia*, G= *C. limetta*, H= *C. limon*

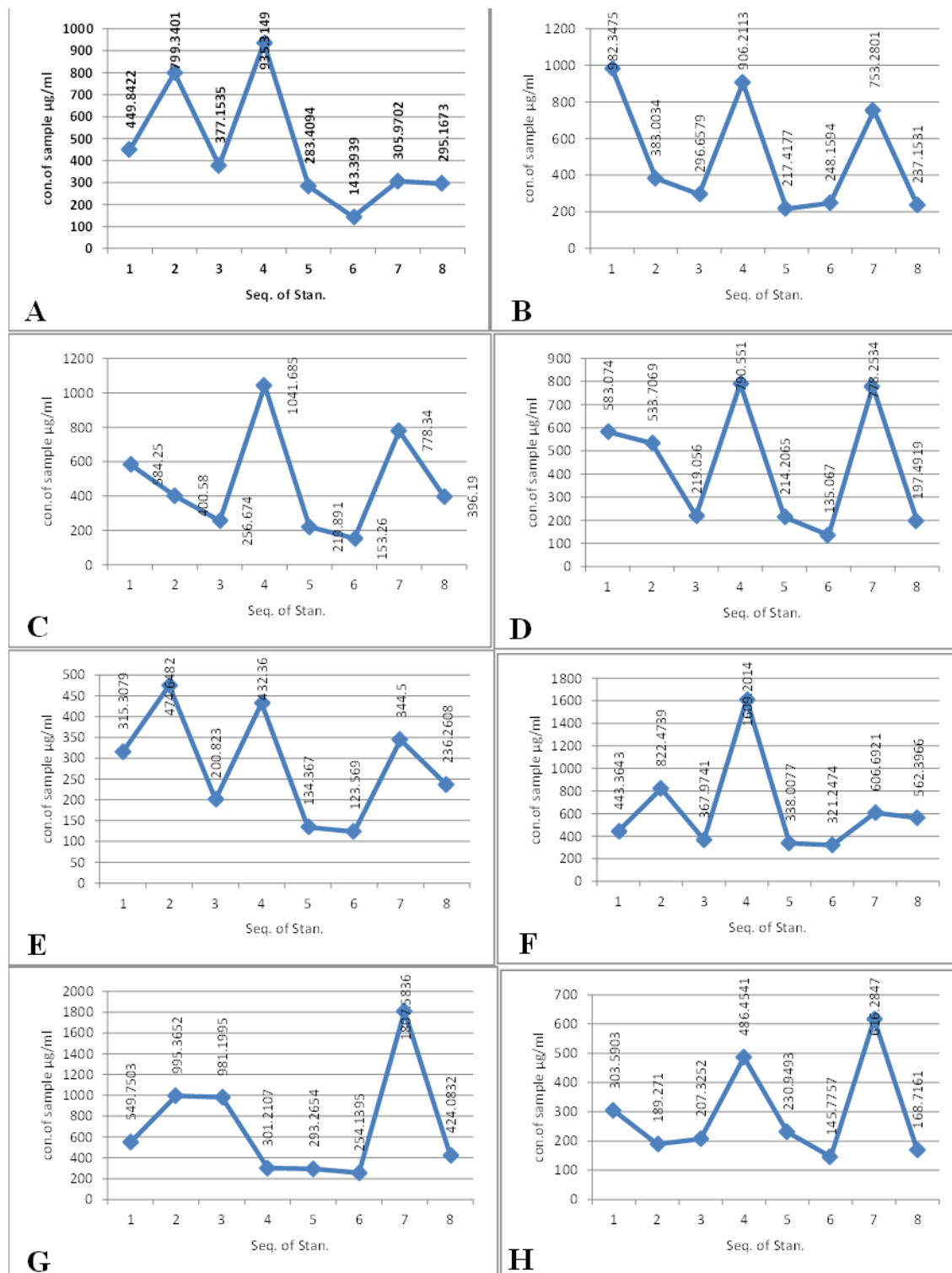


Fig 2.. Essential oils in Citrus species
 A= *C. medica*, B= *C.paradisi*, C= *C. reshni*, D= *C. reticulatavar. clementine*
 E= *C. sinensis*, F= *C.aurantium L. x C. trifoliata*, G= *C.sinensisvar.moro*, H= *C.volkamriana*

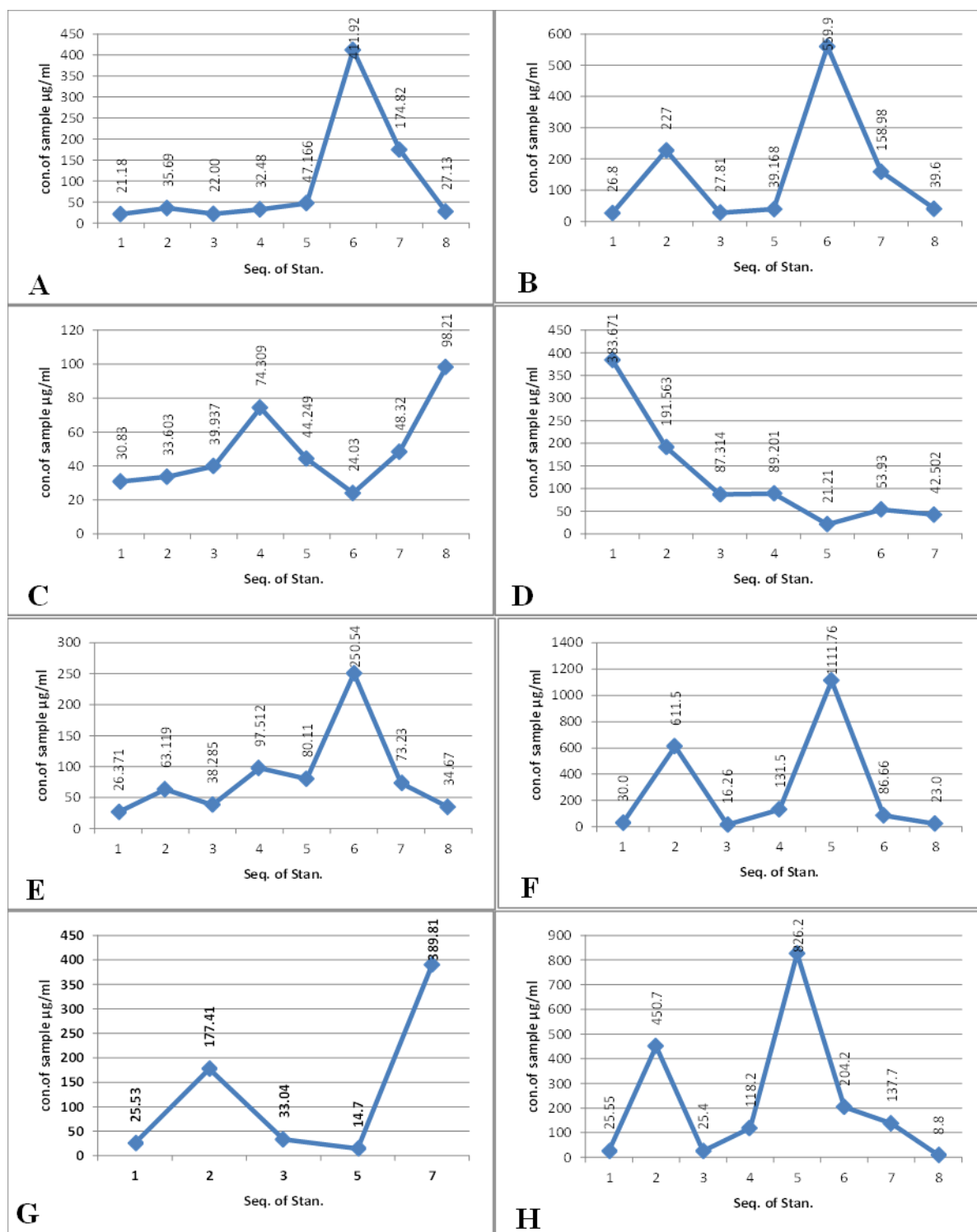


Figure 3. Phenols in the *Citrus* :A= *C. auranifolia* var. *acidica*, B= *C. aurantium*, C= *C. deliciosa*, D= *C. grandis*, E= *C. japonica*, F= *C. latifolia*, G= *C. limetta*, H= *C. limon*

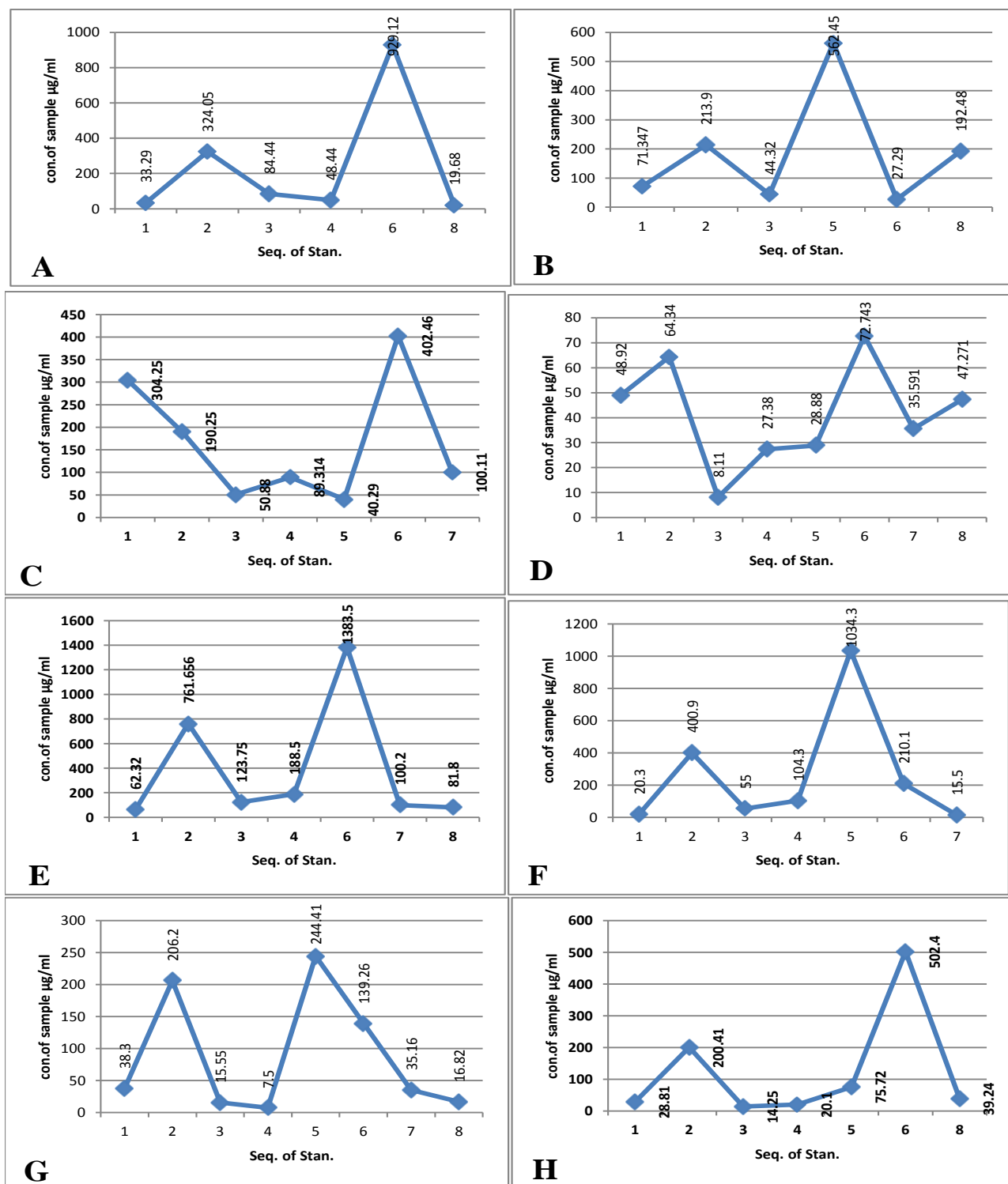


Figure 4. Phenols

A= *C. medica*, B= *C. paradisi*, C= *C. reshni*, D= *C. reticulata* var. *clementine*

E= *C. sinensis*, F= *C. aurantium* L. x *C. trifoliata*, G= *C. sinensis* var. *moro*, H= *C. volkamriana*

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