



Original Article: Investigation of Pharmaceutical and Antioxidant Effects of *Aurantium L.* in North of IRAN

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Received: 14 September 2021

Accepted: 15 April 2022

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Abstract

Phytotherapy is one of the most ancient methods of traditional treatment. Medicinal plants are very important in terms of hygienic and public health, either treatment or prevention of diseases. The genus of *Citrus* belongs to the family of Rutaceae, which grows in the regions of temperate and warm and the shoots of *Citrus* have a drastic odor. Some of them have edible fruit, the others are for treatment of essence extraction. In this research, the organs of *Citrus aurantium L.* containing blossom, leaf, and external rind of immature fruit in Mazandaran and Golestan provinces were collected and tried to investigate the specific composition and medicinal characteristics. The extracted essences of *Citrus aurantium L.* due to different compositions like varied monoterpenes and sesquiterpenes have the effects of expectorant, diuretic, decreasing blood pressure and bacterial and fungal infections, to settle inflammation, polyneuritis, gastroenteritis and the effect of antitumor against Leucémie p383, controlling effect of cell growth of extract in cancers K562, L 1210 and else. Results of this study showed that alkaloid compounds in the fruit peel of *C. Aurantium* with an average of (0.91 - 1.590) in Amol and Gorgan city, respectively, was higher than that of flowers and leaves, which was higher in Gorgan than Amol and. The most amount of total flavonoids in the orange flower of Amol city with an average of 90.92 mg and the most amount of total phenol in the orange flower of Gorgan with an average of 22.7 mg was observed.

Keywords: *Citrus aurantium L.* pharmaceutical effects, Antioxidant effects, Alkaloids

Introduction

Citrus species (Rutaceae), which are widely distributed in tropical and subtropical regions, are the most important fruits of the world (Shan, 2016). *Citrus Aurantium L.* is a medicinal plant belonging to Rutaceae that its other names are *C. Aurantium L.* subsp. *Aurantium* and *C. Aurantium* subsp. *Amara* (L.) Engler and English named Seville orange (Pellati *et al.*, 2007). Different members of the tree are used to treat many diseases as a traditional drug, for example, Asian people use dried fruit and immature fruit for food digestion, and its leaf is used as an antiviral, and also is an essential oil of rind is used for the preparation of drinking and odors (Putzbach, *et al.*, 2007).

C. Aurantium fruit is used in functional foods and medicines due to its multifarious pharmacological effects, including anti-cancer, anti-anguish, anti-diabetic, cardio-protective, anti-obesity, and antioxidant effects (Wang *et al.*, 2019).

In fact, in recent years, plants and their secondary metabolites have attracted much attention from the scientific community about their therapeutic potential (Saleem *et al.*, 2019; Juca *et al.*, 2020). *Citrus* spp. is possessing bioactivities such as antioxidants (Djenane, 2015; Suryawanshi (2011) anti-inflammatory Burt (2004), antimicrobial Salma *et al.* (2016), and is suggested to be for cancer and degenerative diseases (Suryawanshi, 2011). Those bioactivities of *Citrus* spp. are due to the presence of bioactivities of *Citrus* species due to its bioactive

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compounds such as phenolics, flavonoids, essential oil, and vitamins (Anwar, *et al.* 2016; Ejaz, *et al.*, 2006). *Citrus aurantium* is being used by different races in the world. People of China and Caraeib, islands special Haiti used it as a freshness and strengthening agent and Anti-odor (Ferguson, 2002). The bitter orange fruit (*C. Aurantium*) contains several phytochemicals of interest, including *p*-Octopamine and synephrine alkaloids (Pellati *et al.*, 2002). In Turkey, fruit rind is used as a drug for increasing appetite, antipain, disinfectant, and settling inflammation (Gurkan, 2003 Karaca *et al.*, 2007). Fruit is being used increasing appetite, and weight regulation, so fruit slice is being used to settle ophthalmia and relieve muscle pains (Ait Mohamed *et al.*, 2004). *Citrus aurantium* L. Inhibits collecting fats in the body and increases body immunization (Kubo *et al.*, 2005).

In industry, *Citrus aurantium* L. is being used for preparing soap and cosmetic instruments (Ait Mohamed *et al.*, 2004). The fragrance and odor of blossoms could effect on senses, and mental and sexual actions remarkably (Schubert, 2005). *Citrus aurantium* has a lot of vitamin C. and antioxidant compositions for prevention of skin degeneration and effective material of a dihydroxy acid for the prevention of sense (Belleli *et al.*, 2000). In traditional Chinese medicine, the aqueous extracts of *C. Aurantium* fruit were used to treat depression-like symptoms (e.g. Sutar *et al.*, 2018).

A. Quintero and *et al.* (2008) Introduced compositions follow:

1 – Monoterpenes like: lemonin % 77.90, pinen % 3.40, mircen % 1.81 and Trans – ocimen % 1.16.

2 –Sesquiterpenes like: valencen % 0.52.

3- Aldehydes like:Decanal %3.51 , Dodecanal % 0.36 , Geranial % 0.29.

4 – Alcholes like: Neralidol % 0.85 and linalol % 0.89.

5 – Nootkatone only comprises ketones.

Materials and Methods

Selecting and harvesting the plant have been done from two vegetation places in Mazandaran and Golestan provinces (Amol and Gorgan cities, respectively) for one year (2018). New traditional information about methods of collecting eatable organs of *Citrus aurantium* L. and also ways of its

consumption has been obtained from experienced people the blossoms, leaves and immature fruit of *Citrus aurantium* L. have been collected from two mentioned regions and dried under shadow and fit aeration and then have been used of their powders to extracting and identification of their alkaloid measurements.

Preparing Ethanolic Extraction in Plants

In this research, ethanol %70 and percolation were used. Therefore, 50 gr powder of plantlet was placed in the decanter, and then added ethanol 70 graduated. It is going on until wetting the total of plantlet and ethanol was taken in by plantlet and a little remains on the surface of plantlet into percolator, after one hour, the mixture into percolator was replaced and opened its valve until the solution contains extraction and ethanol goes out to drop and then closed the valve and back the solution into the percolator, this process is replicated every one hour, of course, this action prolongs during, 24-72 hours. Therefore, the powder of plantlet could take insolvent better for solving the maximum of ethanol, effective materials.



Fig. 1 Preparation of ethanolic extract using decanter funnel

Alkaloid Test

For testing alkaloid, 0.1 gr of plantlet powder was replaced in a vial and added it 10 ml of acetic acid %10 in ethanol for 4 hours and then is purified the solution, then heated in a heater for reaching the solution volume to 2.5 ml and then adding dense Amounium hydroxide solution drop to drop to constitute sediment. Then the top solution is discarded and the sediment is diluted in sulfuric acid 0.1 and finally, the Absorption was recorded at a wavelength of 600 nm by spectrophotometry UV 2100 (Harborne, 1973).

Flavonoid Assay

To 0.5 ml of the extract, 0.1 ml of 10% Aluminum chloride was poured into methanol and 0.1 ml of Potassium acetate (1M), and 2.8 ml of distilled water were added, after 30 minutes of absorption. Solutions were measured at 415 nm (Chang *et al.*, 2002).

Standard solutions of 5, 2.5, 1.25, and 0.625 mg/ml of quercetin were prepared and all the above steps were repeated on these concentrations. In the end, their adsorption was measured at 415 nm, using the phenol values in standard solutions and light absorption the standard graph was plotted in excel and the equation was used to determine the flavonoid content of plant extracts, and the number of flavonoids present in the extracts was reported based on mg equivalent of quercetin per gram of dried plant powder.

Assessment of Total Phenolics

To 1 ml of methanolic extract was added 1 ml of HCL (6M) and 5 ml of 75% methanol and poured into sealed jars and placed in a 90 ° Water Bath for two hours. During this time, the solutions were shaken several times. After two hours, cooled to room temperature, then with a volume of 10 ml of distilled water, then 1 ml of these solutions was taken, 5 ml of Folin–Ciocalteu reagent (1:10) and 15 ml Na₂CO₃ were added, and then we bring it to a volume of 100 ml and its absorbance was measured at 760 nm (Vinson *et al.*, 1995).

Standard solutions of 5, 2.5, 1.25, and 0.625 mg/ml of gallic acid were prepared. Total phenol assays were performed on different concentrations of gallic acid. The standard diagram was drawn in excel using the amounts of phenol in standard solutions and the light absorption obtained. Acid was reported in one gram of dried plant powder (mg GAE / g DW).

Results

The conclusions of alkaloid measurement of flower, leaf, and fruit of medicinal plant named *Citrus aurantium* L. in Mazandaran and Golestan provinces show that the maximum absorption is by flower and leaf in Mazandaran province and by an immature rind of fruit in Golestan province. The results of this research show that the extraction of the flower of *Citrus aurantium* L. is used to strengthen the heart for the treatment of heart diseases and arteriosclerosis, and also fruit Juice and sour grape juice is mixed that placed on a swell. The leaf is used as a disinfectant and Anti-eclampsia. The flower and leaf are used to treat polyneuritis, ophthalmia, gastroenteritis, body heating, and dysentery. Fruitland also strengthens the heart and abdomen. Essential oil and extraction have been obtained from *Citrus aurantium* L. flower. Leaf and flower are vermicides and relieve digestion.

The results of measuring the amount of total phenol using spectrophotometry by calculating the area under the curve of each sample and measuring its amount in milligrams with an average of three repetitions for each sample showed in Fig.2 that the amount of total phenol in the leaf (7.43 - 2.97 mg) and the skin of unripe fruits (16.41 - 11.45 mg) of Amol city are more than Gorgan, while in the flowers (15.27 - 27.27 mg) the amount of total phenol in Gorgan is more than Amol. The highest amount of total phenol is related to the Gorgan flower.

The results of measuring the amount of total flavonoid content using the spectrophotometric method by calculating the area under the curve and measuring its amount in terms of milligrams with an average of three repetitions for each sample showed in the Fig. 3 that the highest amount of flavonoids was related to flowers (90.92 – 81.71 mg), and in fruit peel (49.91- 24.43 mg) and leaves (57.74- 49.78 mg) were related to Amol and Gorgan habitats, from left to right respectively.

Table1 Comparison of antioxidant & alkaloid levels of leaves, flower and fruit peel of orange in the two studied habitats

	components	Leaf	Flower	Fruit peel
Amol	Alkaloid	1.20 ± 0.210 a	1.020 ± 0.145 a	0.910 ± 0.056 b
Gorgan		0.970 ± 0.075 a	0.780 ± 0.130 a	1.590 ± 0.075 a
Amol	Total phenol	7.43 ± 0.32 a	15.27 ± 0.865 b	16.41 ± 0.434 a
Gorgan		2.97 ± 0.125 b	22.70 ± 0.932 a	11.45 ± 0.262 b
Amol	Total flavonoid	57.74 ± 1.065 a	90.92 ± 1.136 a	49.91 ± 2.0365 a
Gorgan		49.78 ± 0.965 b	81.71 ± 1.234 b	24.43 ± 2.014 b

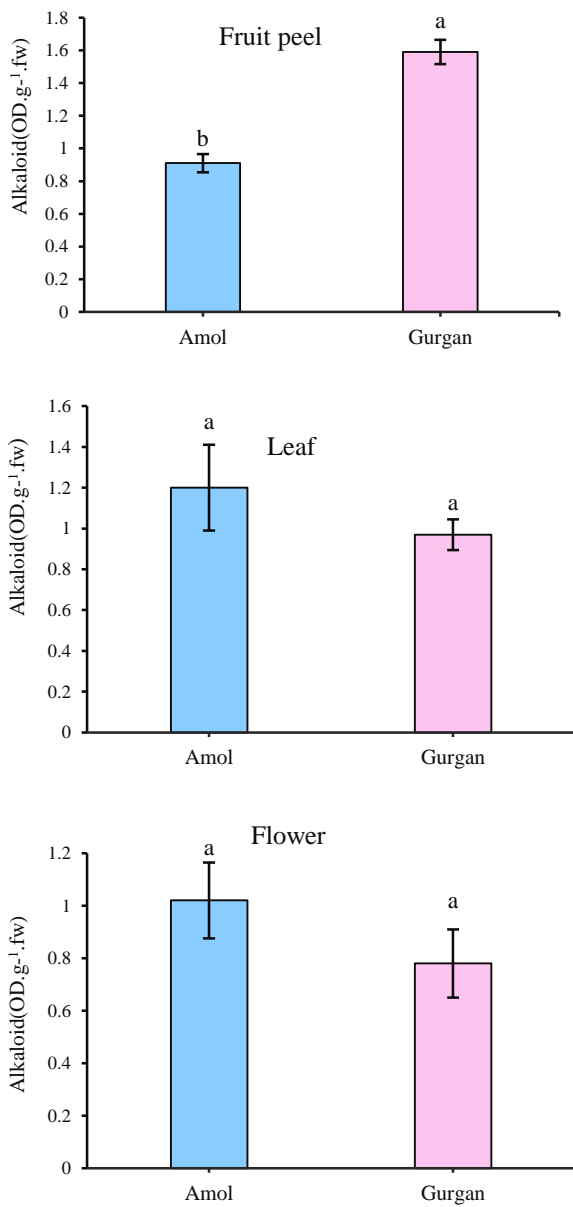


Fig. 2 The comparison of alkaloid measurements of *Citrus aurantium* L. in Amol, and Gorgan cities

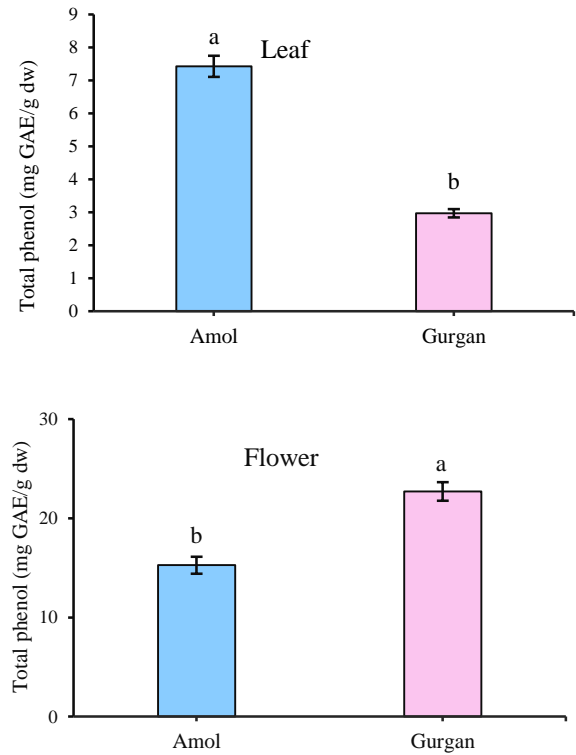
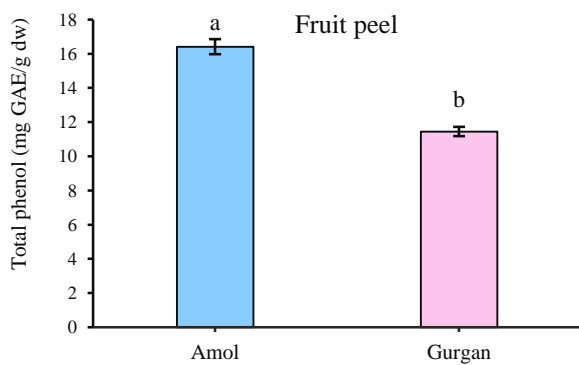
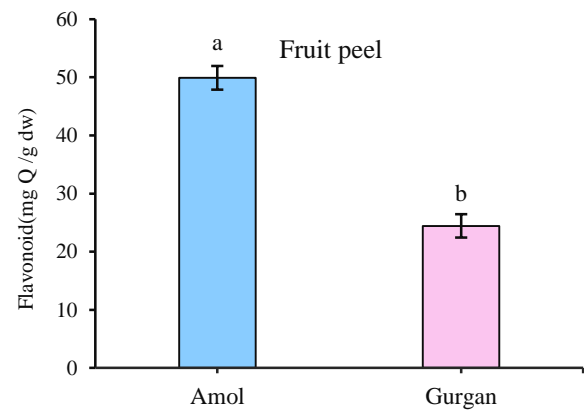
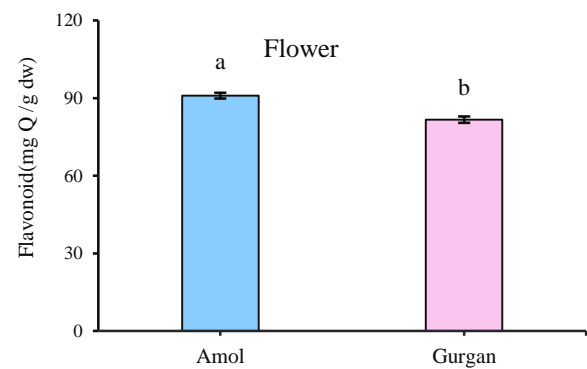


Fig. 3 The comparison of total phenolic content of *Citrus aurantium* L. in Amol, and Gorgan cities.



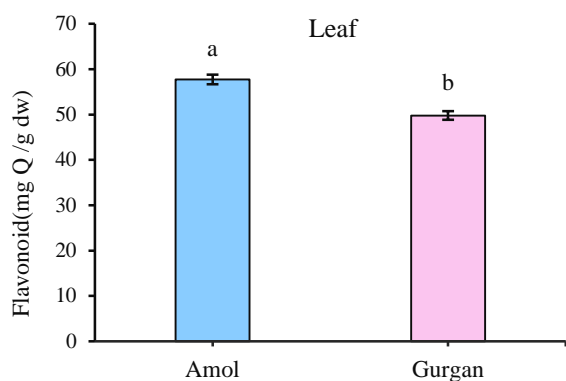


Fig. 4 The comparison of total flavonoid content of *Citrus aurantium* L. in Amol and Gorgan cities.

Discussion

In this study, the number of alkaloid compounds in the fruit peel of *C. Aurantium* with an average of (0.91 - 1.590) in Amol and Gorgan, respectively, was higher than that of flowers and leaves, which was higher in Gorgan than Amol. The most amount of total flavonoids in the orange flower of Amol city with an average of 90.92 mg and the most amount of total phenol in the orange flower of Gorgan with an average of 22.7 mg was observed.

In the same direction Zhao *et al.* (2020) in the study entitled biosynthesis of citrus flavonoids and their health effects reported that abundant flavonoids have been young tissues that are rich in flavonoids & alkaloids. Therefore, fresh young fruits of *C. Aurantium* may be a potential source of dietary supplements with health benefits. However, the phytochemical investigation of the fresh young fruits of *C. Aurantium* has rarely been conducted, however, is rare data from the phytochemical analysis of the fresh young fruits of *C. Aurantium* is available. The chemical composition of *C. Aurantium* contributes to its health-promoting effects (The chemical composition of *C. Aurantium* has many health effects) (Suntar *et al.*, 2018).

Due to the plenty of health-giving secondary metabolites, *C. Aurantium* is also used for the treatment of many diseases like anxiety (Pultrini *et al.*, 2016). Lung and prostate cancers Park *et al.* (2014) and gastrointestinal disorders and obesity (Khan *et al.*, 2017). In this study, the chemical composition of *C. Aurantium* is responsible for health-promoting effects. The chemical composition includes vitamins, minerals, phenolic compounds,

and terpenoids the odor and fragrance of *Citrus aurantium* L. blossom could create remarkable effects on the mental situation, senses, and sexual behaviors especially. Centering is one of the alkaloids in the immature fruit of *Citrus aurantium* L. that has medicinal effects like low blood vein width, high blood pressure, and softening respiratory muscle. Synephrine is in the fruit rind and flesh of citrus plants. Synephrine is the main part of fruit and fruit juice, but the other alkaloids (Ectopamin, Tyramine, N- methyl tyramine, and hordenine) are less important. Centering is similar to ephedrine in terms of structure (Pellati, *et al.*, 2007). In the research of Haj Ammar *et al.*, 2012, Phytochemical screening on the leaves of *Citrus aurantium* showed the presence of different chemical compounds like carbohydrate, phytosterol, saponins, tannins, proteins, essential oils, terpenoids and flavonoids. Other constituents like alkaloids, glycosides and mucilage were absent. In total 35 volatile components were identified, in which sesquiterpene hydrocarbon were found to be the major group of compounds. These were Eucalyptol (1, 8 Cineole - 43.05%), Sabinene (16.65%), β -Linalool (15.25%), α -Terpineol (8.025), α -Pinene (1.34%), β -Myrcene (1.20%), 4-Terpineol (1.11%), β -Pinene (1.01%), D-Limonene (0.97%), O-Cymene (0.88%) and other minor compounds.

Nowadays, food dietary complements prepared with *Citrus aurantium* L. are found for helping to low obesity as an anorectic in markets. But, the complements prepared with ephedra are forbidden by us medicinal and food ministry officials due to heart disorders and other disadvantages (Pittler *et al.*, 2004). This side effect is due to active amines in Ephedra like Ephedrine, Norephedrin, and pseudoephedrine (Abourashed *et al.*, 2003 and Pittler *et al.*, 2004). *Citrus aurantium* L. Immature fruit contains many different active amene agents those are different in replacing hydroxyl situation and number comprises centering Ectopamin, tyramine, N - methyl tyramine and Hordenine (Andrea, 2003).

Conclusion

Citrus aurantium is rich in flavonoids and polyphenolic compounds and alkaloids with numerous pharmacological properties, *C. Aurantium*, is one of the most popular orange fruits, was used as a resource for food and medicine in many countries

for gastrointestinal function disorders, and is 70% ethanolic extract of *C. Aurantium* had a regulatory effect on anti-inflammation. The chemical composition of *C. Aurantium* is responsible for health-promoting effects. The chemical composition includes vitamins, minerals, alkaloids, antioxidants, flavonoids, and phenolic compounds.

Acknowledgement

the authors of the article consider it necessary to express our sincere gratitude and thanks to the management and staff of the laboratory of Mehr zist pajohan amard trading company who assisted in conducting this research.

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