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Cyanidin-3-Glucoside from Litchi: Extraction Techniques and Bioactivity Evaluation

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Author`s Contribution

[TH] Conception and design, [AN] Collection and assembly of data, [HZ, MI] Analysis and interpretation of the data, Statistical expertise, [MA]Final approval and guarantor of the article

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A B S T R A C T

Objectives: The primary objective of this research was to find the lychee extract which shows efficient results for the presence of flavonoids and excellent anti-microbial activity as well.

Methods: Phytochemicals were extracted by using extraction methods (methanol, ethanol and ascorbic acid) from the leaves, fruits and peels of lychee. Phytochemical screening for the presence of terpenoids, saponins, tannins, steroids, resins and flavonoids in these sample extracts Quantification for the presence of total phenolic compounds (TPC) carried out using Folin-Ciocalteu's reagent, which was highest amount in the ethanol extract of fruits. Flavonoids content quantified by using aluminum chloride assay.

Results: The highest amount of phenolic compounds were recorded to be 0.5 mg/g GAE dw in methanol extract of peels and for flavonoids, it was recorded to be 0.27 mg/mL in methanol extracts. Furthermore, the quantity of cyanidin-3-O-glucoside was determined using pH differential method. The highest amount was 0.4 mg/L in methanol extract of peels. Lastly, the anti-microbial activity of the samples was checked against selected bacterial genera; Bacillus spp., Salmonella spp., Citrobacter spp., and E. coli using disk diffusion method. Methanol extracts of fruits showed noticeable zone of inhibition of 36.2 mm against Salmonella spp., which is comparable to the standard antibiotics (Oxidil) i.e., 37 mm.

Conclusion: The study signified that L. chinensis holds great potential in the health industry to treat a variety of infections caused by pathogenic bacteria. In future, we can further explore the variety of biological characteristics i.e., neuroprotective, hepatoprotective, antioxidant and anti-inflammatory activities, exhibited by this plant.

Keywords: L. chinensis, Phytochemicals, Phenolic compounds, Flavonoids, Cyanidin-3-Glucoside, Antimicrobial activity

Introduction

Medicals plants have been providing medicinal and health benefits from ages. Their leaves, seed, branches, fruits and peels have been used as a drug for the treatment of many health issues from the past.[1] *Sage, mint leaves, savory and tansy had been used for the purpose of healing in the middle age. Heleborus odorus, Sennae folium* and *Euphorbium had been used as a mild laxative.*[2] After the discovery of these medicinal plants, the usage of synthetic drugs was reduced and people were more drawn towards natural products and medicine.[3] These plants comprise of many biochemical compounds like alkaloids, polyphenols, glycosides and terpenes in various percentages for various purposes and also exhibit pharmacological activities.[4] These plants have been widely used in the pharmacology to design drugs against different kinds of cancer, malaria, HIV, and Alzheimer.[5]

L. chinensis is the tropical and subtropical plant that belongs to the sapindaceae family and the sole member of the *Litchi* genus.[6] It is cultivated in the South Asian countries such as, China, Indonesia, Thailand, Nepal, Pakistan, Philippines etc. China is most famous in cultivation of *L. chinensis* since many years.[7] Lychee fruit is grown on the evergreen tree which has a height of 10-20 m.[8] It consists of 10-30 cm long leaves. It is the subtropical fruit which comprises of hard peel and the juicy, transparent, flesh like white color fruit pulp.[9] Water is the major constituent of the lychee fruit. In lychee fruit, 76- 90% water is present in the fruit pulp. [10] Mainly, lychee plants contain sugars,

carbohydrates, vitamin A, proteins, amino acids, phenolic compound, fats, minerals, and pectin and flavor compounds. Famous compounds which are found in lychee are alkaloids, Saponins, tannins, terpenoids, steroids, resins, and flavonoids.[11]Flavonoids exert their antibacterial activity through multiple mechanisms that disrupt essential bacterial processes. Firstly, they inhibit nucleic acid synthesis by targeting enzymes involved in DNA replication and transcription, crucial for bacterial growth and reproduction.[12] Secondly, flavonoids disrupt cytoplasmic membrane function by interacting with phospholipids and proteins, leading to increased membrane permeability and leakage of cellular contents, ultimately causing bacterial cell death.[13] Thirdly, these compounds interfere with bacterial energy metabolism, particularly by disrupting the electron transport chain and ATP synthesis pathways in bacterial cells.[14] By targeting these fundamental processes necessary for bacterial survival and proliferation, flavonoids effectively inhibit bacterial growth and exhibit potential as natural antimicrobial agents against a wide range of bacterial pathogens.[15]

In this study, the research was particularly focused on extraction of flavonoid (cyanidin-3-O-glucoside) from *L. chinensis* using different solvents, quantification of total phenolic compounds, flavonoids and C-3-G from leaves, fruits and peels of *L. chinensis* and anti-microbial activity of *L. chinensis* extracts against diverse pathogenic bacterial strains.

Materials and Methods

Collection of *L. chinensis*

L. chinensis fruits and leaves were bought from local vendors in Lahore during summer season, in the months of June and July in the year 2024. The average temperature during these months was 40 ± 5°C.

Preparation of L. chinensis extracts

The lychee fruits and leaves were washed thoroughly with tap water. The fruits peels and leaves left to dry in the open air for 2 weeks. The dried components were grinded into fine powder and stored in the clean and air-tight containers for further analysis.[16] The extraction of each component was carried out using three solvents; ethanol solvent (85 parts ethanol and 15 parts conc. HCl) [17], ascorbic acid solvent (50% methanol, 1.2 M of 37% HCl & 0.04% (w/v) ascorbic acid) [18] and methanol solvent (60% methanol & 40% water). The ratio for solvent extraction was set to be 1:10, which contains 1 part of each sample powder and 10 parts of solvent in a conical flask. The extract flasks incubate overnight at 4 °C in the refrigerator. The filtration performed using Whatman filter paper to separate debris from the extract containing phenolic compounds. Initially, 40 mL of solvent was added but after filtration, 25 mL of extract was obtained. Then, using hot plate and setting the temperature much lower than the boiling point, the extract was concentrated and reduced to 5 mL.[19]

Phytochemical analysis

Screening of sample extracts carried out to detect the presence of phytochemicals. Different colorimetric tests were used for this purpose. For the detection of alkaloids, Wagner's test was used which is considered positive if brownish-red precipitates appear.[20] To detect terpenoids in a sample, copper acetate test was used which is considered positive if greenish color develops in the test tube.[21] To determine the presence of saponins in sample, foam test was used which is considered positive if a stable froth is formed. For determination of tannins in a sample, ferrous chloride test was used which is considered positive if brownish color appears in the test tube. For the detection of steroids, Salkowski's test was used. It is considered positive if a brownish-red ring is formed at the junction separating two layers. For the determination of resins in a sample, acetone solubility test was used which is considered positive by the development of turbidity in the test tube.[19] For detect the presence of flavonoids in sample, Shinoda test was used which is considered positive if orange or red color appears in the test tube.[22]

Total Phenolic Compounds (TPC) Quantification

Folin Ciocalteu's method as described in the literature was used to determine the total phenolic compounds of the sample extracts. A stock solution of gallic acid was prepared with a concentration of 1000 mg/mL. Then, a series of standard solutions of Gallic acid was prepared; 20, 40, 60, 80 and 100 mg/mL. 0.1 mL of standard solution, 0.2 mL of Folin-Ciocalteu's reagent, 0.6 mL 7.5 % sodium carbonate solution and 3.16 mL distilled water such that final volume was 5 mL. Same ratios were used in case of sample extracts but instead of standard solution the sample extracts were used. Lastly, UV-vis spectrophotometry was done at 765 nm wavelength to determine the TPC in lychee sample extracts.[23]

Flavonoid Quantification

For flavonoid quantification, $AlCl_3$ assay was used. 0.3 mL of 10 % $AlCl_3$ and 0.3 mL of 1 M potassium acetate were added in to the solution and incubated for about 30 minutes. These ratios were used for both standard solutions and sample extracts.[24] The standard used in this case was naringenin chalcone with a stock concentration of 1 mg/mL. A series of standard solutions with concentrations of 0.1, 0.2, 0.5 and 1 mg/mL were used to prepare standard curve for flavonoid quantification of our sample extracts. Then, Uv-vis spectrophotometry was carried

out at 370 nm to determine flavonoid content of lychee sample extracts.[25,26]

Cyanidin-3-Glucoside Quantification

To determine cyanidin-3-glucoside quantity in sample extracts, pH differential method was used as described in detail in literature. Two buffers; a 0.025 M potassium chloride buffer with pH 1 and a 0.4 M potassium acetate buffer with pH 4.5, were used. At a lower pH of 1, anthocyanin appears in its most colored form and at a slightly higher pH of 4.5, it becomes colorless. This difference is calculated to determine the quantity of C-3-G, an anthocyanin. Each sample extract was diluted with buffer 1 and 2 respectively after determining the dilution factor of sample extract. Then the absorbance of sample extracts in buffer 1 and 2 was checked at 700 nm and 520 nm.[27,28]

Anti-microbial Activity

To determine the anti-microbial activity, disk-diffusion method was used.[29] 5 µL of each sample extract was loaded onto disks to check their antimicrobial activity against four chosen bacterial strains; *Bacillus* spp., *Salmonella* spp., *Citrobacter* spp., *and E. coli*. For control, 5 µL of antibiotic, Oxidil (250 mg/5 mL), was used. Moreover, nutrient-agar was used for determining the antimicrobial potential of sample extracts.

Results

L. chinensis extracts

The fruits, peels and leaves extracts were prepared in three different solvents. Each component was used to extract phenolic compounds in ethanol, ascorbic acid and methanol extracts.

Phytochemical Analysis of various *L. chinensis* parts in different solvents

Phytochemical tests were conducted on *L. chinensis* fruits, peels and leaves sample extracts prepared in three solvents. The samples showed variable results for the presence of alkaloids, terpenoids, saponins, tannins, steroids, resins and flavonoids. The data obtained from the three extracts of fruits showed variable results for these tests, the methanol and ascorbic acid extracts of fruits sample showed positive results for almost all the tests, except a few tests. The phytochemical screening of peels sample was also conducted for the three extracts. The results obtained for peel extracts showed methanol extract to be positive for almost every test. The leaves sample was also used to prepare three extracts and tested to detect the presence of phytochemicals; methanol extracts tested positive for all the tests except a few (Figure 1).

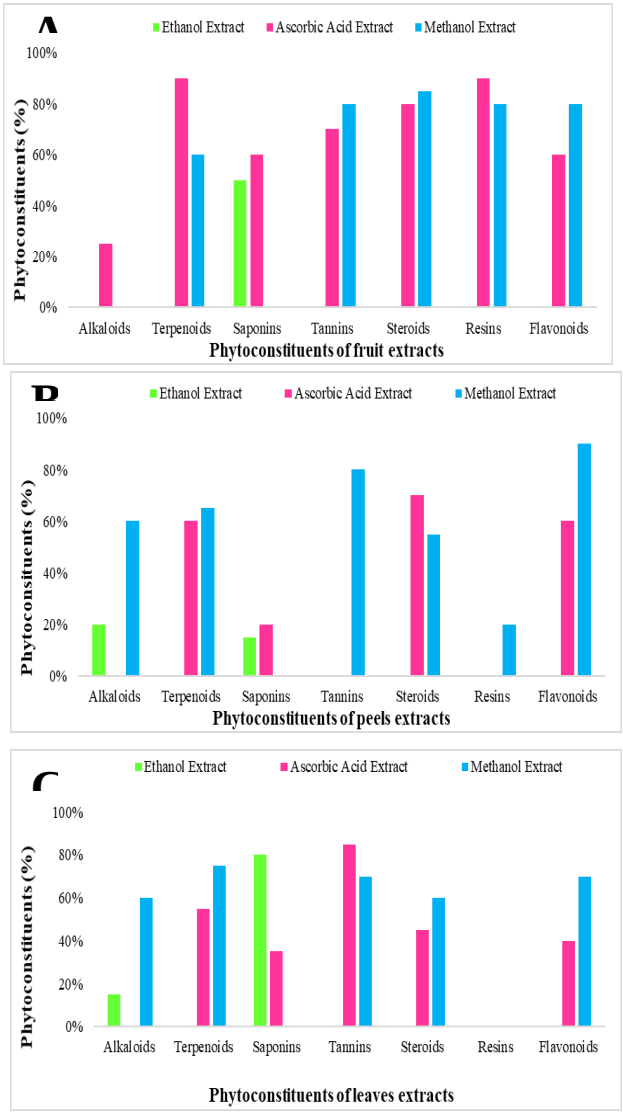

Figure 1: Phytoconstituents of various L. chinensis components. (A) fruit extract, (B) peel extract and (C) leaves extract

Quantification

Phenolic Compounds Quantification

Phenolic compounds quantification carried out by using Folin-Ciocalteu's reagent showed variable amounts of phenolic compounds in each component of L. chinensis among different solvents. Standard curves were prepared for each solvent to find out the total phenolic compounds of the lychee samples in ethanol, methanol and ascorbic acid solvents. The linear regression equations (R2) values were 0.9396 for ethanol, 0.986 for methanol and 0.9922 for ascorbic acid solvents. The highest amount of phenolic compounds was observed in ethanol extract of fruit which was 0.568 mg/mL. Whereas, the lowest quantity of phenolic compounds was observed to be in methanol extract of fruits i.e., 0.132 mg/mL (Figure 2(A)).

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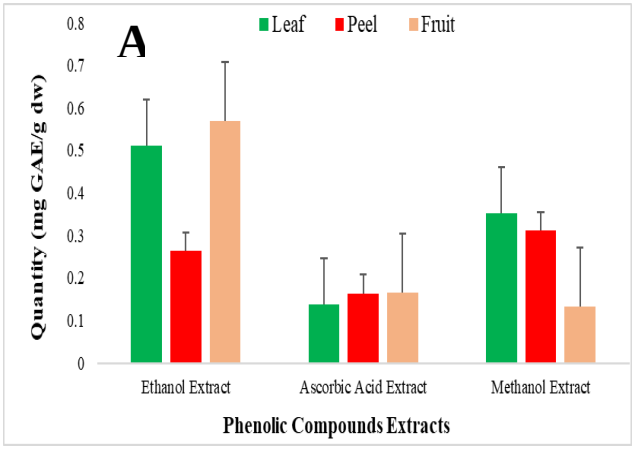

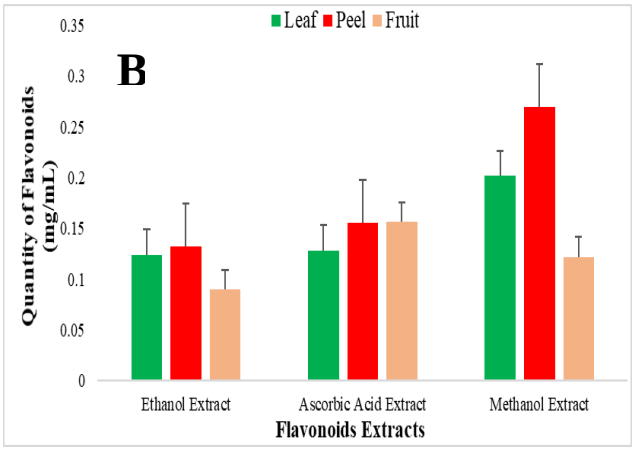

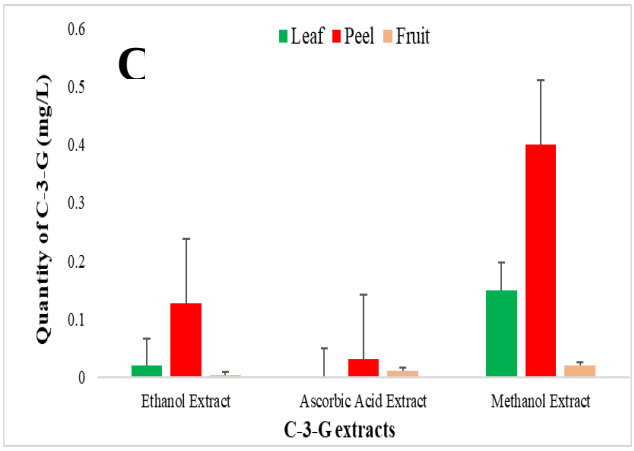

Figure 2: (A) L. chinensis sample extracts quantity of phenolic compounds (B) flavonoid content (C) C-3-G content

Quantification of Flavonoids

The quantification of flavonoid content in the samples, carried out by using Aluminum chloride assay, showed that each sample extract contained different amounts of flavonoid content. Standard curves were prepared for ethanol, methanol and ascorbic acid solvents. The R2 values were 0.8938for ethanol, 0.8343 for methanol and 0.8284 for ascorbic acid solvents. The highest flavonoid content was recorded to be 0.269 mg/mL in methanol extract of peels, while the lowest was observed to be 0.09 mg/mL in the ethanol extract of fruits (Figure 2(B)).

Quantification of Cyanidin-3-Glucoside

The quantification of C-3-G of the sample extracts carried out by using pH differential method showed that each extract contained different amounts of C-3-G. The highest amount of C-3-G was observed to be 0.4 mg/L in the methanol extract of peels, whereas the lowest was observed to be 0.0025 mg/mL in the ascorbic acid extracts of leaves (Figure 2 (C)).

Anti-microbial Activity

The antimicrobial activity of each sample extract was tested against the four selected bacterial genera; *Bacillus* spp., *Salmonella* spp., *Citrobacter* spp., *and E. coli* using disk-diffusion method. The extracts showed different zones of inhibition against each bacterial species.

Anti-microbial Activity of L. chinensis Ethanol Extracts

The ethanol extracts of *L. chinensis* fruits, peels and leaves showed no noticeable zones of inhibition against the chosen bacterial genera. Although, a negligible zone of inhibition of 6 mm was observed by the ethanol extract of leaves. It was neglected as the standard anti-biotic, Oxidil, showed a zone of inhibition of 26.4 mm.

Anti-microbial Activity of L. chinensis Ascorbic Acid Extracts

The ascorbic acid extracts of fruits, peels and leaves showed significantly noticeable zones of inhibition against the chosen strains. The largest zone of inhibition was 25.1 mm by leaves extract, observed against Citrobacter spp. It is comparable to the results of standard, which was recorded to be 24.5 mm. The smallest zone of inhibition was 10.2 mm by fruit extract recorded against E. coli. The overall zones of inhibition by the ascorbic acid extract against the chosen bacterial strains as shown in Figure 3.

Anti-microbial Activity of L. chinensis Methanol Extracts

The methanol extracts of L. chinensis showed the best results in general. The largest zone of inhibition was observed to be 36.2 mm by fruit extract against Salmonella spp. It is comparable to the zone of inhibition of standard anti-biotic, which was recorded as 36.7 mm. The smallest zone of inhibition was observed to be 10 mm against E. coli. The overall zones of inhibition by methanol extracts as shown in Figure 4.

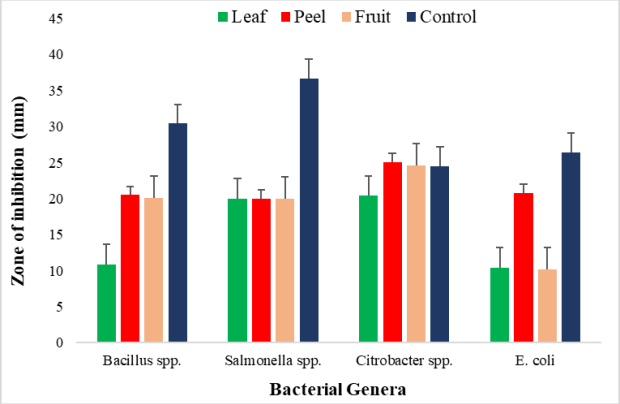

Figure 3: Antimicrobial activity by ascorbic acid extracts

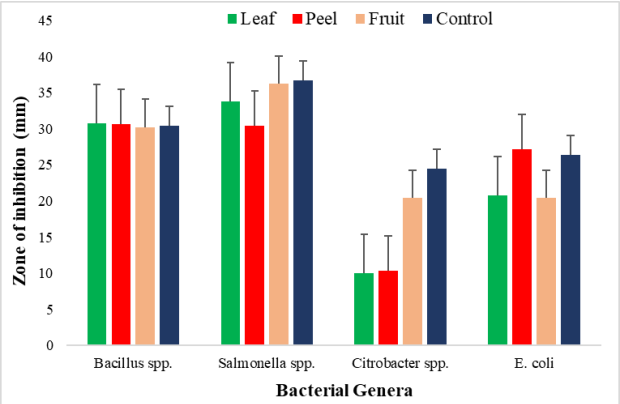

Figure 4: Antimicrobial activity by methanol extracts

Discussion

L. chinensis has enormous number of application in different fields like medicine, nutraceuticals [30] health [31] and pharmacology.[32] It contains different kinds of beneficial compounds like terpenoids, alkaloids, saponins, steroids, tannins, flavonoids and different phenolic compounds.[33] Scientists have reported the presence of alkaloids in peels of lychee but no traces have been observed in fruit pulp.[34] Our research revealed that alkaloid compounds are present in methanol peel and leaf extract. It is also found that a moderate amount of alkaloids is present in ascorbic acid fruit extract. Previous studies have shown the presence of 14 different terpenoid compounds in lychee.[35] Our research demonstrated that fruit, leaf and peel extract in solvents ascorbic acid and methanol show positive results. Scientists have found minute concentration of saponins in lychee seeds by solvent extraction method and percolate.[36,37] Our results showed the presence of saponins in leaf and peel extracts in the solvent ethanol and fruit, leaf and peel extracts in ascorbic acid. The study revealed that tannins are found in peels and fruit pulp of lychee[34]. The phytochemical analysis of our sample showed that tannins are found in all samples in methanol solvents and in leaf and fruit in ascorbic acid solvent. Our phytochemical testing of steroids revealed that steroids are present in all samples (leaf, peel and fruit) in ascorbic acid and methanol solvents. Resins are also found in very minute amount in lychee. As it is only found in fruit extract in ascorbic acid, fruit and peel extract in methanol proved by our results of resin test. The study on identification of flavonoids present in the fruit pericarp stated that after UV, NMR and MS analysis main flavonoids that are present in lychee fruit are proanthocyanidin B 4, proanthocyanidin B 2 and epicatechin.[38] The phytochemical testing of flavonoids on lychee samples showed that all samples in methanol are positive whereas ascorbic acid samples showed the presence of moderate amount of flavonoids.

The quantification of phenolic compounds present in our sample is done by using Folin-Ciocalteu's method. Scientists have demonstrated that *Cannabis* fruit contain total phenolic compounds to be 0.57 ± 0.002 (mg GAE/g dw).[39] Our research showed that ethanol extracts of peels, leaves and fruits contained 0.264, 0.51 and 0.568 mg GAE/g dw. Whereas ascorbic acid extracts of peels, leaves and fruits were found to be 0.164, 0.137 and 0.165 mg GAE/g dw respectively and methanol extracts of peels, leaves and fruits contained 0.312, 0.353 and 0.132 mg GAE/g dw.

The quantification of total flavonoid content present in the leaves of *Ocicum basilicum were checked by using* $AlCl_3$.[40] *Scientists have studied that lychee flower extract in hot water contains* 50.37 ± 3.62 mg/g of dried extract.[41] Our research showed that ethanol extracts of peels, leaves and fruits contained 0.132, 0.123 and 0.09 mg/mL. Whereas ascorbic acid extracts of peels, leaves and fruits were found to be 0.156, 0.128 and 0.156 mg/mL respectively and methanol extracts of peels, leaves and fruits contained 0.269, 0.201 and 0.122 mg/mL.

A study revealed flavonoid content in the *E. uniflora* was found to be 9.19 g% ± 0.0131 (1.43) calculated as quercetin and 17.72 g% ± 0.0253 (1.43) g% calculated as rutin, both determined by DD procedure.[42] Our research showed that the ethanol extracts of peels, leaves and fruits contained 0.127, 0.02 and 0.0034 mg/mL. On the other side, the ascorbic acid extracts of peels, leaves and fruits contained 0.031, 0.0025 and 0.012 mg/mL. Whereas methanol extracts of peels, leaves and fruits contained 0.4, 0.15 and 0.021 mg/mL.

It is reported that the antimicrobial activity of lychee with honey was observed against *staphylococcus auras* by using agar disc diffusion method and the result show about 25.8 mm zone of inhibition.[43] The target compound of this research was Cyanidin-3-Glucoside (C-3-G). C-3-G is particularly known for its antimicrobial activity against *E. coli* by destruction of its cell membrane.[44] C-3-G is also known to target both gram negative

and gram positive bacteria by inhibiting the bacterial cell growth.[45] In our research we used ethanol, ascorbic acid and methanol solvents to carry out solvent extraction from peels, leaves and fruits. In case of ethanol extract, no noticeable zones of inhibition were observed, except for leaves which showed a small zone of inhibition of 6 mm. In case of ascorbic acid extracts, peels showed zones of inhibition of 20.5, 20, 25.1 and 20.8 mm against *Bacillus* spp., *Salmonella* spp., *Citrobacter* spp., and *E. coli* respectively. Whereas leaves showed zones of inhibition of 10.9, 20, 20.4 and 10.4 mm against *Bacillus* spp., *Salmonella* spp., *Citrobacter* spp., and *E. coli* correspondingly. However, fruits showed zones of inhibition of 20.1, 20, 24.6 and 10.2 mm against *Bacillus* spp., *Salmonella* spp., *Citrobacter* spp., and *E. coli* respectively. In case of methanol extracts, peels showed zones of inhibition of 30.6, 30.4, 10.4 and 27.2 mm against *Bacillus* spp., *Salmonella* spp., *Citrobacter* spp., and *E. coli* respectively. Whereas leaves showed zones of inhibition of 30.8, 33.8, 10 and 20.8 mm against *Bacillus* spp., *Salmonella* spp., *Citrobacter* spp., and *E. coli* respectively. However, fruits showed zones of inhibition of 30.2, 36.2, 20.4 and 20.4 against *Bacillus* spp., *Salmonella* spp., *Citrobacter* spp., and *E. coli* respectively.

In recent years, many studies have been conducted to discover the true potential of biological activity of various parts of lychee. In 2022, the peels and seeds are found to be rich in phenolic compounds, particularly flavonoids. They were also found to exhibit potent antihyperglycemic activity.[46] A study conducted in 2025 showed that the seeds of lychee are packed with a flavonoid, proanthocyanidins which has the ability to inhibit breast cancer stem cell activity.[47] In 2021, lychee leaves were shown to exhibit excellent anti-oxidant, anti-inflammatory as well as hepatoprotective activity.[31] Lychee possess many potent compounds which are used in the pharmaceutical industries as well as traditional Chinese medicines.

Conclusions

Extracting cyanidin-3-glucoside (C3G) from lychee isn't just about isolating a compound; it's about unlocking the potential of nature's own defense mechanisms. As a major anthocyanin, C3G is known for its potent antioxidant, anti-inflammatory, anticancer, neuroprotective, and cardio protective effects. However, its successful extraction, purification, and application in pharmaceutical and nutraceuticals formulations require efficient extraction techniques, stability enhancement strategies, and bioavailability improvement approaches.

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