WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 7, 1087-1103. Research Article ISSN 2277–7105

EXTRACTION OF ESSENTIAL OIL FROM *CITRUS LIMON* AND *CITRUS LIMETTA*: CHARACTERISATION AND ANTIOXIDANT ACTIVITY

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Article Received on 10 February 2025,
Revised on 30 Feb. 2025,
Accepted on 20 March 2025
DOI: 10.20959/wjpr20257-36071

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ABSTRACT

Citrus peels contain various bioactive compounds which show therapeutic value. Soxhlet extraction with ethanol as the solvent was used to extract essential oils from *Citrus limon* (lemon) and *Citrus limetta* (mosambi) peel samples. Phytochemical analysis showed alkaloids terpenoids and flavonoids steroids and sugars occurred in both citrus samples but quinine was found specifically in mosambi and phenol-only products and proteins existed only in lemon. The analysis by thin-layer chromatography (TLC) showed different compound separations for *Citrus limon* which had three compounds with R_f values at 0.246, 0.692, 0.815 and *Citrus limetta* contained two compounds with R_f values of 0.307, 0.415. The antioxidant measurements revealed mosambi (276 µg/g) exhibited higher activity than lemon (218 µg/g). Total phenolic content reached 0.814 µg/g in mosambi whereas lemon showed 0.740 µg/g but FRAP results demonstrated stronger reducing power in lemon with 0.692 µg/g compared to mosambi with 0.421 µg/g. The extracted oils received microencapsulation treatment through the sodium alginate method for developing more stable products with planned market applications. Studies revealed distinct phytochemical patterns and antioxidant capabilities between *Citrus lemon* and *Citrus limetta* which demonstrates their practical applications in pharmaceutical development and nutraceutical usage.

KEYWORDS: Citrus peel oil, Phytochemical analysis, Antioxidant activity, Thin-layer chromatography, Microencapsulation.

INTRODUCTION

The global market values citrus fruits as one of the most popular cultivated products because of their therapeutic compounds and their nutritional value as well as industrial use (Dhuique-Mayer *et al.*, 2005). The therapeutic properties of citrus fruits are derived from their bioactive compounds that include flavonoids and alkaloids as well as terpenoids and essential oils (Singh *et al.*, 2023). The waste material from citrus peels contains high levels of bioactive compounds while also exhibiting antimicrobial properties and antioxidant elements and anti-inflammatory capabilities (Saini *et al.*, 2022). Lemon (*Citrus limon*) together with mosambi (*Citrus limetta*) are everyday citrus types with medicinal properties and abundant phytochemical concentrations. Studying bioactive compounds from citrus peels as chemical entities creates new opportunities for drug development, cosmetic manufacturing and food preservation applications.

The important role of antioxidants within citrus peel compounds supports free radical neutralization for prevention of cardiovascular diseases and neurodegenerative conditions and cancer (Hegazy and Ibrahium, 2012). The failure of an organism to maintain equilibrium between reactive oxygen species (ROS) and antioxidant defenses leads to oxidative stress that damages cells and generates chronic illnesses (Lobo *et al.*, 2010). Multiple studies demonstrate that citrus peel extracts hold significant levels of natural antioxidants making them promising options for nutraceutical applications and medical treatment (Baljeet *et al.*, 2015). The antimicrobial properties of citrus peel extracts emerge from their secondary metabolites which include flavonoids terpenoids and alkaloids (Kumar *et al.*, 2014). Studies indicate that citrus peel compounds function as antimicrobial agents which helps scientists develop both natural food preservation methods and new alternative antimicrobial solutions (Sasidharan *et al.*, 2011). The medical advantages of citrus peel compounds increase through their steroid and phenolic chemical composition and thus demand systematic analytic investigation.

The separation and identification of plant extract phytochemicals depends on thin-layer chromatography as a common analytical method. TLC allows researchers to determine retention factor (R_f) values that identify particular bioactive compounds responsible for biological activity (Fried and Sherma, 1999) The actual implementation of citrus peel essential oils faces restrictions because of their unstable nature and high volatility tendency. Sodium alginate technologies serve as microencapsulation methods to improve shelf stability while controlling release of phytochemicals. The process of encapsulation enables bioactive compounds to become more stable which makes them compatible for pharmaceuticals and cosmetics as well as food products (Santos *et al.,* 2015).

The chemical evaluation of citrus peel extracts utilizes thin-layer chromatography (TLC) extensively. The method utilizes retention factor (R_f) values to separate and identify phytochemicals from plant extracts and reveals their biochemical variety. The identification of important phytochemicals via TLC analysis enables pharmaceutical assessment for health-related industrial applications. The practical uses of citrus essential oils face significant limitations because they readily evaporate and become unstable under conditions that include atmospheric factors such as light and oxygen together with temperature variations. Scientists have developed microencapsulation methods to boost the stability and manage the release profiles of bioactive compounds according to Santos *et al.* (2015). Commercial applications benefit from sodium alginate encapsulation as an effective method which maintains essential oils functional characteristics while extending their shelf-life and improving their bio accessibility.

The research examines essential oil extraction from *Citrus limon* and *Citrus limetta* peels followed by phytochemical analysis and antioxidant activity testing and concludes with microencapsulation through sodium alginate encapsulation. A comparison between the antioxidant capabilities and phytochemical distributions of *Citrus lemon* and *Citrus limetta* will demonstrate their value as pharmaceutical and food processing ingredients. The study results will advance the comprehension of citrus peel bioactive elements and their health-enhancing properties along with creating sustainable methods to use citrus waste in industrial applications.

MATERIALS AND METHODS

Collection and Extraction of Oil from the Sample

Citrus limon and *Citrus limetta* were obtained from the local market. Their peels were carefully separated, chopped into small fragments, and left to dry under direct sunlight for one day.

Soxhlet Extraction of the Sample and Phytochemical Screening

Once dried, 5 grams of each peel sample were subjected to Soxhlet extraction using 50 ml of ethanol, ensuring the individual extraction of oils from both citrus varieties. The extracted oils were then filtered and stored for further analysis. Qualitative phytochemical screening was carried out to determine the presence of alkaloids, terpenoids, proteins, sterols, quinones, flavonoids, tannins, saponin and phenolics (Rajan *et al*. 2015).

Characterization of the Extracted Compound

(a) UV–Visible Spectroscopy

The preliminary characterization of the essential oils extracted from *Citrus limon* and *Citrus limetta* was conducted using UV–Visible spectroscopy. The transformation of bioactive compounds within the oils was monitored by measuring their UV–Visible absorption spectra after dilution with deionized water. Spectral recordings were performed using a Labtronics LT -291 UV spectrophotometer, with the samples placed in quartz cuvettes and scanned at a across the wavelength range of 400 to 700 nm. Deionized water was used as the blank control to ensure accurate measurements (Momin *et al.,* 2012).

(b) FTIR

The Fourier Transform Infrared (FTIR) spectroscopy analysis was conducted to identify the functional groups present in the essential oils extracted from *Citrus limon* and *Citrus limetta.* The FTIR spectra were recorded using the KBr pellet method, which involves mixing a small amount of the extracted oil with spectroscopic-grade potassium bromide (KBr) to form a fine powder. This mixture was then compressed into a thin, transparent pellet using a hydraulic press. The prepared KBr pellet was placed in the sample holder of the FTIR spectrometer, and the spectra were recorded in the range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1}. Deionized water was used as a blank to ensure precise spectral readings (Hawkins *et al.,* 2010).

(c) Thin- Layer Chromatography

Thin-layer chromatography (TLC) was utilized as an essential technique to separate and identify the phytochemical constituents present in the extracts of *Citrus limon* and *Citrus limetta* (Sa'adi *et al.,* 2016). A small quantity of each plant extract was carefully applied to a pre-coated silica gel TLC plate (Merck, Germany). The plate was then developed using a solvent system consisting of Toulene and Ethyl Acetate in a ratio of 9:7. After development, the plate was examined under ultraviolet (UV) light to visualize the separated compounds and the R_f value was calculated.

Evaluation of the Antioxidant properties of the Essential oil

(a) Total Antioxidant Activity by Phosphomolybdenum Method

The antioxidant potential of *Citrus limon* and *Citrus limetta* essential oils was evaluated using the phosphomolybdenum method. A total of 0.5 mL of the antioxidant reaction mixture was added to each sample to initiate the reaction. The mixtures were thoroughly homogenized and incubated in a water bath at 50°C for 90 minutes to ensure complete reaction development. Following incubation, the optical density (OD) of each sample was measured at a wavelength of 695 nm using a Labtronics LT 291 UV-Visible spectrophotometer (Prieto *et al.*, 1999).

(b) Total Phenolic Content Determination

The total phenol content of the essential oils were assessed using the Folin-Ciocalteu method, following the procedure described by (Ainsworth and Gillespie, 2007). In this method, 0.5 mL of each extract was combined with 2.5 mL of Folin-Ciocalteu reagent. To this mixture, 2 mL of 7.5% sodium carbonate solution was added. The reaction mixtures were then incubated in the dark for 30 minutes to prevent photo degradation. After the incubation period, the absorbance of the samples was measured at 765 nm using a UV-Visible spectrophotometer. The total phenol content was expressed in terms of gallic acid equivalents (GAE) per mg/g of extract.

(c) FRAP

The ferric reducing antioxidant power (FRAP) assay was conducted to evaluate the antioxidant potential of the oil, following the method described by (Gohari *et al.*, 2011). In this assay, the extracted oils were combined with 1 mL of phosphate buffer and 1 mL of 1% potassium ferricyanide. The mixture was then incubated in a water bath at 50°C for 20 minutes to facilitate the reduction reaction. After the incubation period, the mixture was allowed to cool to room temperature, and 2.5 mL of 10% trichloroacetic acid (TCA) was added to stop the reaction. Subsequently, the solution was blended with 2 mL of distilled water and 0.25 mL of freshly prepared 0.1% ferric chloride solution to enhance colour development. The absorbance of the final solution was measured at 700 nm using a UV-Visible spectrophotometer.

Microencapsulation Technique

The microencapsulation of *Citrus limon* and *Citrus limetta* extracts was carried out using the sodium alginate-calcium chloride ($CaCl_2$) method, as described by (Fadlila *et al.*, 2024). A 2% $CaCl_2$ solution was prepared in distilled water, ensuring complete dissolution. 3% of sodium alginate was incorporated to each plant extract separately and mixed. The mixture was then heated to achieve uniform dispersion. To adjust the consistency, additionally distilled water was added to the solution. The prepared $CaCl_2$ solution was transferred to a vessel, and the sodium alginate-extract mixture was carefully added drop wise using a micropipette. Upon contact with the $CaCl_2$ solution, the droplets formed stable, gel-like beads.

RESULT AND DISCUSSION

The synthesis of gels using natural essential oils has gained significant attention due to their potential therapeutic and pharmaceutical applications. Incorporating these essential oils into a gel formulation using carbopol and sodium alginate enhances their stability and usability for skincare, pharmaceutical, and cosmetic applications. The antioxidant properties of the formulated gel play a crucial role in neutralizing free radicals, potentially offering protective effects against oxidative stress-related conditions.

Extraction and Phytochemical Screening

Soxhlet extraction serves widely as a method to extract bioactive compounds from plant materials while minimizing both extraction efficiency and solvent waste. Ethanol served as the extraction solvent because its solvent characteristics enable the extraction of polar as well as non-polar phytochemicals thereby enhancing the recovery rate of these bioactive compounds (Azwanida, 2015). The extraction method utilized Soxhlet extraction to recycle solvent continuously through *Citrus limon* and *Citrus limetta* peel specimens in order to extract compounds more efficiently. The extraction of flavonoids, alkaloids, phenols, and terpenoids using ethanol as a solvent has been confirmed by Sasidharan *et al.*,(2011) because these compounds possess antioxidant, antimicrobial, and therapeutic functions. The essential oils underwent filtration before scientists analyzed their phytochemical compositions together with their antioxidant properties.

The phytochemical assessment of essential oils obtained from *Citrus limon* and *Citrus limetta* demonstrated multiple bioactive compounds suitable for medical applications and industrial usage. Therapeutic compounds alkaloids and terpenoids and flavonoids and steroids were detected in both citrus extracts based on data shown in Table 1. Alkaloid compounds within extracts demonstrate multiple pharmacological properties mainly anti-inflammatory features with antimicrobial action (Siddiquee *et al.*, 2023). Evidence of flavonoids in citrus essential oils confirms their therapeutic usefulness in treating conditions that result from oxidative stress.

Table 1: Phytochemical Screening Results.

Phytochemical Test	*Citrus limetta* (Mosambi)	*Citrus limon* (Lemon)
Alkaloids	Positive	Positive
Terpenoids	Positive	Positive
Phenol	Negative	Positive
Sugar	Positive	Positive
Saponins	Negative	Negative
Flavonoids	Positive	Positive
Quinine	Positive	Negative
Protein	Negative	Positive
Steroid	Positive	Positive

A specific distribution pattern emerged regarding compound presence in the evaluated *Citrus limetta* and *Citrus limon* samples. The presence of phenols in *Citrus limon* samples marked higher antioxidant potential when these results were compared to the absence of phenols in *Citrus limetta* extracts. Phenolic compounds serve as essential scientific subjects in antioxidant research due to their proven ability to remove free radicals and diminish oxidative stress (Lobo *et al.*, 2010). The substance quinine was present in *Citrus limetta* but absent from *Citrus limon* which indicates different levels of secondary metabolite composition potentially impacting their medical benefits. The absence of proteins in *Citrus limon* while their presence in *Citrus limetta* implies operational restrictions for their employment in nutraceuticals and cosmetics applications. The anti-inflammatory and hormone-regulating properties may be explained by the steroid compounds detected in both extracts. The absence of saponins in the evaluated samples yields restricted foaming capabilities that affect their potential use in pharmaceutical formulations (Saini *et al.*, 2022). Scientific research shows that citrus peels hold important medicinal value thus enabling their application in industrial and natural product development. The phytochemical examination proves that essential oils from *Citrus limon* and *Citrus limetta* have important bioactive materials although they demonstrate minor differences which could shape their particular utilization in pharmaceuticals as well as food production and cosmetics industries.

Characterization of the Extracted Oil

UV-Vis Spectral Analysis

The essential oil spectra from both *Citrus limon* and *Citrus limetta* were assessed using UV-visible spectroscopy over wavelengths from 200–800 nm. The UV spectra reveal intense absorption between 200 to 350 nm which demonstrates the existence of phytochemical compounds including flavonoids terpenoids and phenolic compounds. The peak observed between 200–250 nm in the spectra represents the $\pi \rightarrow \pi^*$ electronic transition in conjugated systems that typically occurs in both flavonoids and phenolic compounds (Saini *et al.*, 2022). During analysis spectra of *Citrus limon* exposed two major peaks at 280 nm and 320 nm indicating polyphenolic compounds present as antioxidants. The chemical content of citrus essential oils particularly flavonoids and limonoids has been extensively studied by Hegazy and Ibrahium (2012) because these compounds contribute to their biological activity. Studies of *Citrus limetta* show it absorbs light at 260 nm as well as 310 nm which supports the existence of bioactive compounds sharing comparable molecular structures.

The UV absorption profiles match between citrus types stay similar but the positioning and peak intensity variations demonstrate differences in their biochemical elements. *Citrus limetta* shows broader absorption bands at 310 nm which suggests elevated flavones and flavonols content because of their antioxidant capacity according to Lobo *et al*. (2010). The intense absorption peaks located at 280 nm within *Citrus limon* indicate that phenolic acids present in higher concentrations compared to other compounds. The spectral data reveals different phytochemical profiles of essential oils since they influence the possibilities for their use in pharmaceutical and nutraceutical products. UV-visible absorption spectra analysis of *Citrus limon* together with *Citrus limetta* demonstrates essential bioactive compounds including flavonoids and phenolics existing in both species. The distinctive spectra demonstrate the distinct chemical makeup of citrus species thus providing opportunities for functional food use along with cosmetic and medicinal applications.

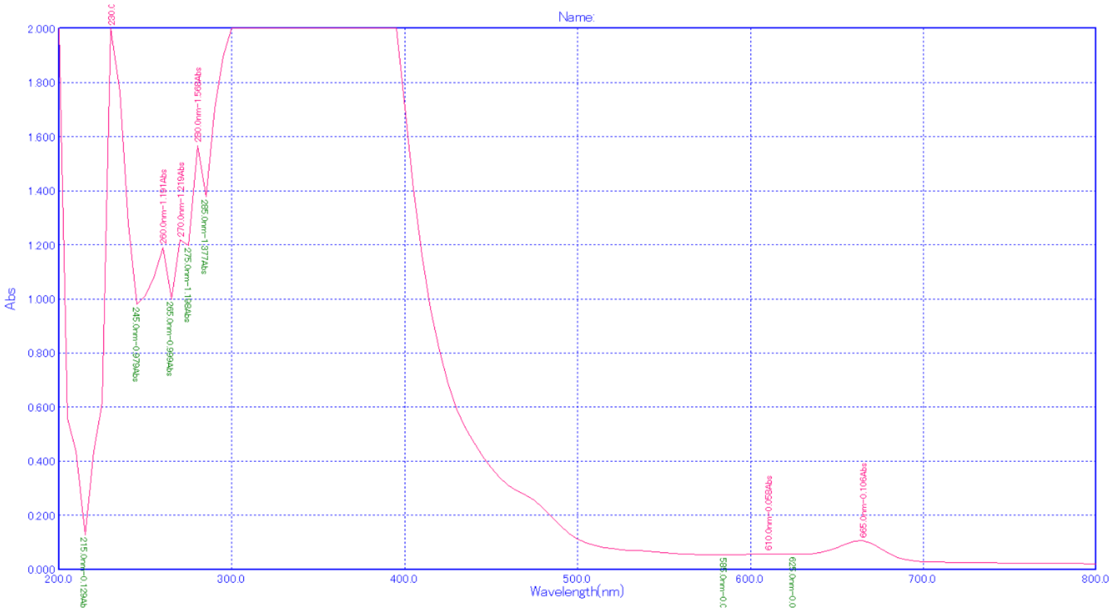

Fig. 1: UV-Vis Spectral Analysis of Citrus Limon.

The Peaks observed at nanometer of 665, 625, 610, 585, 285, 280, 275, 270, 265, 260, 245, 230, 215 were denoting the presence of bioactive compounds in *citrus limon* such as alkaloids, terpenoids, phenol, sugar, flavanoids, protein, steroids.

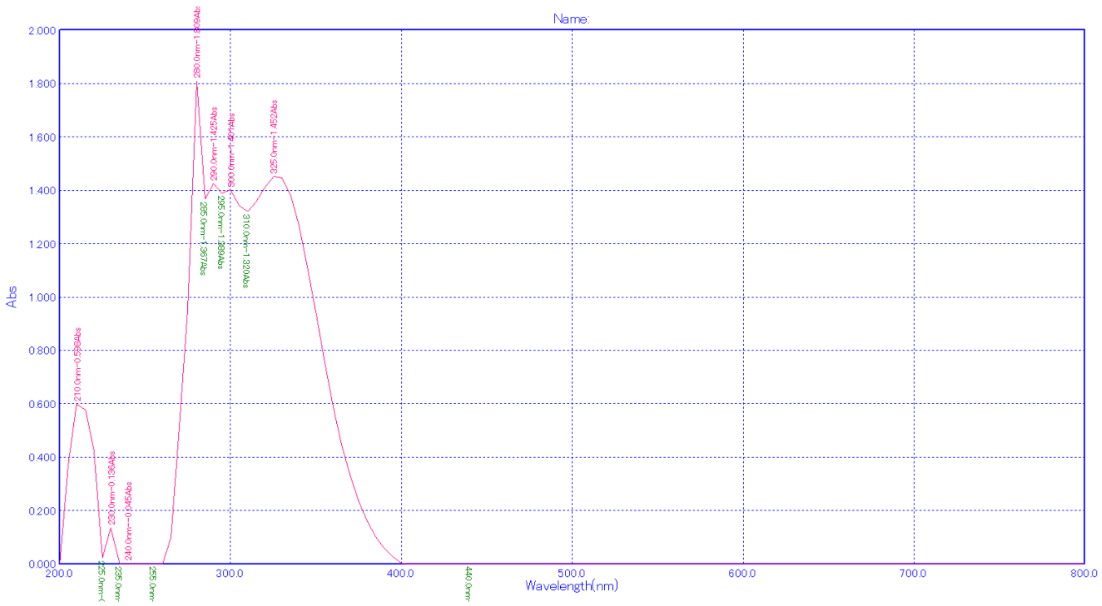

Fig. 2: UV-Vis Spectral Analysis of Citrus Limetta.

The peaks observed at nanometers of 440, 325, 310, 300, 295, 290. 285, 280, 255, 240, 235, 230, 225, 210 were denoting the presence of bioactive compounds in *Citrus limetta* such as alkaloids, terpenoids, sugar, flavanoids, quinine, steroids.

FTIR

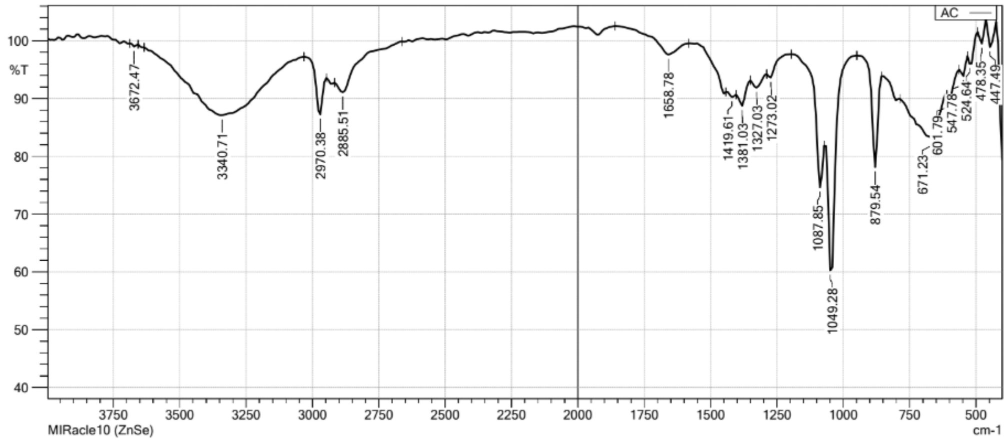

Fig. 3: FTIR of *citrus limetta.*

The functional groups present in the essential oil extracted from the *citrus limetta* were predicted by FTIR. The bonds were determined by interpreting the infrared absorption spectra. The bonds were formed at 3672.47, 3340.71, 2885.51, 1658.78, 1419.61, 1381.03, 1327.03, 1273.02, 1087.85, 1049.28, 671.23, 601.79, 547.78, 524.64, 478.35, 447.49 denoting the presence of water OH Stretch strong, alcohol OH Stretch strong, C-H stretch weak, C=C alkene weak, C=C aromatic, CH3 bend medium, NO2 stretch strong, C-O-C stretch strong , CH3 bend medium , C-F strong, C-F strong, C-CI strong, C-CI strong, C-Br strong, C-Br strong, C-I strong, and C-I strong respectively, the results were coincide with the results of Pharmawati, M., & Wrasiati, 2020.

TLC Analysis

TLC served as a technique to evaluate the phytochemical ingredients in *Citrus limetta* (mosambi) extract together with *Citrus limon* extract. Through the advancement of the mobile phase to 6.5 cm the different bioactive compounds could separate from each other. The mosambi extract produced two distinct bands during TLC that appeared at 2.0 cm and 2.7 cm positions displaying R_f values equal to 0.307 and 0.415 respectively. Phytochemicals such as flavonoids and terpenoids and other polar compounds can be found in the sample because they exhibit unique interactions with the stationary phase (Sasidharan *et al.*, 2011).

The three bands in *Citrus limon* extract appeared at different locations: 1.6 cm, 4.5 cm, and 5.3 cm corresponding to R_f values of 0.246, 0.692, and 0.815. *Citrus limon* contains a higher number of visible bands than *Citrus limetta* which indicates it has a broader diversity in phytochemical content. The band with an R_f value of 0.815 indicates the presence of less polar substances that most likely include essential oil components or non-polar flavonoids whereas the lower R_f values represent more polar compounds like phenolic acids (Wagner & Bladt, 1999). TLC analysis provided effective differentiation of chemical profiles between *Citrus limetta* and *Citrus limon* extracts by showing quantitative variations in phytochemical components. Different chemical properties present in these plants probably lead to their separate biological behaviors through antioxidant effects and antimicrobial action.

Fig. 4: Thin layer chromatography of essential oils extracted from *citrus limetta* and *citrus limon.*

Evaluation of antioxidant properties of extracted essential oils

The antioxidant capabilities of *Citrus limon* and *Citrus limetta* (Mosambi) were determined through the phosphomolybdenum method that measures overall antioxidant potential. Results from the experiment revealed that *Citrus limetta* showed stronger antioxidant properties with a value of 276 µg/g while *Citrus limon* recorded 218 µg/g. The elevated antioxidant properties of *Citrus limetta* relate to its elevated flavonoid and phenolic content that acts as free radical scavenging agents as reported by Singleton *et al.* (1999). Total antioxidant efficacy of *Citrus limetta* depends heavily on its bioactive compounds including limonoids and flavones and ascorbic acid (Sharma *et al.*, 2021).

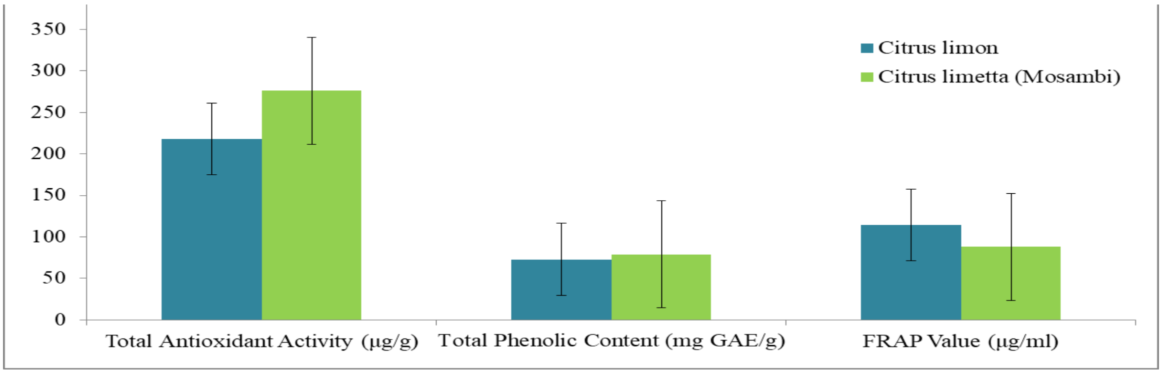

Fig. 5: Graph Showing the Different Antioxidant Potential of essential oils.

The total phenolic content examination revealed that *Citrus limon* possessed 73 mg GAE/g TPC whereas *Citrus limetta* contained 79 mg GAE/g TPC. The elevated phenolic content present in *Citrus limetta* explains its better observed antioxidant activity level. Numerous scientific studies validate the antioxidant functions of phenolic compounds which encompass flavonoids and hydroxycinnamic acids (Dai & Mumper, 2010). The results indicate that *Citrus limetta* provides stronger natural antioxidant protection than *Citrus limon* because phenolic compounds demonstrate extensive oxidative stress inhibition capacity.

The FRAP assay examined the reducing capability of citrus extracts by following the reduction of Fe^{3+} to Fe^{2+}. The evaluation revealed that *Citrus limon* extract provided superior FRAP measurement at 114µg/ml compared to *Citrus limetta* extract with 88µg/ml. The FRAP results show *Citrus limetta* exceeds in total phenolic content and antioxidant ability but *Citrus limon* contains particular reducing agents including ascorbic acid that help in ferric ion reduction (Benzie & Strain, 1996). FRAP results between citrus species reveal distinct antioxidant properties which underline why scientists should evaluate antioxidants through various tests to understand their mechanisms.

Synthesis of Carbopol and Sodium Alginate based Gel

A successful gel formulation with *Citrus limon* and *Citrus limetta* extracts was synthesized through the combination of sodium alginate e and Carbopol together with glycerol. The polymers united to create a matrix that delivered uniform dispersion of the plant extracts. A smooth homogenous gel texture appeared with an excellent application consistency that was easily visible. Glycerol worked as a humectant to improve the gel's hydration capacity while stopping it from drying too quickly (Varshosaz *et al.*, 2006). The successful gel creation indicates the potential of including *Citrus limon*and *Citrus limetta* extracts in this matrix to boost their therapeutic benefits.

Microencapsulation Studies

A successful encapsulation process of *Citrus limon* and *Citrus limetta* extracts occurred by using sodium alginate and calcium chloride ($CaCl_2$) in an ionic gelation method. The addition of alginate-extract mixture into $CaCl_2$ solution produced well-defined spherical beads which confirmed successful bioactive compound encapsulation from the extract sources. The process of bead formation occurs due to calcium ion interaction with alginate molecules that triggers gelation according to the findings of George & Abraham (2006). The encapsulation efficiency depends on sodium alginate concentration as well as extract viscosity and the strength of matrix-compound bindings.

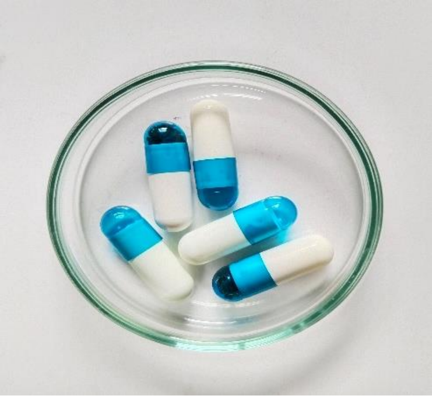

Fig. 6: Microencapsulated sample loaded into capsules.

Bioactive compounds need microencapsulation for environmental stability because the method protects compounds against heat stresses and pH variations and oxidative damage which ultimately extends their biological effectiveness, and it can also significantly extent the shelf life of the product. The prepared beads maintained a uniform and stable structure because the encapsulation process successfully trapped citrus extracts in the alginate matrix.

Alginate proves to be an ideal biopolymer material because it maintains both non-toxic and biodegradable traits while being fully biocompatible thus allowing its usage in food and pharmaceutical industry controlled release applications (Burey *et al.*, 2008). Different extract compositions together with viscosity levels influenced the microencapsulation efficiency outcomes between *Citrus limon* and *Citrus limetta*. Bead formation demonstrates that this procedure proves suitable for stabilizing citrus extracts' antioxidant qualities and phytochemicals that could be implemented in functional foods and therapeutic products (Ribeiro *et al.*, 2020).

CONCLUSION

The essential oils from *citrus limetta* and citrus limon was extracted successfully with evaluation of antioxidant properties and phytochemical distributions. Phytochemical screening tests identified alkaloids along with terpenoids, flavonoids and steroids among the bioactive compounds within the citrus extracts. The antioxidant evaluations through phosphomolybdenum method, total phenol determination and FRAP assay showed *Citrus limetta* yielded higher antioxidant action than *Citrus limon*. UV absorption spectra showed bioactive compounds as present in the extracts through results that TLC analysis supplemented with the detection of diverse phytochemicals. Bioactive compound protection together with controlled release occurs after microencapsulation with sodium alginate and calcium chloride which produces stable beads that boost their potential usage for pharmaceuticals and cosmetics. The experimental results demonstrate that citrus essential oils show promise as natural antioxidant solutions. Studies in the near future should aim to enhance formulation stability alongside bioavailability and storage durability of these substances. The commercial viability of these formulations needs *in vivo* testing along with clinical assessments to demonstrate their therapeutic actions.

ACKNOWLEDGEMENT

We sincerely express our gratitude to Dr.R Renuka, Sri Ramakrishna College of Arts & Science for Womenfor providing guidance and support to conduct this research. We are also deeply thankful to Dr. R Ragunathan, Director and Research Supervisor and Dr. Jesteena Johney, Head of the Department of Centre for Bioscience and Nanoscience Research for granting access to their advanced research facilities, which played a crucial role in carrying out our experimental work efficiently. We would also like to extend our thanks to Ms. V. Abhirami, Microbiologist of the Centre for Bioscience and Nanoscience Research for the technical assistance, cooperation, and valuable discussions that contributed to the smooth execution of our experiments.

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