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Phytochemistry, GC-MS Analysis, physicochemical and *In vitro* Antimicrobial activity of seed and peel of *Citrus sinensis*

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Abstract

Citrus fruits are one of the important fruit trees grown worldwide and appreciated for their refreshing juice and health benefits. The research paper has in depth literature review on the importance of *Citrus* fruits and investigate the numerous therapeutic properties of *Citrus* fruits. The research paper summarizes the findings of the phenolic content, Antioxidant activity of extracts of *Citrus* fruits on 10 samples. The research made to estimate the mineral composition (g 100-1), proximate composition (%) and food energy (g Cal-1), phytonutrients content of *Citrus* species on dry weight basis expressed as mg/100g , Vitamin composition of *Citrus* juice expressed as mg/100g for 5 species and tabulated the data with graphical representation. Finally, paper concluded the importance of *Citrus* fruits on the basis of results of antioxidant activity, total phenolic and total flavonoid contents of *Citrus* leaves. The research also find out the different parameter by vitro antimicrobial activity the oil samples of seeds and peels of orange and analysis the findings.

Citrus fruits are the main fruit trees grown throughout the world and are well – appreciated for their refreshing juice and health benefits. Numerous therapeutic properties have been attributed to *Citrus* fruits, like anticancer, antiviral, anti-tumor, anti-inflammatory activities, and effects on capillary fragility as well as an ability to inhabit platelet aggregation. Though there were many studies on antioxidant and antibacterial effect of juice and edible parts, there are meager literature on the wastes of *Citrus* fruits of lemon and oranges of different varieties.

The clinical use of lemon and orange are investigated by identification of multiple constituents Alkaloids, polysaccharides, lectins, glycosides, phenolic compounds, flavonoids, anthocyanins, tannins, and sterols for antioxidants properties and d-limonene for better pharma-

cokinetic and pharmacodynamics properties[3,29]. The *Citrus* peel wastes are rich in nutrients and contain many phytochemicals. These wastes can be effectively disposed by manufacturing by-product from them. The hydro-distillation and GC-MS analysis on by-product of orange peels made to find the aromatic compounds in the peel oil. The parameters of oil like limonene (86.75%), linalool (1.96%), α-pinene (1.63%), trance-limonene oxide (1.39%), and γ-terpinene (1.03%) confirm its utilization as food preservative[1,6,7,8]. The food, cosmetic and chemical industries use The Cold pressed and microwave roasting of orange *(Citrus sinensis*) seeds oils studied and found the availability of fatty acids : linoleic, palmitic and oleic acids, phenolics, α-tocopherol, ß-sitosterol conclude the application of oil in the food, cosmetic or chemical industries[9,14,15,29].

The experiment of CSE, MAE and UAE and SC-CO_2 extraction claim that antioxidant activity (DPPH method) and its correlation to TPC, TFC or individual flavonoids. Further the findings of total carotenoids, total carotenoids (19.01 mg/kg), total phenolic compounds (4.43 g/kg), α-tocopherol (135.65 mg/kg) and phytosterols (1304.2 mg/kg) form Extracted from orange (*Citrus sinensis*) seeds determine their antioxidant activity[20,23]. Phytochemicals and assess antioxidant capacity from *Citrus* processing by-product and essential oils were tested *in vitro* for their anticandidial activity[10,19]. Further Flavonoid-rich chloroform fraction (FRCF), microwave assisted extraction, MAE and supercritical CO_2 extraction, SC-CO_2) on the total phenol contents, total flavonoid contents, individual flavonoids, vitamin C and antioxidant activity of orange peel confirm the antioxidenet activity[24-26,28].

The present study has shown the usefulness of the extraction methodologies adopted for efficient extraction, processing and utilization of these *Citrus* fruit peel wastes and to characterize the phytochemicals, antioxidant property and antibacterial activities of fruit peel wastes of lemon and oranges.

Collection and identification of Citrus fruit peels :

Peels of *Citrus* fruits was collected from local *Citrus* juice shop. Further the *Citrus* Seed oil extracted by hydro distillation (3-4 hours extraction) and kept in fridge. The experimental process to find Physico-chemical parameters mentioned in Table-1.

Collection and preparation of sample seed & peel oil:

Orange seed oil : Seed manually separated and dry in air, grinded on electrical grinder machine approx. 1.5 kg of *Citrus* sinensis seed kept in flask. The n-hexane added as extracting solvent and find oil by rotary evaporated method.

Orange peel oil : Peels washed with double distilled water and dried in an oven at (105°C ± 2) for two days to constant weight. The dried peels were ashed in a porcelain container placed in furnace for 6 h by stepwise increase of the temperature up to 500°C. The ashed samples were homogenized in porcelain mortar and grinder and filtered. Then same method as above for oil extraction.

Table-1. Experimental process to find Physico-chemical parameters of the oil samples

SNo.	Investigation Method	Parameters and Process
1	Physicochemical and Phytochemical Investigation of *Citrus* EO	Including refractive index, optical rotation, specific gravity, color, odor and solubility.
2	GC-MS Analysis of *Citrus* EO	Screening of bioactive compounds
3	DPPH Assay Antioxidant potential methanolic and shaken well.	500 µL essential oil added in 3 ml of 0.002%

Table-2. *In vitro* Antimicrobial Activity the oil samples of seeds and peels of orange

SNo.	Investigation	Parameter to Checked
1	Physical properties of the oils	Refractive index ,specific gravity, flash and smoke points, cloud and pour points and viscosity test
2	Chemical properties of the oils	acid value (AV), free fatty acid (FFA), Iodine value (IV), saponification value (SV) and peroxide value (PV)
3	Determination of fatty acid composition	Fatty Acid composition
4	Total fatty matter (TFM)	determined by the petroleum spirit extraction method
5	Determination of total alkali	Total alkali obtained by method of AOAC (1990).
6	Foamability test	Measure height of the foam in the solution

Statistical analysis :

The oil samples analyses were performed for three times, and the results were reported as mean values ± SD with 95% confidence level. All experimental value are summarized in different table heading from Table-3 to Table-10.

Table-3. Physico-chemical parameters of the oil samples.

Variable	Orange seed oil	Orange peel oil
pH	4.5	5.5
Colour	Golden yellowish	Brownish-yellow
Percentage yield (%)	40	32
Specific density (g/cm^3)	0.95	0.75
Cloud point (degree C)	14	15
Pour point (degree C)	7.5	9
Viscosity 100°C (CST)	3.25	0.95
Refractive Index	1.4	1.48
Acid value (mgKOH/g)	23.5	26.1
Peroxide value (mgKOH/g)	18.2	5.5
Free fatty acid (% as oleic acid)	11.5	111.5
Saponification value (mgKOH/g)	221.5	42.5

The experimental values of Orange seed and peel oil are summarized in above tables addressed that excess of fatty acids make Seed oil is more acidic than the peel oil. Carotenoids responsible for yellow colour and higher saponification value make it suitable for to use in edible product. The observations like the smoke point, flash point and free fatty acid content of the oils have a linear relationship and peroxide value serves as a common indicator of lipid oxidation.

Table-4. Phytonutrients content of *Citrus* species on dry weight basis expressed as mg/100g.

Species	Alkaloids	Flavonoids	Tannins	Phenols	Saponins
Blanco C. reticulata	0.4±0.15	0.3±0.05	0.03±0.01	0.035±0.02	0.035±0.02
C. aurantifolia	0.35±0.15	0.3±0.05	0.05±0.01	0.025±0.01	0.25±0.1
C. limonum	0.6±0.25	0.6±0.10	0.02±0.01	0.055±0.01	0.5±0.05
C. vitis	0.65±0.15	0.25±0.1	0.03±0.15	0.09±0.01	0.25±0.05
C. sinensis	0.6±0.15	0.2±0.05	0.04±0.05	0.015±0.01	0.06±0.02

Table-5. Proximate composition (%) and food energy (g Cal^{-1}) values of *Citrus* species

Species	Moisture	Crude protein	Crude fiber	Ash
C. reticulata	5.6±0.15	11.9±0.02	5.35±0.15	4.5±0.15
C. aurantifolia	6.1±0.25	10.5±0.15	6.25±0.25	7.6±0.15
C. limonum	6.5±0.15	14.5±0.15	6.85±0.15	5.5±0.15
C. vitis	6.35±0.25	12.95±0.25	7.4±0.25	5.2±0.15
C. sinensis	4.5±1.15	18.15±0.25	5.66±0.2	5.52±0.25

Table-6. Mineral composition (g 100^{-1}) *Citrus* species

Species	P	K	Mg	Na	Ca
C. reticulata	0.3±0.15	0.48±0.22	0.5±0.12	0.35±0.25	2.5±0.25
C. aurantifolia	0.3±0.2	1.5±0.15	0.4±0.15	0.38±0.1	2.9±0.15
C. limonum	0.4±0.25	0.4±0.15	0.35±0.01	0.35±0.02	2.2±0.15
C. vitis	0.25±0.15	0.3±0.02	0.31±0.15	0.3±0.15	3.5±0.15
C. sinensis	0.45±0.25	0.85±0.1	0.35±0.1	0.34±0.25	2.12±0.15

Table-7. Vitamin composition of *Citrus* juice expressed as mg/100g

Species	Ascorbic Acid	Thiamine	Riboflavin	Niacin
	Vitamin C	Vitamin B_1	Vitamin B_1	Nicotinic acid
C. reticulata	32.5±0.25	0.15±0.1	0.02±0.1	0.45±0.11
C. aurantifolia	23.5±0.15	0.15±0.15	0.05±0.15	0.04±0.2
C. limonum	62.5±0.2	0.9±0.2	0.03±0.08	0.15±0.35
C. vitis	35.89±0.25	0.1±0.01	0.08±0.15	0.34±0.2
C. sinensis	20.5±0.2	0.06±0.1	0.15±0.15	0.35±0.1

Table-8. Percentage yield of *Citrus* essential oils by fabricated unit

Citrus species Essential oils (EO)	Raw material Input (g)	Time extract (minutes)	Oil volume (ml)	Productivity (ml/2000g)%
C. paradisi	2000	200	8.8	0.44
C. sinensis var. Malta	2000	220	7.5	0.375
C. reticulata var. Mandarin	2000	215	6.5	0.325
C. sinensisavar Mousami	2000	240	6.2	0.31
C. reticulata var. Tangerine	2000	225	5.8	0.29

Table-9. The phenolic content and Antioxidant activity of extracts of *Citrus* fruits

Sample	Total phenolic content mg GA/mL	IC_{50} (mg/mL)
Lemon peel	0.5±0.008	19.85±2
Lemon juice	0.35±0.02	78.15±5.5
Orange peel	0.48±0.025	20.2±0.25
Orange juice	0.45±0.015	6.5±0.5

Table-10. Total phenolic and total flavonoid contents of *Citrus* leaves

Extracts	Total phenolic content, mg, Gallic acid equivalents/g DM	Flsvonoids, mg Quercetin equivalents/g DM
Aqueous extract		
C. cleamentina	124.85±0.25	46.8±2.5
C. aurantifolia	105.95±2.5	38.2±1.5
C. limon	98.52±3.05	37.85±1.5
C. navel	85.68±8.1	20.5±2
C. hamlin	85.25±7.5	19.2±2.5
Methanolic extract		
C. cleamentina	11.5±0.5	8.2±0.5
C. limon	4.5±0.75	3±0.35
C. navel	7.5±0.65	4.1±0.4
C. grandis	3.1±0.08	1.05±0.2

Further the different Physicochemical value of Orange seed & peel Oil obtain from experiments are visualize in graphical representation from Figures-1 to Figure-6.

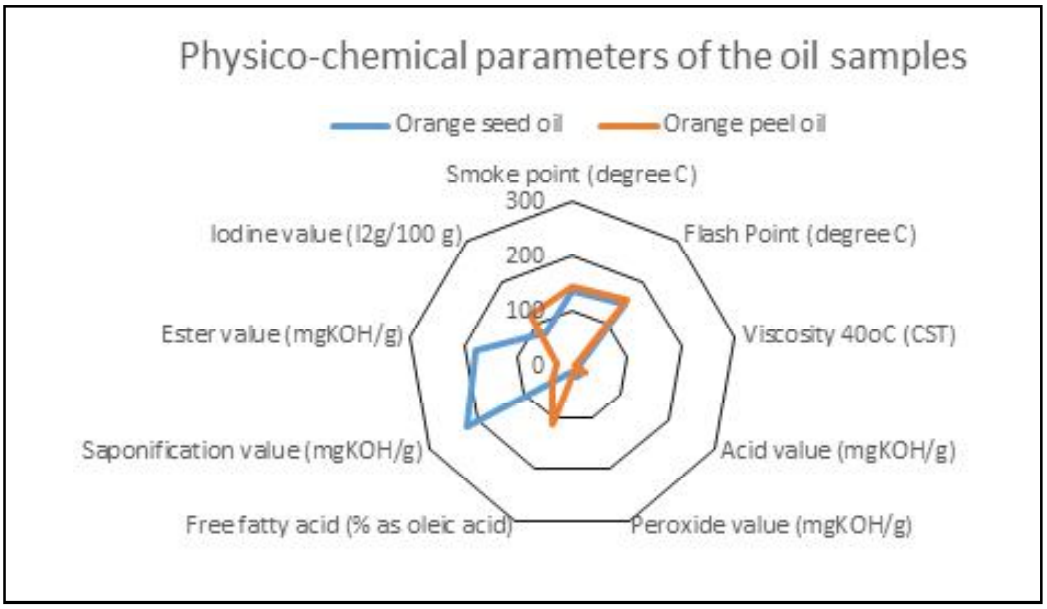

Figure-1. Graphical re-presentation of Physico-chemical parameters of the oil samples of orange seed and peel

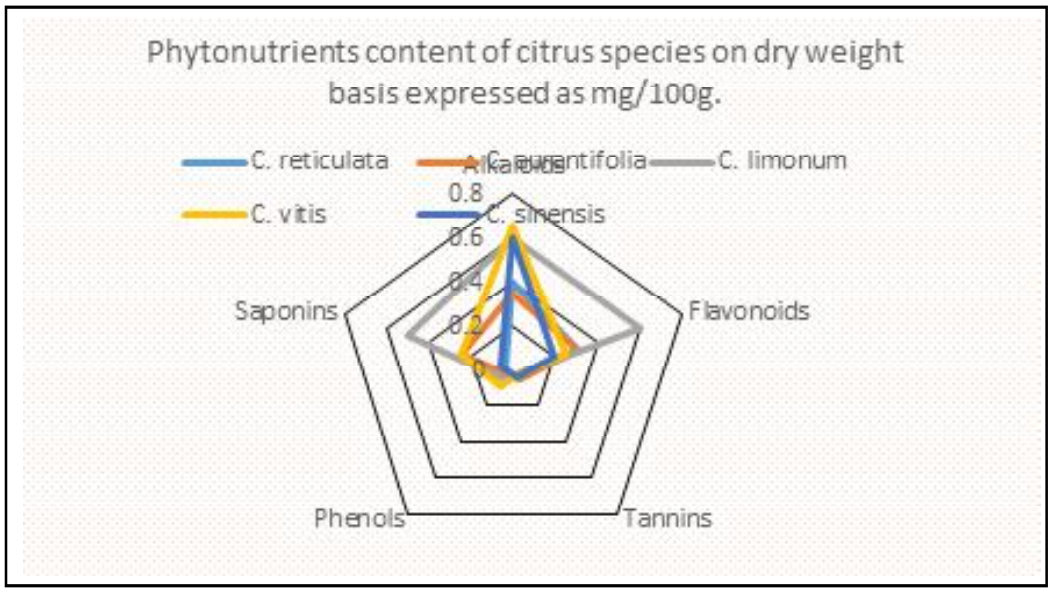

Figure-2: Graphical re-presentation of Phytonutrients content of citrus species

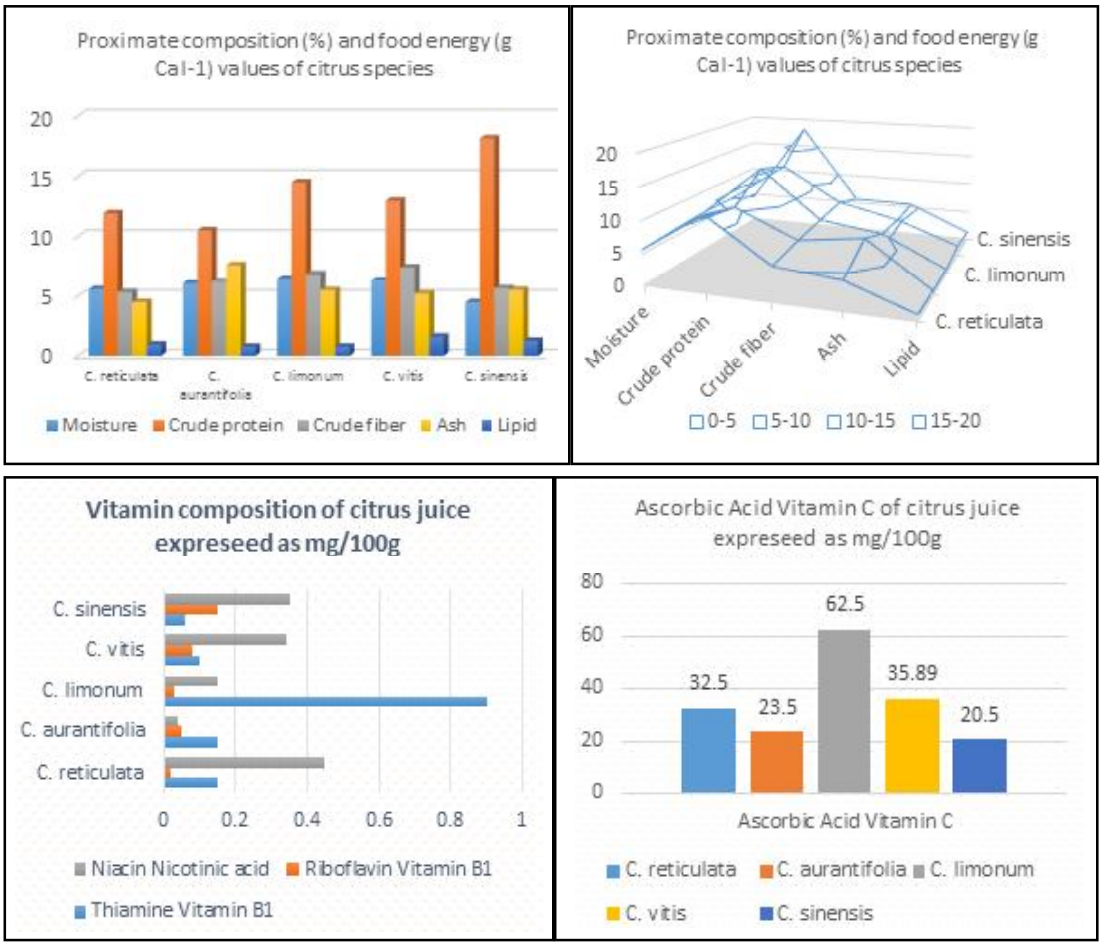

Figure-3. Graphical re-presentation of Chemical Composition of Citrus species

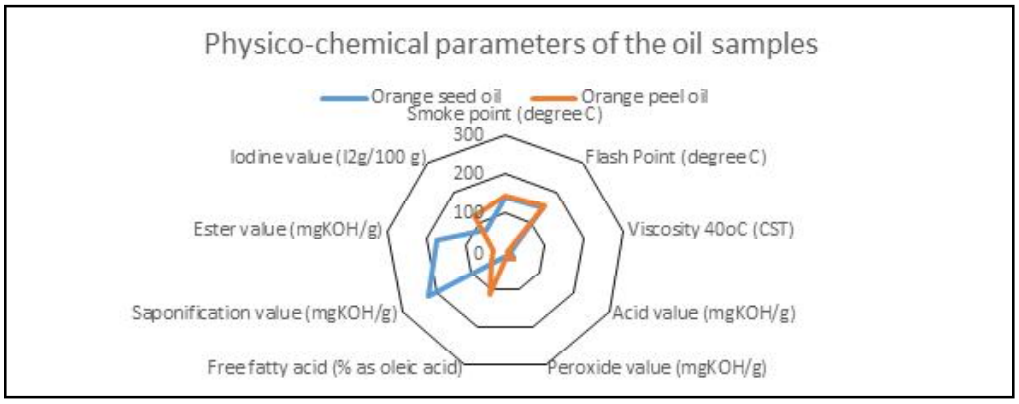

Figure-4. Physico-chemical parameters of the oil samples of Orange seed and peel oil

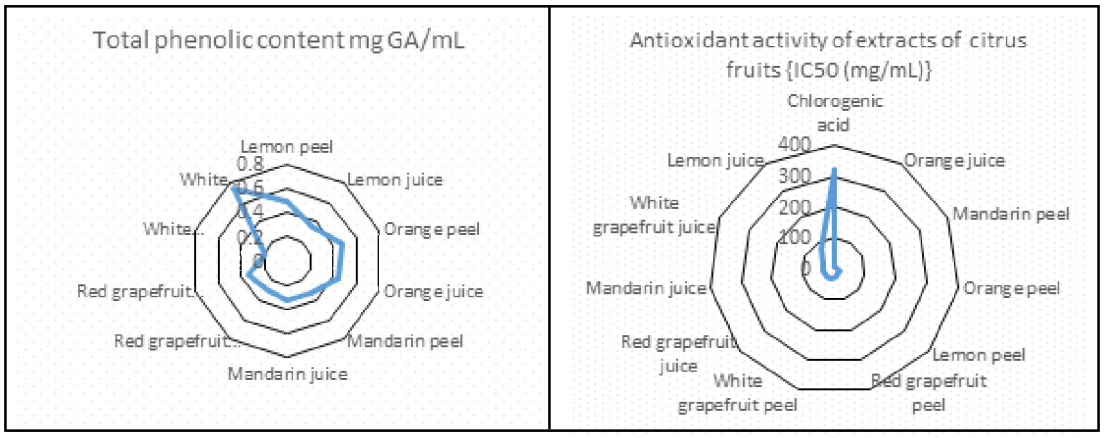

Figure-5. Total phenolic content and antioxidant activity of citrus fruits.

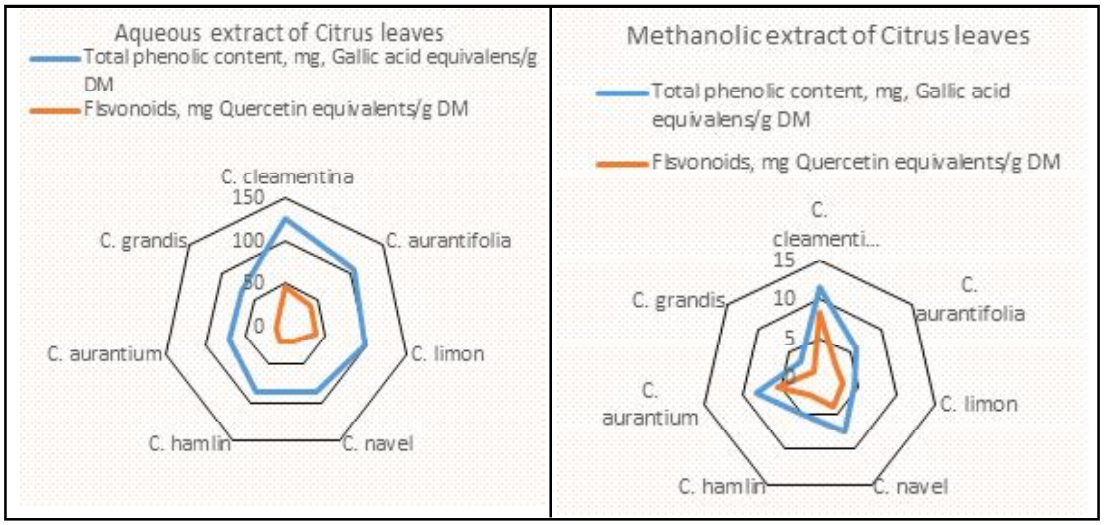

Figure-6. Aqueous and Methanolic Extract Citrus leaves propoerty

The research on the seed and peel of Citrus Sinensis had made in different angle, the physicochemical and phytochemical investigation, GC-MS Analysis and DPPH Assay Antioxidant potential. The values of Physico-chemical parameters of the oil samples of Orange peel oil is higher than Orange seed oil (pH value 5.5 in respect of 4.5 , Acid value (mgKOH/g) is 26.1 in respect of 23.5). Total phenolic and total flavonoid contents of Citrus leaves measure by aqueous extract and Methanolic extract along with other 6 parameter, further finding are summarized in tabulated and graphical representation for ease of understanding. The results of our study showed that *Citrus sinensis, C. limetta* and *C. limon* peel oil have the probability to be applied as a natural

constituent of food preservations, cosmetics and medi-cines as they exhibit a strong antioxidant, antibacterial and antifungal activity against food borne pathogens. In conclusion, the Chemical analysis of essential oil extracted from *Citrus sinensis, C. limetta* and *C. limon* showed in research able to justify the importance of the citrus seed oil.

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Conflict of interest :

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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