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EXTRACTION OF NATURAL ANTIOXIDANT FROM LEMON PEELS, KINETIC AND ANTIOXIDANTS CAPACITY

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Abstract :-

An experimental study was conducted to examine how particle size and solvent concentration affect the extraction process of lemon peels using ethanol-water solutions. The study also measured the total polyphenol content and antioxidant activity of the extracts. It was found that the process is particularly sensitive to particle size, with different fractions showing varying levels of confusion and frustration. The impact of solvent concentration on the system was found to be minimal. The total polyphenol content and antioxidant activity of the extracts were determined. Lemon peels are a cost-effective byproduct that shows promise for use in food and cosmetic industries due to their natural antioxidant properties.

***Keywords**: natural antioxidants, polyphenols, extraction, lemon peels.*

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INTRODUCTION :

➢ **Introduction :-**

Today, oxidative stress is a serious issue in modern society. Factors like air pollution, smoking, UV radiation, and lifestyle choices can cause oxidative stress in cells, which is linked to various health problems such as dermatitis, melanomas, and skin aging. One important use of natural plant extracts in cosmetics is their ability to act as antioxidants. Unlike some other types of antioxidants, natural ones are less likely to cause allergies, making them ideal for creating hypoallergic cosmetics. Another useful trait of natural extracts is their antimicrobial properties. While the antioxidant and antimicrobial effects of different plant extracts are not yet fully understood, new research is emerging, focusing on previously unstudied plants and materials. Oxidative damage to fats and oils in food leads to unpleasant smells and tastes, reduces nutritional value, and can create harmful compounds. To prevent this, adding antioxidants is essential to maintain flavor, color, and prevent the breakdown of vitamins.

Citrus residues are a great source of natural antioxidants because citrus is an important crop, with production reaching about 80 million tons each year. In the food industry, citrus is mainly used for making fresh juice or citrus-based drinks. The by-products from processing citrus can be a good source of natural flavonoids, especially since a lot of peel is generated during processing. Citrus peels also have a high level of phenolic compounds. Additionally, while flavonoids are found in many plants, there are several types, like flavanones, flavanone glycosides, and polymethoxylated flavones, that are unique to citrus and are not commonly found in other plants. Typically, valuable natural materials are extracted using organic solvents. However, some of these solvents can be toxic, and the extraction process can be harsh. For this reason, food-grade ethanol is often used instead of methanol when extracting phenolic compounds from citrus peels. Different researchers have studied how various factors, such as how the peel samples are prepared, whether extraction is repeated, the type and concentration of solvents used, and the temperature, affect the extraction process.

The purpose of this research is to explore the potential of using lemon peel extracts as a natural source of antioxidants. It also aims to measure the amount of phenolic compounds found in the lemon peels, compare the results from different solvent extraction methods, and assess the antioxidant activity of the resulting extracts.

For this purpose, the extraction process of lemon peels using ethanol-in-water solutions is studied. The impact of ethanol concentration and particle size on the total amount of extract, the quantity of phenolic compounds extracted, and the antioxidant activity of the extracts is examined.

Used in Cosmetic:-

Cosmetics are products designed to enhance skin appearance and body fragrance, available in forms like creams, lotions, and powders. They serve to clean, protect, and hydrate the skin. Today, consumers tend to prefer products that are gentle and safe for their skin. Many pharmaceutical companies have introduced "cosmeceuticals," which combine the properties of cosmetics and pharmaceuticals. These products not only improve beauty but also provide biological benefits to the skin. In particular, antioxidant-based cosmetics help protect the skin from damage caused by ultraviolet (UV) radiation and harmful free radicals. Antioxidant compounds are valuable to the pharmaceutical and cosmetic industries because they help neutralize free radicals. However, many antioxidants are unstable, which can create challenges during cosmetic formulation. Therefore, choosing the right type and concentration of antioxidants is crucial. In recent years, natural antioxidants from plants have become more popular than synthetic ones, as plant extracts typically contain a blend of bioactive compounds that work together synergistically. This combination often results in improved effectiveness and reduced toxicity in cosmetic products.

Natural antioxidants:-

Antioxidants can be classified into two main types: primary or natural antioxidants and secondary or synthetic antioxidants. Primary antioxidants include mineral-based ones like selenium, copper, iron, zinc, and manganese, as well as vitamins such as C and E, and compounds known as phyto-antioxidants. These minerals often act as cofactors for enzymatic antioxidants. Secondary or synthetic antioxidants work by trapping free radicals and stopping the chain reaction that causes oxidative damage. Some common examples of secondary antioxidants include synthetic compounds designed for this specific purpose.

➢ **Introduction of Lemon:-**

- Biological Source: Lemon consists of the fresh or dried fruit and peel of Citrus limon (L)Burm. f.
- Family: Rutaceae
- Genus: Citrus
- Species: C. limon (L.) Burm. f.

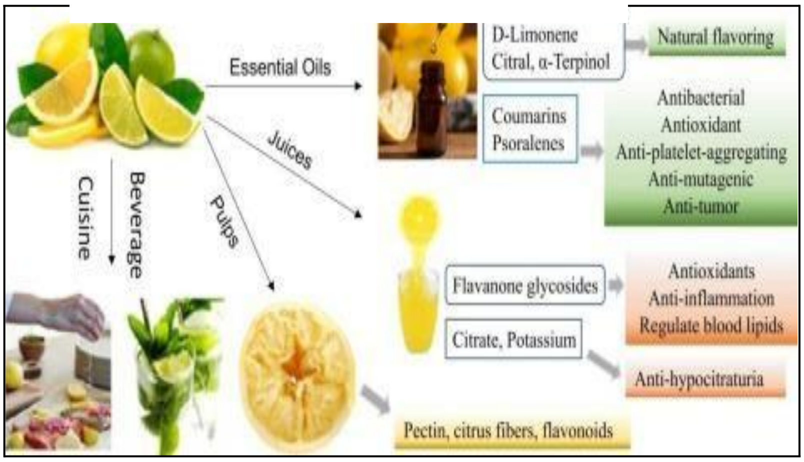
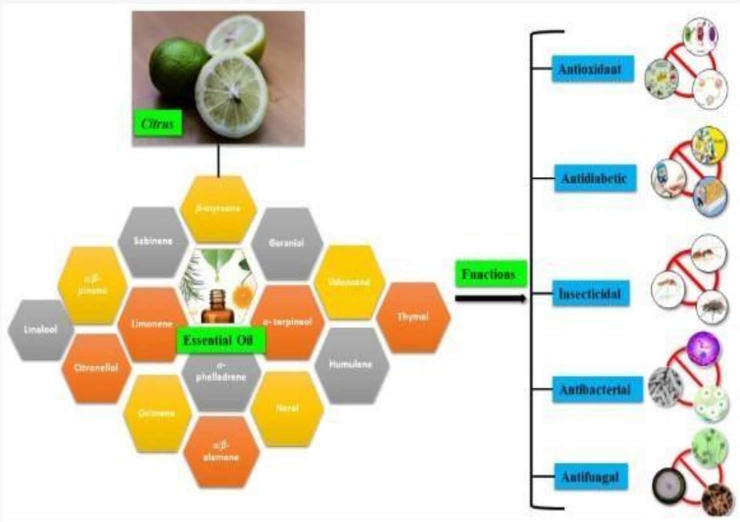


Figure 1. *Citrus* essential oils (CEOs) and their various functions.



Plant Part Used

- Fruits (mainly juice and peel)
- Leaves (for essential oils)
- Seeds (contain fixed oils)

Chemical Constituents

- Citric acid
- Vitamin C (Ascorbic acid)
- Flavonoids – Hesperidin, Eriocitrin, Diosmin
- Essential oils – Limonene, Citral, Linalool
- Pectin, Sugars, and Minerals

Uses / Importance

- Acts as a natural antioxidant and flavoring agent.
- Used in cosmetics, pharmaceuticals, and food industries.
- Possesses antimicrobial, anti-inflammatory, and skin-protective properties.

➔ **Antioxidant Constituents in Lemon**

1. Vitamin C (Ascorbic Acid)

Flavonoids (Polyphenolic compounds) Key antioxidant flavonoids include:

- Hesperidin
- Eriocitrin
- Naringin
- Diosmin
- Rutin

2. Phenolic Compounds
3. Essential Oils
4. Carotenoids (like β-carotene)

EXPERIMENTAL:

- **Plant materials**

Lemons were bought from a local fruit market.
The country of origin for the lemons is Greece, but they were sold in Sofia, Bulgaria. All the fruits were of good quality and free from any damage or imperfections. The fruits were peeled by hand. The part removed was the pericarp, which includes the outer and middle layers of the peel. The peeled material was placed in paper bags and dried under normal conditions to reduce the moisture content from 85% to 10% of the total mass. The dried lemon peels were kept under normal conditions before any additional processing. Later, the peels were ground and passed through vibrating metal sieves with mesh sizes of 2.5 mm, 2 mm, and 1 mm.

- **Chemicals**

Folin-Chiocalteu reagent, Sodium carbonate, and Gallic acid were bought from Sigma Chemical Co. The ethanol used was 96% PA, which was provided by Valerus, a company based in Bulgaria.

- **Extraction procedure**

The process of extracting was studied in both liquid and solid forms.
As the extraction happens, the amount of substance in the liquid part goes up, and at the same time, the solid part gets lighter. For each group, several samples were made. Each sample had exactly Ig (with a small error of ±0.0005 g) of dry, crushed lemon peel, packed into a piece of cheesecloth. These were also weighed. The weighing was done using a Sartorius balance, which can measure up to 0.1 mg precisely. The solvent used was a mix of ethanol and distilled water in three different strengths: 20%, 50%, and 70% ethanol. The ratio of solid to liquid was either 0.05 or 1 gram of lemon peel per 20 cubic centimeters of ethanol and water. Once the samples were ready, they were put into a lab shaker called THUS 2 and mixed with the solvent. This was the start of the extraction. Samples were taken at different times, between 3 and 80 minutes. The solid part was removed, dried, and weighed. The liquid parts were also dried at 50°C in a heated oven until they stopped losing weight. The results of these experiments, in the form of graphs showing how much was extracted over time, are shown in Figure 1. After the drying process, the total amount of extract was measured by weighing.

- **Total polyphenols content**

It is believed that the Total Polyphenols Content (TPPC) serves as a general indicator for estimating antioxidant capacity.
Since the molecular weight of the polyphenol mixture is not known, TPPC is often expressed as Gallic Acid Equivalent (TPPC aut). The photometric method, which relies on the color change of phenolic compounds when reacted with Folin-Ciocalteu reagent, is widely used for measuring total polyphenols. This method is favored for its simplicity and dependable results.

- **Antioxidant activity determination**

Other methods for determination of the antioxidant activity are FRAP (Ferric Reducing Antioxidant Power), ORAC (Oxygen Radical Absorbance Capacity) or TEAC (Trolox Equivalent Antioxidant Capacity). As it is underlined in the literature, all these methods give quite different results and the choice of suitable method for antioxidant activity determination is of great importance. A method largely applied is the DPPH method because of its simplicity and stable results. It is based on the discoloration reaction between nitrogen electron (from DPPH) and hydrogen atom of hydroxyl group (from antioxidant substance). An inconvenience of this method is its light sensitivity. For this reason, the reaction must be carried out in the dark and in a non-alkaline medium. In our study we applied this method using a spectrophotometer Helios B (Unicam, USA). To compare the AOA of different materials we used the IC parameter. It represents the amount of antioxidant sample, which inhibits 50% of the initial concentration of DPPH.

- **Different methods are used:-**

☐ **Spectrophotometric methods**

1. **DPPH radical scavenging assay:** This is a simple and commonly used test where an antioxidant helps change the stable purple DPPH radical into a lighter yellow or white form.
   The amount of color decrease is measured at 517 nm, and this helps find out how good the antioxidant is, with less color meaning more antioxidant activity.
2. **ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay:** The ABTS assay measures how well an antioxidant works by checking how much of the ABTS radical stays

after it reacts with the antioxidant.
3. FRAP (Ferric Reducing Antioxidant Power) assay
4. CUPRAC (Cupric Reducing Antioxidant Capacity) assay
5. Folin-Ciocalteu (FC) method

- **Other method:-**

1. Electrochemical methods
2. Chromatographic methods
3. Hydrogen atom transfer (HAT) based assays

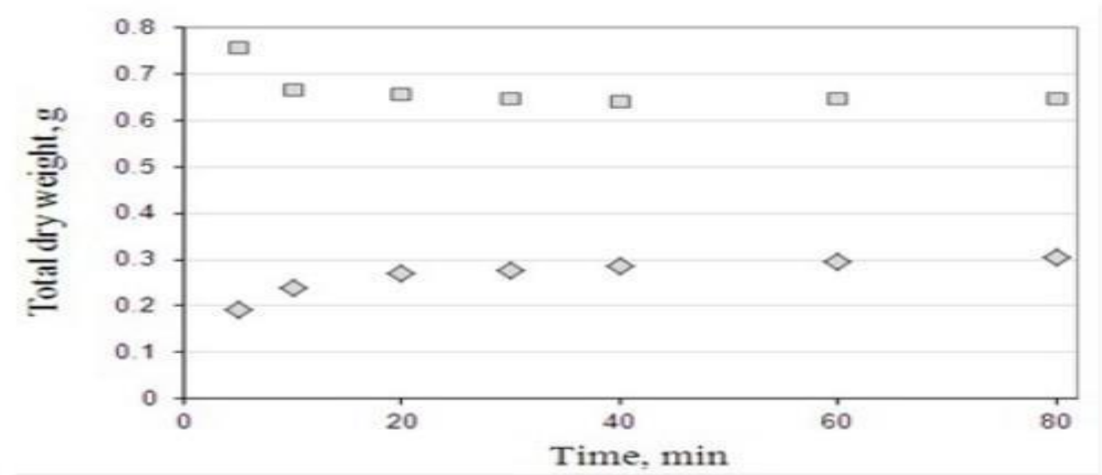
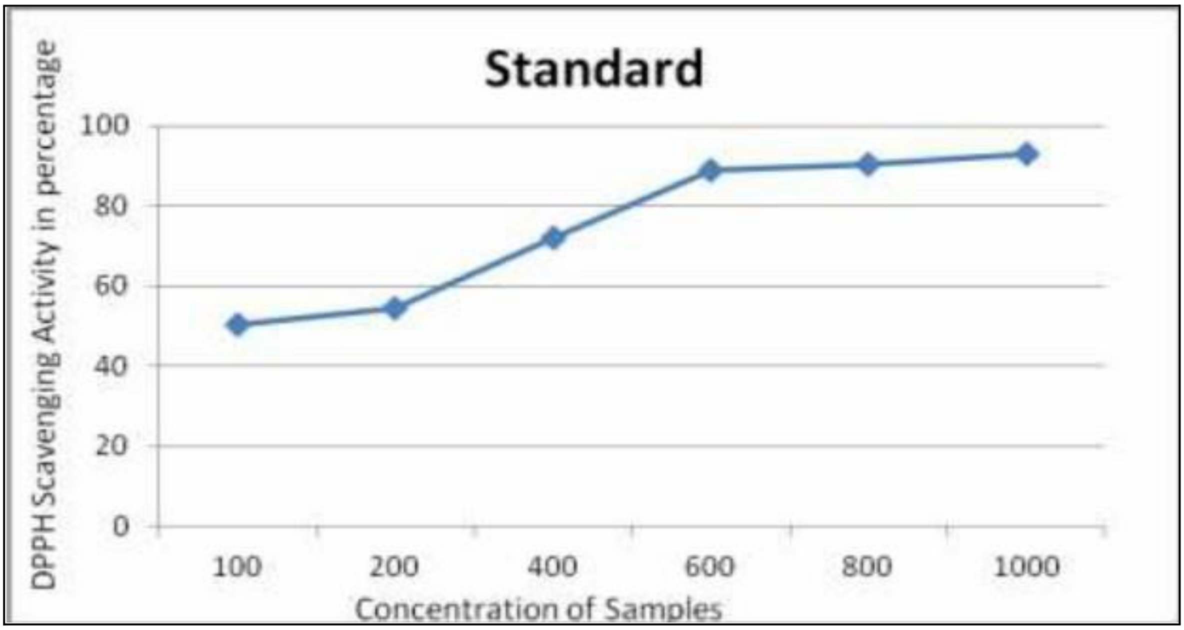


Fig. 1. Experimental kinetic curve at solid-to-liquid ratio 0.05 g cm^{-3}, particle size d < 1 mm and 50 % ethanol concentration. □– solid phase; ◇– liquid extract.



RESULTS AND DISCUSSION:

- **Extraction kinetics:-**

The process of extracting compounds from dried lemon peels was studied over time.
The total dry weight was measured in both the solid and liquid phases. Figure 1 shows typical kinetic curves for the fraction of lemon peel particles smaller than 1 mm, using a 30% ethanol-in-water solution. The ratio of solid to liquid was 1 gram of raw material to 20 cubic centimeters of solvent.

The kinetic curve has three main sections: the first part is steep and shows the rapid dissolution of substances that are easily accessible on the surface of the particles.
The middle part reflects the simultaneous release of substances from both the remaining surface and the inside of the solid material, which is controlled by both external and internal diffusion.
The final, gradually increasing part of the curve indicates the complete removal of soluble substances from the surface, with the process now being controlled by internal diffusion.

Definition
Extraction kinetics refers to the rate and mechanism by which antioxidant compounds are transferred from the solid lemon peel into a liquid solvent during extraction.
It helps determine:

A. How quickly antioxidants are extracted.
B. The time required to reach maximum yield.
C. The effect of factors like temperature, solvent type, and particle size.

- **Effect of particle size:-**

To take a look at the impact of debris size experiments with 50% ethanol concentration and special sieve fractions described in the experimental segment were carried out. the total dry extract weight became investigated. The outcomes are provided in Fig. 2. it's far glaring that the best results were obtained for the fraction <1 mm. which can be explained with improved strong segment surface improving the segment touch. For the largest sieve fraction used (2.5 mm) the method becomes finished until one hundred twenty min. It turned out that the full extract's quantity slowly has a tendency to the values reached at 60-80 min for fraction among 1 and a couple of mm and between 2 and 2.five. mm. Our consequences verify different authors' experimental information about the positive effect of debris demolition on the polyphenols extraction.

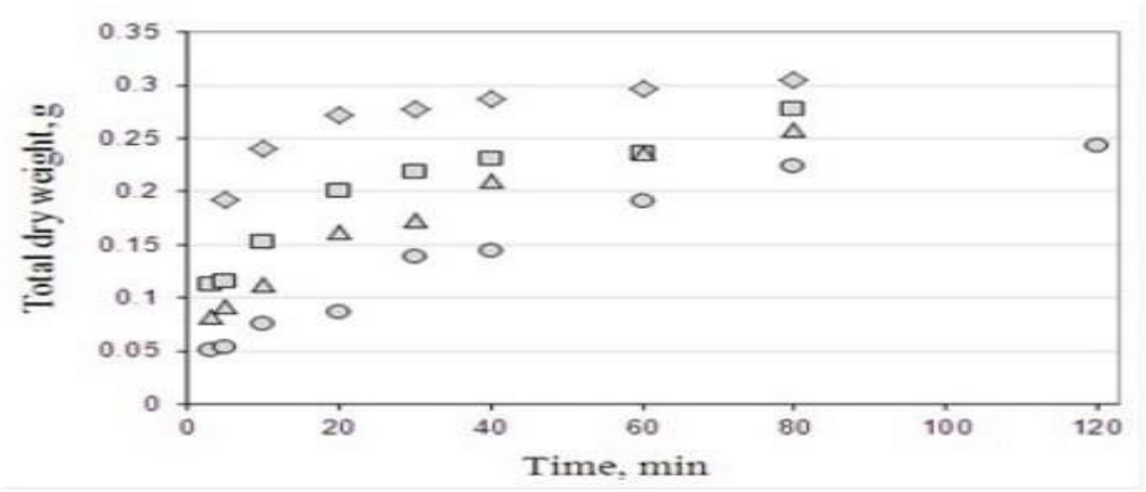


Fig. 2. Influence of the particle size on the extract amount: ◇- < 1 *mm*; □ - 1 *mm* < d_p < 2 *mm* ; △- 2 *mm* < d_p < 2.5 *mm*; ○- d_p > 2.5 *mm*.

- **Effect of the solvent concentration :-**

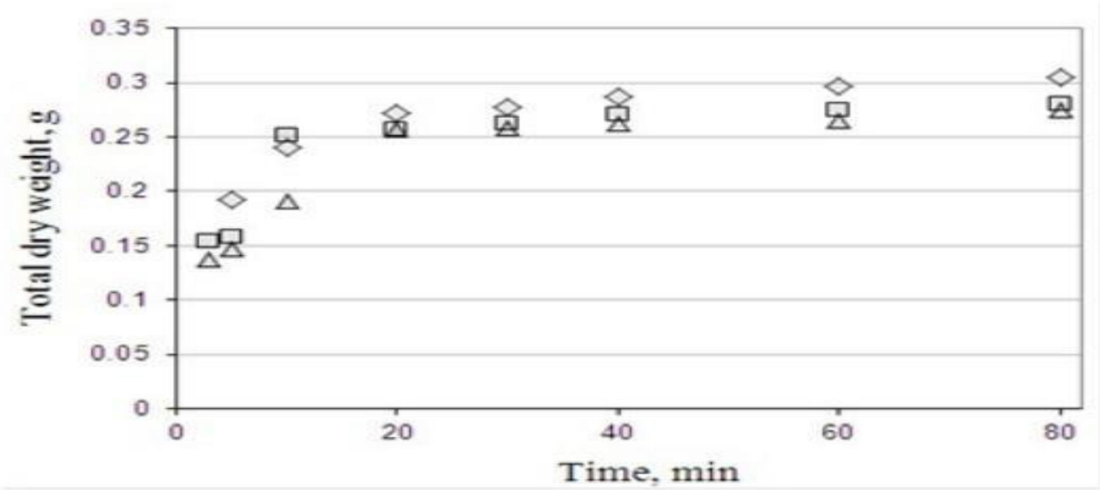


Fig. 3. Effect of the solvent concentration on total dry extract: ◇ - 50 % ethanol, □– 20 % ethanol, △ - 70 % ethanol.

- **Total polyphenols content and antioxidant activity**

Table 1. Lemon peels extracts: TPPC and AOA.

Ethanol concentration (% vol.)	Particle size (mm)	$TPPC_{GA}$ (mg l^{-1})	IC50% (mg l^{-1}) sample	$IC50\%_{AA}$ (mg ml^{-1})
20	<1	832.73	80.52	0.19
20	1-2	779.09	86.94	0.18
70	<1	224.55	46.87	0.33
70	1-2	202.73	46.18	0.33
50	<1	928.18	40.4	0.38
50	1-2	821.82	40.13	0.382
50	>2.5	454.55	33.35	0.46

The results are summarized in Table 1.
It can be seen that the extracts made with 70% ethanol have about four times less total polyphenols compared to those made with 20% and 50% ethanol.
When looking at the total dry extracts, the particles smaller than 1 mm show the highest total polyphenol content. As the particle size increases, the total polyphenol content decreases, even when using the same ethanol concentration of 50%.

There is no clear connection between the amount of phenolics and the antioxidant activity.

This finding matches results from other researchers on malts, citrus waste, and plant extracts **[1].**

A comparison of the TPPC and AOA data for lemon peels with other plant extracts is shown in Fig. 4.
It is clear that lemon peels have higher total polyphenol content and reasonable antioxidant activity. These results make lemon peels a promising and affordable source of natural antioxidants for use in the food and cosmetic industries.

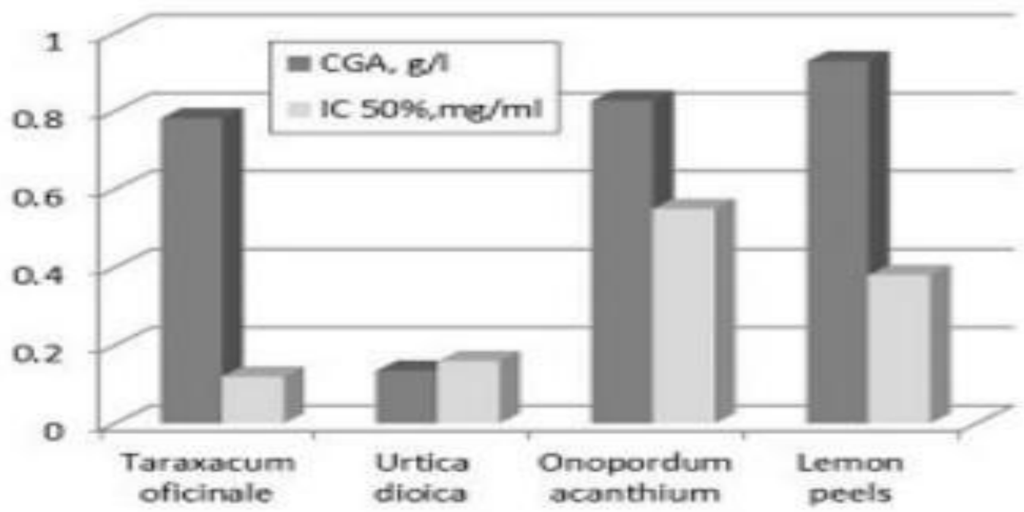


Fig. 4. Comparison between TPPC and AOA for three medicinal plants and the lemon peels [9, 10, this work].

CONCLUSIONS:

The study looked into using lemon peels as a natural source of antioxidants.
The research examined how well lemon peels can release antioxidants when mixed with ethanol and water, both in solid and liquid forms. It considered how the size of the peels and the strength of the solvent affect the process. It was found that the size of the peels, based on how finely they were ground, had a noticeable effect on the amount of extract obtained, while the concentration of ethanol had a small or almost no impact. The total amount of polyphenols and the antioxidant activity of the extracts were measured. However, there was no clear link between these two measurements. When compared to three other medicinal plants, lemon peels show promise as a good natural antioxidant source for use in food and cosmetic products.
Various antioxidant test methods can be used parallel to determine antioxidant activity because each method will give different results.

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