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STUDY ON AQUEOUS EXTRACT OF *Citrus limon L.* PEEL

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Abstract : The principal components detected in lemon peel aqueous extract are γ-terpinene, terpinolene, d-limonene, and citral. In this study characterization is carried out by UV visible spectroscopy and FT-IR analysis gives functional groups present (=C−H) 3022.25cm^{-1}, (C-H) 2918.10cm^{-1}, (C=C)780.90cm^{-1} ,(>C=C<) 3195.19 cm^{-1} which is sp^2 carbons, (CHO) 2357.23 cm^{-1} and 2935.37 cm^{-1} was observed. Ascorbic acid estimated is 210.4mg /g dw by DNPH method. Presence of Phenol, flavanoids, steroids got to know by phytochemical analysis. 78.26% of Radical scavenging activity reported at 1000 µg /ml.

Index Terms - lemon peel , DNPH ,DPPH ,ascorbic acid ,functional groups.

INTRODUCTION

Lemon (*Citrus limon L.*) is used in a variety of cuisines, including soft drinks, alcoholic beverages, and jams. Citric acid, ascorbic acid, minerals, and flavonoids are among the nutrients found in lemons. Flavonoid chemicals are found in all vascular plants and are a significant group of naturally occurring compounds in the plant world. Citrus, as well as other fruits, vegetables, nuts, seeds, grains, tea, and wine, contain flavonoids, and the normal Western diet contains about 1 gram of mixed flavonoids (Miyake *et.al.*, 1997). Lemon is a valuable medicinal plant of the Rutaceae family. It is grown primarily for its alkaloids, which have anticancer and antibacterial properties in crude extracts from various sections. Flavonoids have the ability to alter enzyme activity and act as direct antioxidants and free radical scavengers, stop cells from multiplying. (Dhanavade *et.al.*, 2011).

Antioxidants are compounds that may neutralise free radicals by absorbing or giving an electron to remove the free radical species' unpaired state. They protect other molecules in the body from destructive oxidation processes by interacting with free radicals, primarily reactive oxygen species (ROS), the most toxic result of the aerobic environment, at low concentrations relative to the oxidisable substrate. As the beneficial antioxidant molecule is oxidised, there is a continuing need to replace antioxidant supplies, either endogenously or by supplementation, during these processes (Akhila *et.al.*,2009).

D-limonene is utilised as an insect-killing ingredient in insecticides and as a "green" biodegradable and non-toxic solvent in the oleo chemical, wax, resin, paint, and glue industries, in addition to these food, cosmetic, pharmaceutical and d pharmacological applications. As a result, D-limonene is gaining popularity as a green solvent in analytical chemistry and in the extraction of lipids from microalgae or plant matrices (Lopresto *et.al.*, 2014).

Both the pharmaceutical and food sectors have shown a growing interest in the valorization of phenolic substances such as flavonoids and phenolic acids in recent decades. Lemon (*Citrus limon*) peels, which account for 50–65 percent of the total weight of the fruit, are a good source of phenolic compounds such as phenolic acids (ferulic, p-coumaric, and sinapic acids) and flavonoids (flavanones, flavonols, flavones), which have been linked to antimicrobial, anticancer, and antioxidant activities. High temperatures or oxidation can cause phenolic compounds to deteriorate. As a result, a significant proportion of polyphenols may be lost during the drying process. The majority of studies on Citrus waste have looked at the impact of freeze-drying and hot air-drying on polyphenol concentration (Papoutsis *et.al.*, 2017).

However, the majority of these extraction procedures have drawbacks, such as chemical degradation caused by high temperatures or ultrasonic power, mass transfer resistance, extended extraction durations, huge volumes of solvents, or health risks. In other circumstances, the extraction methods' equipment is also difficult to use and/or expensive. Despite their efficiency, their usage at industrial scale raises questions about cost, toxicity, and safety, with water being the preferred solvent for high volume extraction. As a result, scientific efforts should be focused on ways to improve aqueous extraction efficiency, as well as the development of safer, more efficient, energy-efficient, and sustainable extraction processes for citrus waste reuse, which can also benefit the food industry in terms of effectiveness, profitability, time, and solvent consumption. These extraction technologies have the potential to bring value to the citrus processing sector while also setting a precedent for cleaner production of high-demand phenolic compounds (Gómez *et.al.*,2019).

The aims of this study are to know photochemical analysis, evaluate the antioxidant property, characterization and ascorbic acid estimation from the extract sample.

RESEARCH METHODOLOGY

A. Materials.

Lemon used for juice purpose were collected from Alva's juice centre ,Moodubidri , Karnataka, India on January 5th 2022,then the used lemon peel separated and cleaned in distilled water for 10 min in room temperature. After that kept it for air drying in hot air oven for 75ºC for 3days then powdered.

B. Characterization

In this study the aqueous extract was subjected to UV- visible spectroscopy in double beam spectrophotometer: 2202.The scan range from 200-600nm. Additionally, Fourier Transform Infrared Spectroscopy in BRUKER is used to detect functional groups in the extract sample in this study the aqueous extract was subjected to UV- visible spectroscopy in double beam spectrophotometer: 2202. The scan range from 200-600 nm. Additionally, Fourier Transform Infrared Spectroscopy in BRUKER is used to detect functional groups in the extract sample.

C. Aqueous extraction.

Aqueous extract from dry lemon powder is obtained via solvent extraction in a soxhlet system. A glass extractor is sandwiched between a circular bottom flask at the bottom and a bulb condenser at the top of this soxhlet device. A firm matrix of seeds is inserted into the thimble in the glass thimble holder. The extracting solvent was first held in the round-bottom distillation flask, which was heated to 65°C using an electro thermal heating mantle. As the solvent vapour rose to the condenser, it condensed and accumulated inside the extractor (Ali et al., 2015).

D. Phytochemical analysis.

The following approach was used to look for phytochemicals in the extract (Diksha et.al., 2018).

1. Alkaloids were determined by mixing 5 mL of extract with 2 mL of HCl. Mayer's Wagner reagent was added to this.
2. Tannins were tested using 5mL of extract and a few drops of 1% Lead acetate.
3. Saponins were determined by diluting the 5ml extract with distilled water and vigorously agitating it.
4. Glycosides were determined by mixing the crude extract with 2 mL of chloroform. After that, 2ml of concentrated H_2SO_4 was cautiously added and gently shaken. (Yadav *et.al.*,2011).
5. Steroid test was done when crude extract was combined with 2 mL chloroform and concentrated H_2SO_4 was added sideways. (Yadav *et.al.*,2011).
6. Phenols were determined by combining crude extract with 2% $FeCl_3$.

E. Determination of antioxidant property by DPPH method.

The assay is carried out using a slightly modified version of the technique reported by (Arun *et.al.*, 2012).The DPPH method is used to conduct the test, which results in a high absorbance at 517nm. The reaction is performed with freshly generated 0.0043% 2,2-diphenyl-1-picrylhydrazyl in ethanol and a 1 mg/ml Standard Ascorbic acid solution. The material is divided into different concentrations in individual test tubes, and the volume is increased to 2ml using ethanol. 2ml DPPH is added to each test tube and incubated for 30 minutes in the dark. Because DPPH is light sensitive, this test must be carried out in complete darkness.

As a control, 2ml 2,2-diphenyl-1-picrylhydrazyl with 2ml ethanol is used. The OD is measured at 520nm after incubation. The scavenging activity % is computed as follows:

$$\% \text{ Radical scavenging activity} = (A_0 - A_S)/ A_0 * 100$$

Where, A_0 = Absorbance of control,
A_S = Absorbance of sample/ standard

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F .Estimation of ascorbic acid by 2,4-Dinitrophenylhydrazin method.

Fill a series of test tubes with a 10-100 mg standard dehydroascorbic solution. Similarly Pipette out separate aliquots of brominated sample extract (0.5-1.5ml). Add distilled water to each tube to bring the total volume to 3ml. To each tube, add 1ml of DNPH reagent and 1-2 drops of thiourea. Create a blank in the same way as before, but this time use water instead of the ascorbic acid solution.

Incubate at 37°C for 3 hours after properly mixing the contents of the tubes. After incubation, add 7 mL of 65 percent sulphuric acid to dissolve the orange red osazone crystals. At 540nm, measure the absorbance (Roe *et.al.*, 1944).

RESULTS AND DISCUSSION

Characterization:

In this study the aqueous extract was subjected to UV visible spectroscopy, the peak exited in 280nm it says the presence of aldehyde group (Casale *et.al.*,2007).

Then it sent to sent to FT-IR analysis, from that (=C−H) 3022.25cm^{-1}, (C-H) 2918.10cm^{-1}, (C=C)780.90cm^{-1} ,(>C=C<) 3195.19 cm^{-1} which is sp^2 carbons, (CHO) 2357.23 cm^{-1} and 2935.37 cm^{-1} shown in fig (a) and (b)

The principal components detected in lemon peel aqueous extract are γ-terpinene, terpinolene , d-limonene, and citral (Nguyen, 2009).

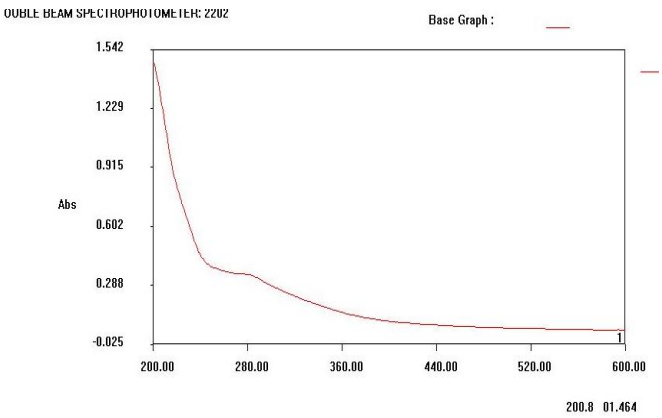

Fig (a) UV-visible spectrum analysis of aqueous extract of *Citrus limon L.* Peel

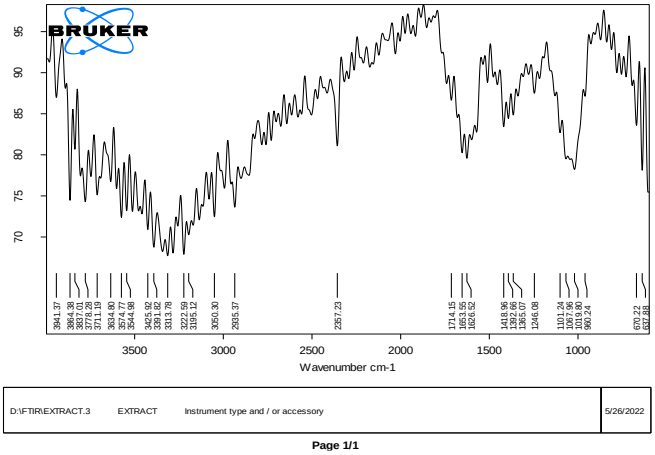

Fig (b) FTIR analysis for aqueous extract of *Citrus limon L.* Peel

Table 01: Phytochemical screening of aqueous extract of *Citrus limon L.* Peel.

Phytochemicals	Aqueous extract of *Citrus limon .L*
Phenols	+
Flavonoids	+
Saponins	–
Steroids	+
Glycosides	+
Tannins	–
Alkaloids	–

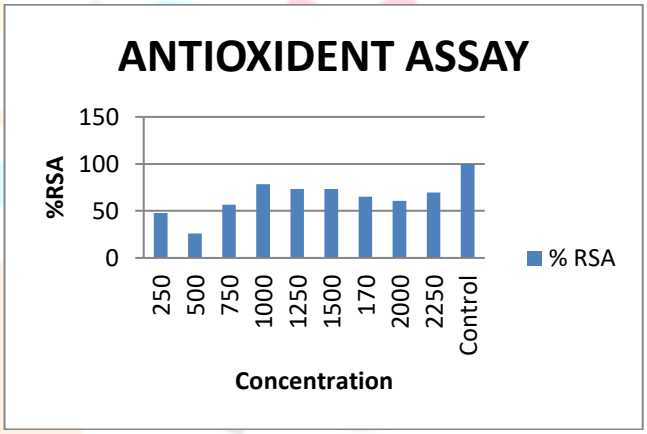

Fig (c): DPPH Radical scavenging activity of Aqueous extract of *Citrus limon L. Peel* extract

Phytochemical analysis.

The phytochemical screening was done for qualitatively determine the different phytochemicals present in the Table 01. Indicates the different phytochemicals present in the aqueous extract of *Citrus limon L.* peel extract. The positive sigh indicates the presence and the negative sign indicates the absence of the phytochemical. The present study reveals the presence of Phenol, flavanoids, steroids and glycosides. Alkaloids, saponins, tannins were absent in the aqueous extract of *Citrus limon L. Peel.*

Antioxidant assay

Percentage free radical scavenging by the DPPH method in which *Citrus limon L.* peel of concentrations 250 µg, 500 µg, 750µg, 1000 µg, 12500 µg,1500 µg , 1750 µg ,200 µg and 2250 µg showed % scavenging activity of 47.8, 26.0, 56.5, 78.26, 73.46, 73.46, 65.0, 60.8 and 69.5 respectively.

The % free radical scavenging activity of *Citrus limon L. Peel* extract is given in fig (c) and Ascorbic acid is taken as standard which shows 100% Radical scavenging activity.

The extracts produced with 50 percent acetone had the best antioxidant activity in the DPPH experiment. Water and pure acetone extracts exhibited the lowest antioxidant capabilities, followed by methanol (Papoutsis *et.al.*2017).

Estimation of Ascorbic acid:

The Mean ascorbic acid estimated by 2,4-Dinitrophenylhydrazine method is 210.4mg /g dw from *Citrus limon L.* aqueous extract .Fresh lemon peel has a concentration of 288.96 mg/g dw of ascorbic acid. When compared to fresh samples, the ascorbic acid level of dried peels reduced. It might be due to the lengthy drying process (Tekgül *et.al.*, 2018).

Conclusion

Citrus limon .L peel waste management is a issue, from this peel waste more useful outcome can be produced .It have elements such as are γ-terpinene, terpinolene, d-limonene, and citral along with that it also having good antioxidant quality so we can use in pharmaceutical, food additive, skin care and perfume industry. Clinical trials have shown that d-limonene can dissolve cholesterol-containing gallstones. It has also been used to relieve heartburn and gastroesophageal reflux due to its stomach acid neutralising action and maintenance of proper peristalsis (GERD).

References

Akhila, S., Bindu, A. R., Bindu, K., & Aleykutty, N. A. (2009). Comparative evaluation of extracts of Citrus limon burm peel for antioxidant activity. *Journal of Young Pharmacists*, *1*(2), 136-140.

Ali, M. A., Al-Hattab, T. A., & Al-Hydary, I. A. (2015). Extraction of date palm seed oil (Phoenix dactylifera) by soxhlet apparatus. *International Journal of Advances in Engineering & Technology*, *8*(3), 261.

Dhanavade, M. J., Jalkute, C. B., Ghosh, J. S., & Sonawane, K. D. (2011). Study antimicrobial activity of lemon (Citrus lemon L.) peel extract. *British Journal of pharmacology and Toxicology*, *2*(3), 119-122.

Diksha, G. R., and Dr. Nibha. (2018)Formulation and Evaluation of foot cream from *Ficus glomerata* extract. *Indo- American Journal of Pharmaceutical Research*.:8(09):93-100

Gómez-Mejía, E., Rosales-Conrado, N., León-González, M. E., & Madrid, Y. (2019). Citrus peels waste as a source of value-added compounds: Extraction and quantification of bioactive polyphenols. *Food chemistry*, *295*, 289-299.

Lopresto, C. G., Petrillo, F., Casazza, A. A., Aliakbarian, B., Perego, P., & Calabrò, V. (2014). A non-conventional method to extract D-limonene from waste lemon peels and comparison with traditional Soxhlet extraction. *Separation and Purification Technology*, *137*, 13-20.

Miyake, Y., Yamamoto, K., Morimitsu, Y., & Osawa, T. (1997). Isolation of C-glucosylflavone from lemon peel and antioxidative activity of flavonoid compounds in lemon fruit. *Journal of agricultural and food chemistry*, *45*(12), 4619-4623.

Nguyen, H., Campi, E. M., Jackson, W. R., & Patti, A. F. (2009). Effect of oxidative deterioration on flavour and aroma components of lemon oil. *Food Chemistry*, *112*(2), 388-393.

Papoutsis, K., Pristijono, P., Golding, J. B., Stathopoulos, C. E., Bowyer, M. C., Scarlett, C. J., & Vuong, Q. V. (2017). Effect of vacuum-drying, hot air-drying and freeze-drying on polyphenols and antioxidant capacity of lemon (Citrus limon) pomace aqueous extracts. *International Journal of Food Science & Technology*, *52*(4), 880-887.

Yadav, R. N. S., & Agarwala, M. (2011). Phytochemical analysis of some medicinal plants. Journal of phytology, 3(12).

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