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Bioactive Potentials of *Citrus aurantifolia* Leaf Extracts: A Solvent-Based Investigation into Antioxidant, Antibacterial, and Cytotoxic Activities

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Abstract: *Citrus aurantifolia* is a medicinal plant widely recognized for its therapeutic properties in traditional medicine. Its leaves, in particular, are known to possess bioactive compounds with potential pharmacological relevance. This study aimed to evaluate the antioxidant, antibacterial, and cytotoxic properties of *C. aurantifolia* leaf extracts obtained using four different solvents viz. hexane, diethyl ether, isopropanol and ethanol, highlighting the influence of solvent polarity on bioactivity. The antioxidant potential was assessed using DPPH radical scavenging assay, antibacterial efficacy was tested via the agar well diffusion method against five bacterial strains, and cytotoxicity was evaluated using the brine shrimp lethality bioassay. Statistical analyses including ANOVA, Tukey's HSD, and Pearson's correlation were performed to determine significance and correlations between extract concentration and bioactivity. Results revealed that the hexane extract exhibited the highest antioxidant activity with an IC_{50} of 3 mg/ml, followed by ethanol and isopropanol extracts. Among the antibacterial tests, hexane and isopropanol extracts showed consistent and highest mean inhibition, followed by ethanol extract. Cytotoxic studies demonstrated strong lethality for the diethyl ether extract (LC_{90} = 22 µg/ml), indicating potent bioactivity. Correlation analysis confirmed a positive relationship between extract concentration and antibacterial activity. It is concluded that *C. aurantifolia* leaf extracts showed promising antioxidant, antibacterial, and cytotoxic properties, with bioactivity varying significantly based on the solvent used.

Keywords: Key lime, Medicinal plant, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Citrus aurantifolia*, Anti-microbial, Diethyl ether extract, Isopropanol extract, DPPH

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Introduction

Natural products have long been recognized as a rich source of therapeutic agents, particularly antioxidants that counteract the harmful effects of reactive oxygen (ROS) and nitrogen species (RNS),

which contribute to diseases such as cancer, cardiovascular disorders, and neuro-degeneration (Paudel *et al*., 2014). Synthetic antioxidants, though effective, carry potential carcinogenic risks, driving the search for safer, naturally-derived alternatives. Concurrently, the rise of antibiotic-resistant pathogens underscores the urgent need for novel antimicrobials. Evaluating plant extracts for antioxidant and antibacterial efficacy thus meets a dual need, namely, safeguarding against oxidative stress while addressing evolving infectious threats.

Brine shrimp lethality bioassays, used as a preliminary screen for cytotoxicity, have proven valuable in drug discovery and toxicological assessment, especially due to their simplicity, cost-effectiveness, and correlation with antitumor activity in higher models (Mbwambo *et al*., 2007). Compounds exhibiting notable brine shrimp lethality (low LC_{90}) often advance to more specific cancer or antimicrobial screens (Rahman and Islam, 2013). Conducting integrated studies, simultaneously assessing antioxidant potential, antibacterial activity, and brine shrimp cyto-toxicity provides a comprehensive profile of a plant extract's pharmacological promise.

Citrus aurantifolia, a small shrubby tree of the Rutaceae family, typically reaches up to 5 m and is characterized by thorny branches, ovate leaves, and yellowish-white flowers (Enejoh *et al*., 2015). Botanically, it is a Southeast Asian hybrid, distinguished by its aromatic, highly acidic green fruit that ripens to yellow and bears numerous seeds. Phytochemical investigations of the leaves have identified a rich array of bioactive constituents, including flavonoids (apigenin, quercetin, kaempferol, nobiletin, rutin, hesperetin, naringenin), limonoids, phenolic acids, terpenoids, coumarins, and essential oils rich in D-limonene, neral, and linalool (Loizzo *et al*., 2012). GC–MS studies confirm the leaf essential oil is a D-limonene chemotype accounting for approxi-mately 57.8% of its composition (Ibrahim *et al*., 2019).

Traditionally, *C. aurantifolia* leaves have been used in decoctions or poultices to treat headaches, fever, hypertension, coughs, sore throats, and digestive upsets, while the juice has been used as an antiscorbutic, diuretic, and febrifuge (Khare, 2008). Modern pharmacological studies validate several of these traditional claims: the leaf essential oil demonstrated significant antihyper-glycemic activity in alloxan-induced diabetic rats lowering blood glucose, improving lipid profiles, and enhancing hepatic glycogen (Ibrahim *et al*., 2019). Essential oils displayed potent antioxidant capacity and hypolipidemic effect (Lin *et al*., 2019). Anti-cholinesterase activity was observed in both essential oils and hexane leaf extracts supporting potential neuroprotective effects. Additional pharmacological gains have been reported, including antibacterial, antiplatelet, anti-inflammatory, anticancer, and anti-obesity properties, confirming that *C. aurantifolia* leaves are a valuable source of bioactive compounds with multiple therapeutic potentials (Narang and Jiraungkoorskul, 2016).

Despite well-documented antioxidant and neuroprotective properties, a comprehensive analysis across antioxidant, antibacterial, and cytotoxic activities using various extraction solvents remains lacking. Studies have demonstrated effective antibacterial activity from aqueous and ethanolic leaf extracts against pathogens like *Salmonella* and *E. coli* (Mohammed *et al*., 2016), and notable cytotoxic effects in brine shrimp and human cancer cell lines, with leaf extracts comparable to cyclophosphamide in potency (Ajaiyeoba *et al*., 2016). However, the bioactive profile is highly influenced by solvent polarity. Therefore, a solvent-based comparative investigation is critical to determine extraction methodologies that maximize bioefficacy and safety. The present research addresses this gap by systematically evaluating bioactivities across hexane, diethyl ether, isopropanol, and ethanol extracts, providing essential insights for pharmacological development.

Materials and Methods

Collection of plant material

The plant was selected based on traditional knowledge of indigenous people. Leaves of *Citrus aurantifolia* were collected from home garden located at Thirumal Nagar, Perumalpuram, Tirunelveli district between December and January 2023. The collected leaves were identified and authenticated by a botanist in the Department of Botany, St. Xavier's College, Palayamkottai, Tirunelveli, India.

Extraction

Collected lime leaves were cut into pieces and dried in a shady area for about 4 days. After complete drying, it was coarsely powdered and weighed. Powdered leaves were extracted by maceration method with each of the four solvents such as hexane, diethyl ether, isopropanol and ethanol. With occasional shaking, it was allowed to remain in airtight condition for 2 days. Then the extracts were filtered and evaporated to dryness to yield the dry extracts (Longbap *et al*., 2018).

Phytochemical screening

Preliminary qualitative analysis was performed on the four extracts with standard methods to find the presence of various secondary metabolites (Banu and Cathrine, 2015; Mital and Jha, 2021).

Antibacterial activity

The test bacterial cultures, *Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Streptococcus pyogenes* were obtained from Sadakathullah Appa College – Microbiology Lab, Tirunelveli. Antibacterial activity was assessed by agar well diffusion method. In brief, Muller-Hinton Agar was dissolved in distilled water and autoclaved at 121° C for 20 min. Under sterilized conditions, the agar was plated in Petri plates and bacterial cultures swabbed using cotton buds. Seven wells were made for five concentrations (20 mg, 10 mg, 5 mg, 2.5 mg, and 1.25 mg) of *C. Aurantifolia* leaf extracts, one for negative control (1% v/v DMSO) and the other for standard antibiotic (1 mg/ml streptomycin). After 24 h, the zone of inhibition (mm) was measured for each concentration using a scale. The same procedure was repeated for all the bacterial species (Magaldi *et al*., 2004).

Antioxidant activity

The free radical scavenging activity of the leaf extracts was determined using DPPH assay as described in an earlier work with few modifications (Karimi *et al*., 2012). 200 µl of each extract (hexane, diethyl ether, isopropanol and ethanol) of *Citrus aurantifolia* leaves at different concentrations (20 mg, 10 mg, 5 mg, 2.5 mg, and 1.25 mg) was mixed with 1 ml solution of 0.15 mM 1,1 -diphenyl-2-hydrazyl (6 mg of DPPH in 100 ml of ethanol) and incubated for 30 min in the dark condition. The absorbance of the mixture was read using visible spectrophotometer at 521 nm. Ascorbic acid was used as standard. Free radical scavenging activity was calculated with the formula: % inhibition = {(A0 – A1)/A0} × 100, where, A0 is the absorbance of the control reaction and A1 is the absorbance in the presence of the sample.

Cytotoxic activity

Brine shrimp (*Artemia salina*) lethality assay was carried out by a standard protocol with minor modifications (Sarah *et al*., 2017). *Artemia salina* eggs were allowed to hatch in simulated sea water (30 g crystal salt in 1 L water) and exposed to natural light. The nauplii hatched within 24-30 h. 1 ml plant extract (concentrations 10 mg/ml, 1 mg/ml, 100 µg/ml, 10 µg/ml and 1 µg/ml) containing 10 nauplii and 1 ml of seawater was prepared. 1 ml distilled water with 1 ml of sea water containing 10 nauplii served as negative control. The number of dead nauplii was counted after 24 h and the percentage of lethality was calculated as: % Death = [Number of dead nauplii/ (Number of dead nauplii + Number of live nauplii)] × 100.

Statistical analysis

The statistical analysis for evaluating the antibacterial activity was done using SPSS (v. 16.0) software. Levene's test of equality of error

Table1: Phytochemical profiling of *Citrus aurantifolia* lea fextracts

Compounds	Hexane	Diethyl ether	Isopropanol	Ethanol
Alkaloids	+	-	-	++
Steroids	+++	-	-	+
Terpenoids	+	++	+	-
Flavonoids	++	+	+	-
Phenols	+	-	-	++
Saponins	+++	+	-	+++
Carbohydrates	-	-	+	-
Proteins	-	-	-	-
Gumsand mucilages	-	+	+	-
Oilsandfats	+	-	-	++
Tannins	++	+	+	+++
Glycosides	-	-	+	-

variances was first employed to verify the assumption of homogeneity in variances among the treatment groups. One-way ANOVA followed by Tukey's HSD post hoc test was used to compare the mean zones of inhibition across different extracts, concentrations, and bacterial strains and the results expressed in Mean ± S.E. Pearson's correlation test was performed to assess the relationship between extract concentration and zone of inhibition. IC_{50} for DPPH assay and LC_{90} for brine shrimp lethality assay were determined in MS-Excel and SPSS, respectively.

Results

Phytochemicals in C. aurantifolia leaf extracts

The phytochemical profiling of *Citrus aurantifolia* leaf extracts (Table 1) using four different solvents: hexane, diethyl ether, isopropanol, and ethanol highlighted the presence and relative intensity of various bioactive compounds such as alkaloids, steroids, terpenoids, flavonoids, phenols, saponins, carbohydrates, proteins, gums and mucilage, oils and fats, tannins, and glycosides. The results were indicated semi-quantitatively using symbols: + (low), ++ (moderate), and +++ (high intensity), with - denoting absence. Among the extracts, hexane and ethanol showed the broadest phytochemical presence. Hexane exhibited high levels of steroids and saponins, and moderate levels of flavonoids and tannins. Ethanol, on the other hand, was rich in tannins and saponins and moderately positive for alkaloids and phenols. Isopropanol showed limited but notable presence, especially of glycosides and flavonoids, while diethyl ether predominantly extracted terpenoids and tannins. Carbohydrates and proteins were largely absent in all solvents.

Antibacterial effect of C. aurantifolia leaf extracts

The antibacterial activity of *Citrus aurantifolia* leaf extracts using different solvents hexane, diethyl ether, isopropanol, and ethanol was measured by the zone of inhibition (in mm) against five bacterial strains: *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Staphylococcus aureus*, and *Escherichia coli*. In all extracts, the standard antibiotic showed consistently large zones of inhibition (33–40 mm), while the negative control (1% DMSO) showed no inhibition, confirming the activity was due to the plant extracts. Among the solvents, the hexane extract (Table 2) demonstrated broad anti-bacterial activity, particularly against *S. pyogenes* and *E. coli*, and showed good inhibition even at lower concentrations. However, *S. aureus* showed no sensitivity to hexane extracts.

The diethyl ether (Table 3) and isopropanol extracts (Table 4) also showed notable antibacterial effects, with isopropanol producing more consistent results across all five bacterial strains even at lower concentrations suggesting better solubility or extractability of active

Table2: Zones of inhibition formed by *Citrus aurantifolia* leaf hexane extract

Bacteria	Zone of inhibition (mm)						
	Standard	Control	20 mg	10 mg	5 mg	2.5 mg	1.25 mg
S.pyogenes	39	-	20	19	16	13	11
P. aeruginosa	38	-	21	17	15	13	-
B.cereus	32	-	19	16	14	11	-
S. aureus	34	-	-	-	-	-	-
E. coli	40	-	19	16	15	13	12

Table 3: Zones of inhibition formed by *Citrus aurantifolia* leaf diethyl ether extract

Bacteria	Zone of inhibition (mm)						
	Standard	Control	20 mg	10 mg	5 mg	2.5 mg	1.25 mg
S.pyogenes	39	-	16	13	12	12	-
P. aeruginosa	37	-	17	16	14	14	-
B.cereus	33	-	18	15	14	-	-
S. aureus	34	-	-	-	-	-	-
E. coli	39	-	20	12	11	11	-

Table 4: Zones of inhibition formed by *Citrus aurantifolia* leaf isopropanol extract

Bacteria	Zone of inhibition (mm)						
	Standard	Control	20 mg	10 mg	5 mg	2.5 mg	1.25 mg
S.pyogenes	39	-	17	16	15	15	13
P. aeruginosa	38	-	16	15	15	14	14
B.cereus	33	-	12	12	12	11	11
S. aureus	34	-	16	16	12	11	11
E. coli	40	-	16	14	13	13	12

Table 5: Zones of inhibition formed by *Citrus aurantifolia* leaf ethanol extract

Bacteria	Zone of inhibition (mm)						
	Standard	Control	20 mg	10 mg	5 mg	2.5 mg	1.25 mg
S.pyogenes	38	-	16	-	-	-	-
P. aeruginosa	36	-	18	15	14	12	12
B.cereus	32	-	18	15	14	12	11
S. aureus	34	-	14	14	13	13	12
E. coli	39	-	15	14	12	11	-

components in this solvent. The ethanol extract (Table 5), though effective, showed a narrower range of inhibition and reduced activity against *S. pyogenes* and *P. aeruginosa*. Overall, isopropanol and hexane extracts appeared to be the most potent in terms of broad-spectrum antibacterial activity, while diethyl ether and ethanol exhibited moderate effectiveness.

Levene's test of equality of error variances to check if the variance in the zone of inhibition is equal across groups revealed a non-significant P-value (sig = 0.662) suggesting homogeneity of variances, meaning statistical comparisons among groups are valid. Tukey comparison between extracts compared the mean zone of inhibition among four different solvent extracts of *C. aurantifolia*. Diethyl ether extract showed the lowest mean inhibition (8.6 ± 1.52), followed by ethanol (11 ± 1.18), hexane (11.2 ± 1.52) and isopropanol (13.68 ± 0.39), while the standard

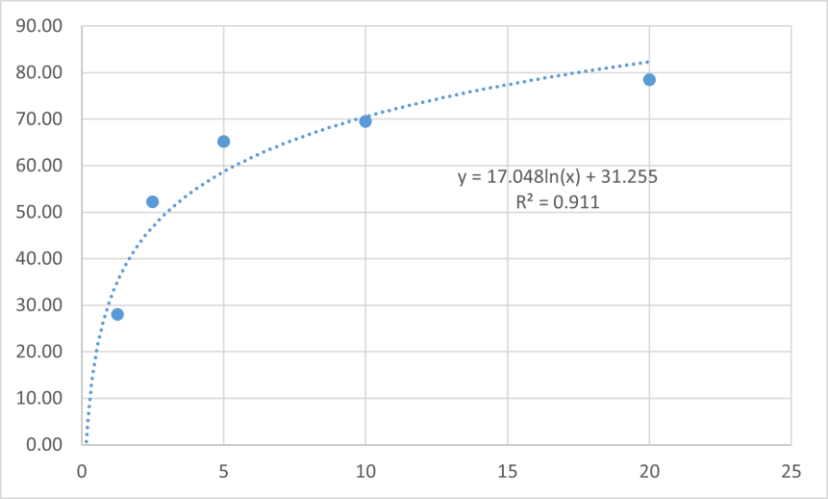

Fig. 1: DPPH radical inhibition by *C. aurantifolia* leaf hexane extract.

streptomycin had the highest (36.4 ± 0.63), indicating strong activity from the standard antibacterial agents. Tukey comparison between concentrations compared the antibacterial effects across extract concentrations from 1.25 mg to 20 mg. The highest concentration (20 mg) had a significantly greater inhibition zone (15.4 ± 1.27), followed by 10 mg (12.75 ± 1.28), 5 mg (11.55 ± 1.15), 2.5 mg (9.95 ± 1.17) and 1.25 mg (5.95 ± 1.37), demonstrating a dose-dependent increase in antibacterial activity. Tukey comparison between bacterial strains compared the extract's effect on different bacterial species. *P. aeruginosa* showed the most susceptibility with largest mean zone of inhibition (17.54 ± 2.07). *E. coli* (16.96 ± 2.29), *S. pyogenes* (15.79 ± 2.50) and *B. cereus* (15.21 ± 1.92) showed moderate susceptibility. *S. aureus* (11.17± 2.48) was the least susceptible, with smallest inhibition zones. A statistically significant positive correlation ($r = 0.365$, $P<0.01$) existed between the concentration of the extract and the inhibition zone, indicating higher concentrations led to stronger antibacterial effects.

Antioxidant potential of C. aurantifolia leaf extracts

The effectiveness of antioxidant activity of *Citrus aurantifolia* leaf extracts in various solvents (hexane, diethyl ether, isopropanol and ethanol) was evaluated based on percentage of free radical scavenging activity at increasing concentrations and the IC_{50} value, which represents the concentration needed to inhibit 50% of free radicals. A lower IC_{50} indicated higher antioxidant potential. Among the extracts, the hexane extract (Fig. 1) exhibited the highest antioxidant activity with an IC_{50} of 3 mg/ml, showing a steep increase in activity from 28.03% at 1.25 mg to 78.50% at 20 mg. The ethanol extract (Fig. 2) and isopropanol extract (Fig. 3) followed, with an IC_{50} of 7.49 mg/ml and 8.783, respectively, while the diethyl ether extract (Fig. 4) showed the weakest activity, having an IC_{50} of 49.45 mg/ml, with relatively low scavenging percentages even at higher concentrations.

In contrast, ascorbic acid (Fig. 5), used as the standard antioxidant, demonstrated superior scavenging activity at significantly lower concentrations, with values ranging from 82.72% at 12.5 mg to 88.89% at 200 mg, and an IC_{50} value significantly less than 9 µg/ml, which confirms its high antioxidant potency. These findings suggest that among the plant extracts, hexane was the most effective solvent for extracting antioxidant compounds from *Citrus aurantifolia* leaves, likely due to better solubility of certain non-polar antioxidant compounds. Isopropanol and ethanol

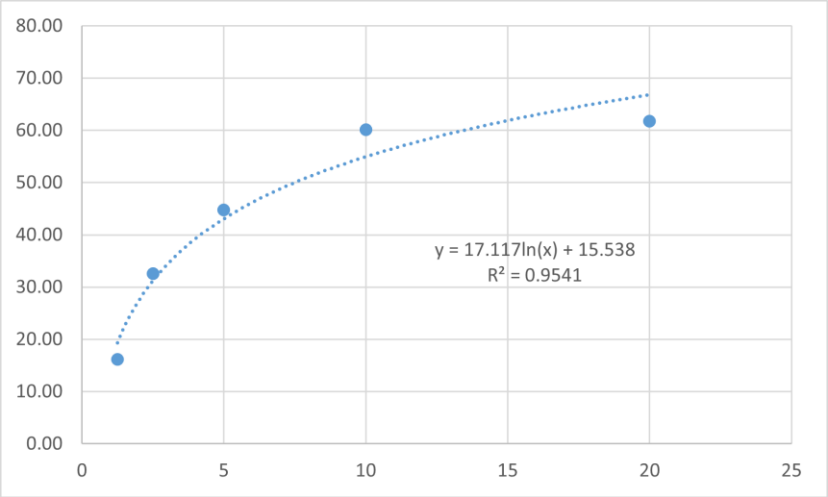

Fig. 2: DPPH radical inhibition by *C. aurantifolia* leaf ethanol extract.

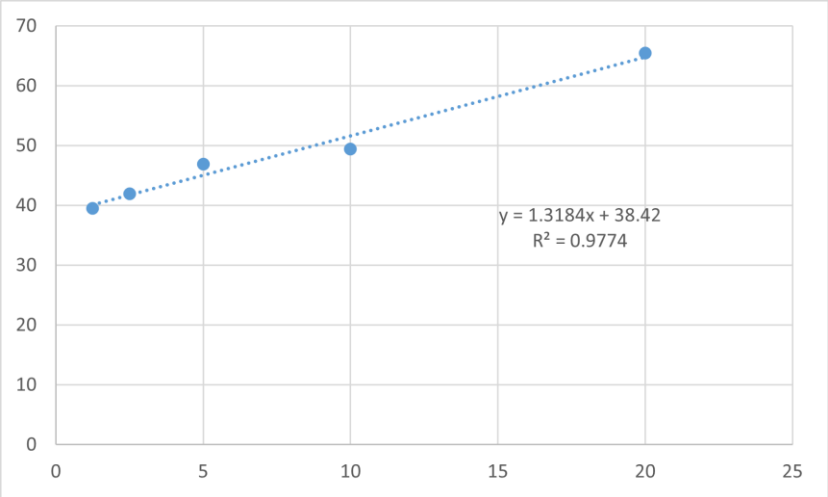

Fig. 3: DPPH radical inhibition by *C. aurantifolia* leaf isopropanol extract.

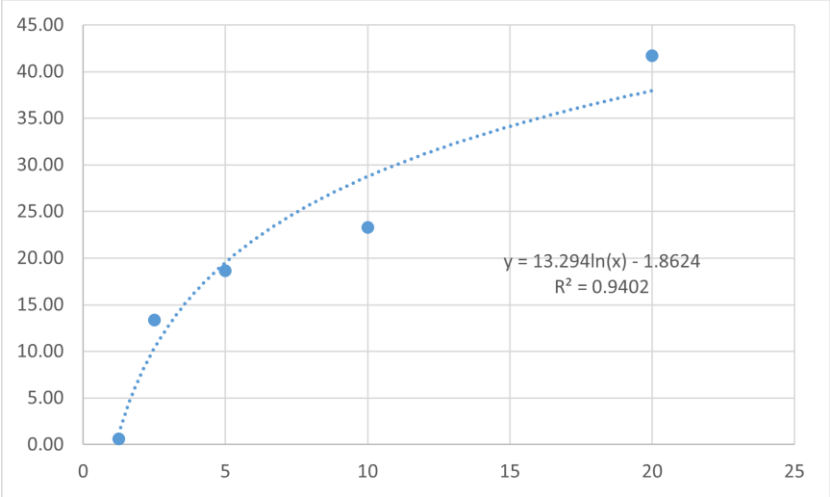

Fig. 4: DPPH radical inhibition by *C. aurantifolia* leaf diethyl ether extract.

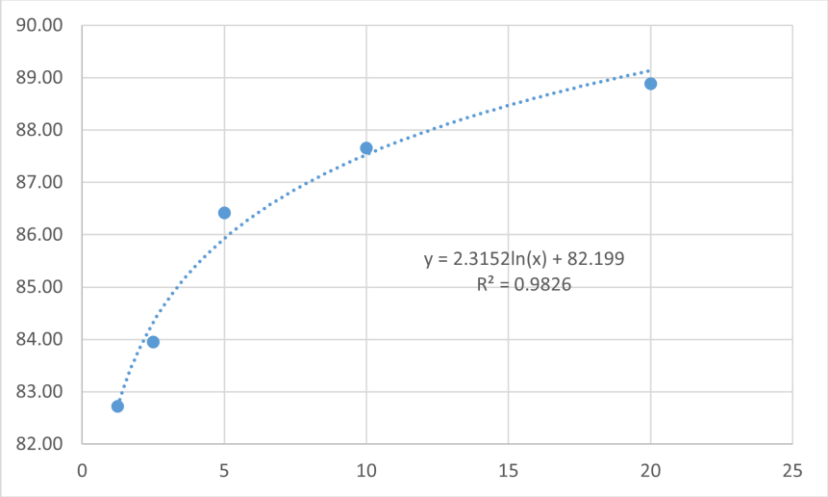

Fig. 5: DPPH radical inhibition by ascorbic acid.

Table 6: Deaths of brine shrimp in *C. aurantifolia* leaf diethyl ether extract

Concentration (µg/ml)	Number of live nauplii (Day1)	Number of live nauplii (after 24 h)	Calculated % death
0	10	10	0
1	10	3	70
10	10	2	80
100	10	0	100
1000	10	0	100
10000	10	0	100

Table 7: Deaths of brine shrimp in *C. aurantifolia* leaf hexane extract

Concentration (µg/ml)	Number of live nauplii (Day1)	Number of live nauplii (after24 h)	Calculated % death
0	10	10	0
1	10	6	40
10	10	3	70
100	10	2	80
1000	10	1	90
10000	10	1	90

extracted moderately active compounds, whereas diethyl ether, with intermediate polarity, was the least effective.

Cytotoxicity of C. aurantifolia leaf extracts

The cytotoxic effects of *Citrus aurantifolia* leaf extracts prepared in hexane, diethyl ether, isopropanol, and ethanol solvents were tested using the brine shrimp lethality assay, a widely accepted method for preliminary toxicity screening. The toxicity was indicated by the percentage of dead nauplii after 24 h, and LC_{90} values (the concentration required to kill 90% of nauplii) were used to compare the potency of each extract. The diethyl ether extract (Table 6) showed the highest cytotoxicity, with an LC_{90} of only 22 µg/ml, indicating that even low concentrations were lethal to the brine shrimp. At 1 µg/ml, 70% mortality was observed, and 100% mortality occurred at concentrations of 100 µg/ml and above. This suggests the presence of potent bioactive compounds in the diethyl ether extract with strong cytotoxic potential.

In comparison, the hexane extract (Table 7) was the least toxic, with a very high LC_{90} value of

Table 8: Deaths of brine shrimp in *C. aurantifolia* leaf isopropanol extract

Concentration (µg/ml)	Number of live nauplii (Day1)	Number of live nauplii (after24 h)	Calculated % death
0	10	10	0
1	10	10	0
10	10	3	70
100	10	2	80
1000	10	1	90
10000	10	0	100

Table 9: Deaths of brine shrimp in *C. aurantifolia* leaf ethanol extract

Concentration (µg/ml)	Number of live nauplii (Day1)	Number of live nauplii (after24 h)	Calculated % death
0	10	7	30
1	10	3	70
10	10	2	80
100	10	2	80
1000	10	0	100
10000	10	0	100

8907 µg/ml, indicating that even at 10,000 µg/ml, the mortality rate was limited to 90%. The isopropanol (Table 8) and ethanol extracts (Table 9) showed moderate cytotoxicity, with LC_{90} values of 905.251 µg/ml and 245.857 µg/ml, respectively. Both showed increased mortality with rising concentrations, but not as sharply as the diethyl ether extract. For instance, the ethanol extract caused 70% mortality at 1 µg/ml and reached 100% by 1000 µg/ml. These findings imply that while all extracts exhibited some level of toxicity, the diethyl ether extract is the most potent, and the hexane extract is the safest in terms of brine shrimp lethality.

Discussion

Citrus aurantifolia is a small evergreen shrub in the Rutaceae family, recognized not just for its flavourful fruits but also for its biologically active leaves that thrive in warm climates. The leaves contain various secondary metabolites including flavonoids, phenolics, terpenoids, limonoids, and essential oils that collectively contribute to their medicinal potential (Indriyani *et al*., 2023).

The phytochemical screening of *Citrus aurantifolia* leaves consistently demonstrates a diverse profile of bioactive metabolites, corroborating previous studies. Qualitative tests reveal significant levels of tannins, terpenoids, flavonoids, phenolics, saponins, steroids, and alkaloids, similar to earlier reports (Okwu *et al*., 2007). Quantitative analyses further support that the methanolic and aqueous leaf extracts contain high phenolic content and flavonoid contentwith flavonoids such as apigenin, quercetin, rutin, kaempferol, nobiletin, and hesperetin identified via HPLC alongside acetylcholinesterase-inhibiting monoterpenes and sesquiterpenes (Loizzo *et al*., 2012). Research also confirms that leaves are rich in not only flavonoids and phenolics, but also terpenoids, limonoids, and alkaloids (Indriyani *et al*., 2023). A comprehensive phytochemical investigation aligns with GC–MS data. Overall, the phytochemical profiling validates the presence of multiple high-value compounds, laying a robust foundation for observed antioxidant, antibacterial, and cytotoxic properties associated with solvent-specific extracts of *C. aurantifolia* leaves.

Natural compounds that are derived from microbial, animals, or plants attracts the attention of many researchers (Gyawali and Ibrahim, 2014. Moloney, 2016). These compounds have exhibited

promising results in overcoming the emergence of antibiotic resistance in bacterial pathogens (Rossiter *et al*., 2017). In the present study, four extracts obtained from *C.aurantifolia* leaf were tested for antibacterial activity gainst *Escherichia coli*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pyogenes.* Similar studies have reported that the hexane extract of fruit peels of *C. aurantifolia* exhibited important activity against isoniazid, streptomycin or ethambutol mono-resistant *M. tuberculosis* strains (Camacho-Corona *et al*., 2008). Another study that analysed the inhibition of *Streptococcus mutans* growth by methanol extract, hexane, ethyl acetate, and water fractions of *Citrus aurantifolia* peel have proved hexane fraction as the most active (Jeffrey *et al*., 2020). Plant derivative compounds including phytochemicals have even been employed to treat various infectious disease and have shown interesting antimicrobial activity against several human pathogens. Some of these compounds show both intrinsic antibacterial activity and antibiotic resistance modifying activities. Some of them while not effective by themselves as antibiotics, when combined with antibiotics, they can overcome antibiotic resistance in bacteria (Khameneh *et al*., 2019). This study revealed that phytochemical screening and antibacterial activity was noted significantly in hexane extract of *C. aurantifolia* leaves when compared to other extracts. Therefore, it is evident that more the bioactive compounds of plant origin, better the antibacterial activity was observed.

The free radical scavenging activity determined by DPPH assay was compared. Due to the presence of multiple -OH group in their structures, phenolic compounds and flavonoids have been described to be associated with antioxidative action in biological organisms, acting as scavengers of free radicals (Rice-Evans *et al*., 1997; Jørgensen *et al*., 1999). Naringin, one of the flavonoids in lime juice has showed chemo-preventive effect in an experiment and has proved mild anti-oxidative effects on lipid peroxidation (Jeon *et al*., 2001). Flavonoids showed different antioxidant effects in various studies of *C. aurantifolia*. Antioxidant activity of *C. aurantifolia* leaf and peel extracts quoted that all flavonoids in the extract have had antioxidant role and increasing their concentrations caused antioxidant role to be elevated (Boshtam *et al*., 2011). A study on the antioxidant capacity of peel essential oils of three citrus species has documented *Citrus aurantifolia* with highest radical scavenging activity on ABTS assay (Loizzo *et al*., 2012).

The degree of lethality of brine shrimp shown by the extracts was directly proportional to the concentration of the extracts ranging from the lowest to the highest concentration. All the four extracts of *C. aurantifolia* leaf showed cytotoxicity that more than 50% lethality occurred within 24 h with the concentrations taken. In a study of cytotoxic activities of different fractions of *Citrus aurantifolia* peel, LC_{50} of chloroform extract (228.14 µg/ml) was found to show highest cytotoxicity (Ghosh *et al*., 2020). The stem bark and leaf extracts of *C. aurantifolia* were strongly toxic on brine shrimps than cyclophosphamide and also it was noted that *C. aurantifolia* leaf extract had cytotoxicity on Hep-2c cancer cells comparable to cyclophosphamide (Ajaiyeoba *et al*., 2016).

Conclusion

The present work demonstrates that *Citrus aurantifolia* leaf extracts possess notable bioactive properties, with solvent selection playing a critical role in modulating their antioxidant, antibacterial, and cytotoxic efficacy. Among the tested solvents, hexane and ethanol extracts showed the highest antioxidant efficiency; isopropanol, hexane and ethanol extracts brought out the best antibacterial activity, while the diethyl ether extract exhibited remarkable cytotoxic potential in the brine shrimp assay. The results validate traditional claims regarding the medicinal utility of lime leaves and highlight their potential as a natural source of therapeutic agents. The positive correlation between extract concentration and zone of inhibition further strengthens the case for dose-dependent efficacy. Overall, the findings pave the

way for further phytochemical characterization and *in vivo* studies to explore and harness these bioactivities for pharmacological applications.

Ethical Statement

The authors confirm that all ethical issues have been dealt with the research ethics guidelines provided by the Vector Biology and Pharmacology Research Centre, Department of Zoology, St. Xavier's College (Autonomous), Palayamkottai, Tirunelveli, Tamil Nadu, India.

Author Contributions

Petchiyammal R.M.: conceptualized and designed the study, involved in acquisition of data and analysis and interpretation of data; *Mabel Parimala S.*: drafted the article and revised it critically for important intellectual content. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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