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Bioprospecting of *Coccinia grandis* (L.) Voigt leaf: A wild nutraceutical of Bangladesh

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ABSTRACT

Coccinia grandis (L.) Voigt leaf is a widely consumed leafy vegetable in Bangladesh for a variety of medical purposes. The methanolic leaf extract was investigated for its phytochemical as well as nutraceutical (antioxidant, anti-inflammatory, cytotoxic, and antibacterial) properties to substantiate its traditional uses. It contains bioactive elements such as alkaloids, glycocides, steroids, tannins, phenols, terpenoids, quinines, phlobatanins and protein but lacks flavonoids, saponins, anthraquinone, and coumarin, according to phytochemical analysis. Leaf extract has strong antioxidant (IC_{50} = 37.334 µg/ml), anti-inflammatory (IC_{50} = 51.87 µg/ml), and cytotoxic (LC_{50} = 41.49 µg/ml) activities, according to biological tests. It also had modest antibacterial activity (8 to 12 mm) against *Bacillus subtilis, Bacillus cereus, Escherichia coli*, and *Salmonella typhi,* with a maximum inhibition zone of 12 mm (250 µg/ml) against *Bacillus subtilis*.

Keywords: *Coccinia grandis,* antioxidant activity, anti-inflammatory activity, cytotoxic activity, antibacterial activity.

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INTRODUCTION

Bioprospecting is the process of discovering and utilizing biologically active compounds found in nature for various purposes, including the development of new medicines, food additives, and cosmetic products. A food product or component that offers health benefits beyond basic nutrition is referred to as a nutraceutical. The nutraceutical value of a food can be determined by evaluating its nutrient content and potential health benefits such as antioxidant, anti-inflammatory, or anti-cancer properties (Chen, 2000).

The concept of using wild foods as medicine was first introduced through traditional Chinese medicine and food therapies. For centuries, people have consumed wild plant species, including those that are not cultivated or kept current, for their therapeutic properties (Tardío et al., 2006). These wild vegetables, including blooms, young stems, and leaves, are rich in flavonoids, carotenoids, polyphenols, isothiocyanates, allylic sulfides, phytosterols, monoterpenes, linoleic acid, dietary fiber, vitamins, and minerals. They have been shown to have anti-anemic, anti-diabetic, anti-hypertensive, and anti-cancer activities, as well as improve intestinal health and lower the risk of cardiovascular disease (Bonet and Valles, 2002; Aslam et al., 2020).

Coccinia grandis (L.) Voigt, commonly referred to as "Diabetes Shak," is a widely consumed wild leafy vegetable in Bangladesh (Facciola, 1990). This plant is versatile and can be eaten raw, in a salad, or cooked in a curry when it is tender (Orech et al., 2005; Ramachandran and Subramaniam, 1983). In addition, traditional medicinal systems such as Ayurveda, Unani, and Siddha have used *C. grandis* for treating skin diseases, gastrointestinal disturbances, diabetes, urinary tract infections, respiratory tract problems, and joint pain (Mollik et al., 2010; Gautam et al., 2014).

With the growing interest in natural products for health and wellness, the bioprospecting of *C. grandis* has become more important. This study aims to explore the bioprospecting of *C. grandis*, focusing on its nutraceutical properties, such as phytochemical, antioxidant, anti-

inflammatory, cytotoxic, and antibacterial properties. The study will provide valuable insights into the potential health benefits of this plant and the possibilities for sustainable use of natural resources in the development of functional food and healthcare products.

MATERIALS AND METHODS

Collection of plant material

Leaf samples of *C. grandis* were collected from KEPZ, Karnafully, Anowara, and Chittagong districts. A voucher specimen (16-2019_KEPZ) was placed for future use at the Department of Botany, Chittagong University Herbarium (CTGUH), where the plant was identified.

Preparation of extract

Natural plant leaves were cleaned, chopped, allowed to dry at room temperature, then ground into a coarse powder and kept for further study in an airtight container. To obtain the dark-colored extract, the methanolic extract was filtered using Whatman No. 1 filter paper and dried under a vacuum at temperatures below 50°C.

Phytochemical group test

Phytochemical screenings for alkaloids, flavonoids, glycosides, saponins, steroids, tannins, terpenoids, and anthraquinones, quinine, phenol, coumarin, phlobatannins and protein were conducted in accordance with published methods.

For alkaloids, 5 g of fresh, finely chopped and pasted plant material was moistened with 10 ml of 2% HCl before being cooked in a water bath at 60°C for one hour. The extract was filtered through Whatman No. 1 filter paper after cooling. A tiny groove slide was coated with two drops of the extract and one drop of the alkaloid-detecting agent (Mayer's, Wagner's, Dragendroff's, Hager's and Tannic acid) for each test and observed Yellow creamish or white-cloudy, Brownish red, orange, yellow and reddish precipitation respectively (Aplin and Cannon, 1971).

For flavonoids, A fraction of the raw powdered plant material was boiled in 10 ml of ethyl acetate for three minutes over a steam bath. One milliliter of weak ammonia solution and four milliliters of the filtrate were combined with the mixture after filtering. The presence of a yellow color denoted a successful test for flavonoids (Edeoga et al., 2005).

For glycosides, 0.5 g of crude powder was dissolved in 5 ml of methanol. In a test tube, 10 ml of 50% HCl was added to 2 ml of methanolic extract. It was then cooked in a bath of boiling water for 30 min. After adding 5 ml of Fehling's solution, the mixture was cooked for 5 min. As proof that glycosides were present, a brick-red precipitate was used (Harbrone, 1973).

For saponins, 2 g of raw powder were cooked with 20 ml of distilled water in a water bath before being filtered. To create a stable, long-lasting foam, 10 ml of filtrate was combined with 5 ml of distilled water and forcefully shaken. The presence of saponins is indicated by persistent foam (Kapoor et al., 1969).

For steroids, in 5 ml of methanol, 0.5 g of crude powder was dissolved. A test tube was filled with 10 ml of chloroform, 1 ml of the extract, and an equivalent volume of pure sulfuric acid. The sulphuric acid layer appears yellow with green fluorescence as the higher layer turns red. This suggested that steroids were present (Kolawole et al., 2006).

For tannins, in an assay tube, 0.5 g of the crude powdered samples were boiled in 10 ml of distilled water before being filtered. A few drops of the ferric chloride reagent were applied to the filtrate. The presence of tannins was determined by the presence of a blue-black precipitate (Harbrone, 1973).

For terpenoids, in 5 ml of methanol, 0.5 g of crude powder was dissolved. In a test tube, 5 ml of the extract and 2 ml of chloroform were combined. To create a layer, seven milliliters of carefully diluted sulfuric acid were added to the mixture. When terpenoids are present, an interface with a reddish-brown color develops (Kolawole et al., 2006).

For anthraquinone, magnesium acetate was added to a solution of 2 ml. Pink color formation indicates the presence of anthraquinones (Sofowara, 1993).

For quinine, 1 ml of the extract was combined with 1 ml of concentrated sulfuric acid, and the mixture was given some time to acquire color. The development of red color indicates the presence of Quinine (Sofowara, 1993).

For phenol, 1 ml of the extract was mixed with 1 ml of alcohol, and then a few drops of a neutral ferric chloride solution were added. Blue or green color formation indicates the presence of phenol (Soloway and Wilen 1952).

For coumarin, 1 ml of the extract was combined with 1 ml of 10% NaOH, and the mixture was allowed to sit for a while. Yellow color formation indicates the existence of coumarin (Sofowara, 1993).

For phlobatannins, the aqueous plant extract was combined and heated with a 1% hydrochloric acid solution. The formation of a red precipitate indicates the presence of phlobatannnins (Edeoga et al., 2005).

For protein, a 2% copper sulphate solution was combined with a few drops of crude extract. The addition of 1 ml of ethanol was followed by the addition of more potassium hydroxide pellets. Green formation indicates the presence of protein (Gahan, 1984).

The presence of relative metabolites in *C. grandis* leaf was expressed by the "+" sign ("+" minimum to "+++", the highest quantity) to indicate the presence in degrees. The absence of the metabolite was denoted by the "-" sign.

Antioxidant activity test

In a separate test tube, 3 ml of each *C. grandis* leaf extract and Ascorbic Acid (50 µg/mL, 100 µg/mL, 150, 200 and 250 µg/ml) and 3 ml of DPPH solution were employed as the test groups (Brand-Williams et al., 1995). Additionally, 3 ml of DPPH solution and 3 ml of methanol were used as a negative control, while 3 ml of ascorbic acid (50, 100, 150, 200 and 250 µg/ml) and 3 ml of DPPH solution were used as a positive control. These solution mixtures were kept in dark for 30 minutes. The absorbance was measured at 517 nm using UV-visible spectrophotometer. The scavenging activity against DPPH was calculated using the following equation:

$$\text{Scavenging activity (\%)} = \left(\frac{A-B}{A}\right)\times 100$$

Where,
A = Absorbance of control (DPPH solution + methanol).
B = Absorbance of test / standard (DPPH solution + extract / standard).

Anti-inflammatory activity test

In a separate test tube, 2 ml of each plant extract (50, 100, 150, 200 and 250 µg/ml) and 2 ml of a solution of 5% egg albumin were employed as the test groups. Additionally, 2 ml of 5% egg albumin and 2 ml of methanol were used as a negative control, while 2 ml of acetyl salicylic acid (50, 100, 150, 200 and 250 µg/ml) and 2 ml of 5% egg albumin were used as a positive control. The pH of the reaction mixtures was adjusted at 5.6 ± 0.2 by 1N HCl. All the reaction mixtures were heated for 20 minutes at 57°C. After cooling and filtering, absorbance was measured at 660 nm using UV-visible spectrophotometer (Mizushima and Kobayashi, 1968). The anti-inflammatory activity was calculated by using the following equation:

$$\text{\% of inhibition} = \left(\frac{A-B}{A}\right)\times 100$$

Where,
A = Absorbance of control (albumin solution + methanol)
B = Absorbance of test / standard group (albumin solution + extract / standard)

Cytotoxic activity test

Artificial seawater for the cytotoxic test was made by mixing 1 L of distilled water with 38 g of pure NaCl. A 1000 ml beaker of saline water was added with artemia salina cysts, which were then cultured at room temperature with constant oxygen supplied by a pump unit. 48 to 72 hours later, the cysts began to hatch. Five test tubes each containing 1ml of each plant extract (50, 100, 150, 200 and 250 µg/mL) with 4 ml sea water and 10 nauplii were taken. Vincristine sulphate was taken as standard, and the results were expressed as LC_{50} in µg/ml. The percent mortality of nauplii was determined by counting the number of dead nauplii after 24 h. The LC_{50} was determined by exposing nauplii to five different concentrations (Meyer et al., 1982). The percentage of mortality of nauplii was calculated for each concentration by the following equation:

$$\text{\% of Mortality} = \left(\frac{\mathrm{Nt}}{\mathrm{No}}\right) \times 100$$

Where,
N_t = Number of dead nauplii
N_o = Number of taken nauplii

Antibacterial activity test

For this study, four human pathogenic bacteria *Salmonella typhi* (ATCC 19430), *Bacillus cereus* (ATCC 14579), *Bacillus subtilis* (ATCC 19659), and *Escherichia coli* (ATCC 25925) were selected. To ensure optimum growth, the bacterial cultures were kept at 35 ± 2°C. The inoculum concentration was established by OD, and the bacterial suspensions were created using a progressive dilution approach. Standard ampicillin discs served as the positive control, and empty discs soaked in the solvent served as the negative control. Test discs (Whatman filter paper, grade 17, diameter 8 mm) were made by dipping them in various concentrations of plant extract (50, 100, 150, 200 and 250 µg/ml), then letting them air dry. The test discs, the standard discs and the control discs were placed gently on the marked zones in the bacterial plates. The antibacterial activities were expressed in mm of the diameter of the inhibition zone surroundings the discs compared to the control (Bauer et al., 1966).

RESULTS

Phytochemical group profile

The qualitative phytochemical results are shown in Tables 1 and 2. Table 1 demonstrates how the leaf reacted differently to several reagent types depending on the presence of alkaloids. Here Wagner's reagent (W) and Tannic acid reagent (T) showed the highest result (+++). On the other hand, Hager's reagent (H) showed a moderate (++) result and Dragendroff's reagent (D) and Mayer's reagent showed the lowest (+) result.

It also revealed that glycosides, steroids, tannins, terpenoids, quinines, phenol, phlobatanins and protein

Table 1. Qualitative test of alkaloids of *C. grandis* (leaf).

Plant part	Qualitative estimation of alkaloids by different reagents				
	D	H	M	W	T
Leaf	+	++	+	+++	+++

Note: Name of the reagents, D = Dragendroff's reagent, H = Hager's reagent, M = Mayer's reagent, W = Wagner's reagent and T = Tannic acid reagent.

Table 2. Qualitative test of twelve other phytochemicals of *C. grandis* (leaf).

Plant part used	Phytochemicals (% of coloration)											
	Flv.	Gly.	Sap.	Ste.	Tan.	Ter.	Ant.	Qui.	Phe.	Cou.	Phl.	Pro.
Leaf	-	+	-	+	+	+	-	+	+	-	+	+

Note: Flv.= Flavonoids, Gly. = Glycosides, Sap. = Saponins, Ste. = Steroids, Tan. = Tannins, Ter. = Terpenoids, Ant. = Anthraquinone, Qui. = Quinine, Phe. = Phenol, Cou. = Coumarin, Phl. = Phlobatanins, Pro. = Protein.

were present but flavonoids, saponins, anthraquinone, and coumarin were absent in the leaf of the plant studied (Table 2).

Antioxidant activity

Data on the antioxidant activity of *C. grandis* leaf extract are shown in Figure 1. In the current investigation, the scavenging activity of ascorbic acid was found to be 54.27 ± 0.004%, 60.19 ± 0.004%, 77.74± 0.0004%, 88.01 ± 0.0008% and 94.08 ± 0.0004% at the consecutive concentrations of 50, 100, 150, 200 and 250 µg/ml. The IC_{50} value of ascorbic acid (standard) is 34.31 µg/ml. The scavenging activities of *C. grandis* leaf extract were 52.27 ± 0.0018%, 61.12 ± 0.0060%, 69.07 ± 0.0018%, 74.31 ± 0.0024% and 87.66 ± 0.0062%. The IC_{50} value of *C. grandis* leaf extract was recorded as 37.334 µg/ml.

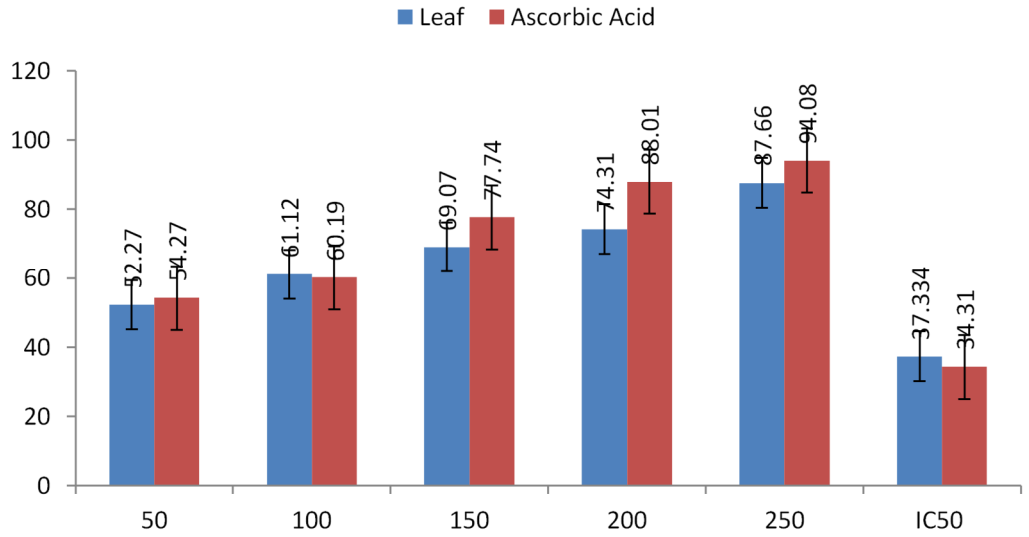

Figure 1. Comparison of antioxidant activities of ascorbic acid and *C. grandis.*

Anti-inflammatory activity

The anti-inflammatory effects of *C. grandis* leaf extract are shown in Figure 2. When a protein is denatured, its biological activity is lost. Inflammation is brought on by protein denaturation. As part of the anti-inflammatory investigation, it was investigated if plant extract may lessen protein denaturation.

The percentage (%) of inhibition activity caused by acetylsalicylic acid are 48.55 ± 0.004%, 67.88 ± 0.004%,

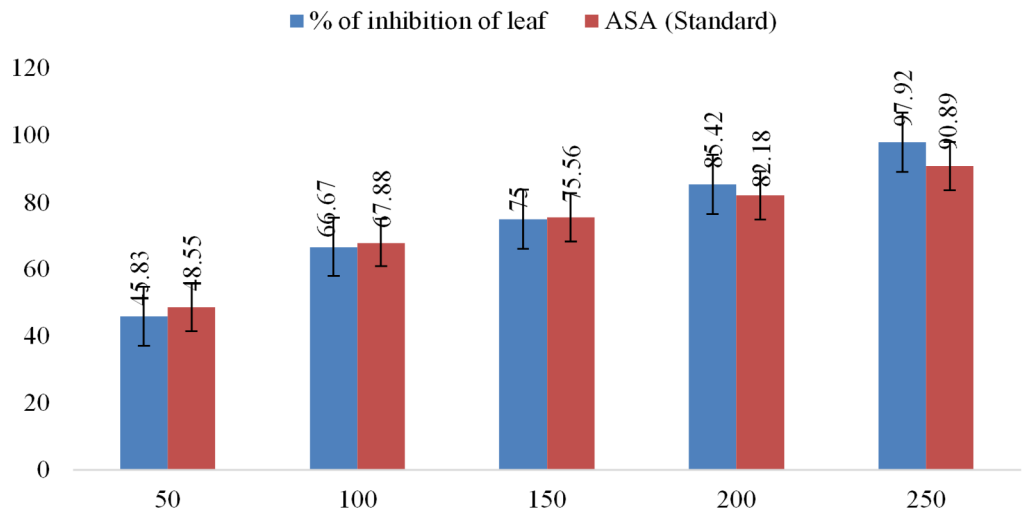

Figure 2. Comparison of anti-inflammatory activities standard and *C. grandis*.

75.56 ± 0.0004%, 82.18 ± 0.0008% and 90.89 ± 0.0004% at the five concentrations 50, 100, 150, 200 and 250 µg/mL, consequently. The IC_{50} value of acetylsalicylic acid was recorded as 32.84 µg/ml. The anti-inflammatory activity (%) of leaf extract was 45.83 ± 0.0003%, 66.67 ± 0.0003%, 75.00 ± 0.0006%, 85.42 ± 0.0009%, 97.92 ± 0.0003% at the same previous concentrations, where the highest percent of inhibition activity was 97.92 ± 0.0003% at the concentration of 250 µg/ml, and the lowest one was 45.83 ± 0.0003% at the concentration of 50 µg/mL. IC_{50} value of leaf extract and acetylsalicylic acid was recorded as 51.87 and 32.84 µg/ml, respectively.

Cytotoxic activity

The crude extracts of *C. grandis* leaf were subjected to brine shrimp lethality bioassay and lethality (in percent %) at different concentrations has been presented. The records of cytotoxic activity among the different concentrations of the leaves extract were taken after 24 h. LC_{50} was calculated by using a probit analysis chart. After 24 h, the mortality rate caused by the leaf extract at the five concentrations 50, 100, 150, 200 and 250 µg/ml were 81, 88, 93, 96 and 100% respectively, and the value of LC_{50} of *C. grandis* leaf extract was 41.49 µg/ml (Table 3).

Table 3. Cytotoxic activities of *C. grandis* leaf extract.

Concentration		Nauplii		Mortality		LC_{50} value (µg/ml)	
						Extract	Standard
µg/ml	LogC	Taken	Dead	Percentage	Probits	41.49	0.30
50	1.699	27	22	81	5.88		
100	2.000	24	21	88	6.18		
150	2.176	27	25	93	6.48		
200	2.301	23	22	96	6.75		
250	2.398	25	25	100	7.33		

Antibacterial activity

Antimicrobial refers to any substance, whether natural, semi-natural, or synthetic, that eradicates microorganisms or prevents their growth with minimal to no negative effects on the host. Any action that either stops bacteria from growing or reproducing or kills them is referred to as antibacterial activity. By measuring the inhibition zone, we find out the minimum and maximum antibacterial properties of a plant. The antibacterial activities of the extracts from the test samples in terms of the diameters of inhibition zones were recorded.

Ampicillin (standard) exerted inhibition zone of 16, 15, 22 and 16 mm against *B. subtillis*, *E coli*, *S. typhi* and *B. cereus* respectively. While leaf extracts at each dose inhibited *B. subtilis*, 2 doses (200 to 250) inhibited *E. coli*; 3 doses (150-250) inhibited *S. typhi* and 4 doses (100 to 250) inhibited *B. cereus* and exerted inhibition zone (Figure 3). The range of zone of inhibition recorded were 8 to 12 mm against *B. subtilis*; 8 to 9 mm against *E. coli*;

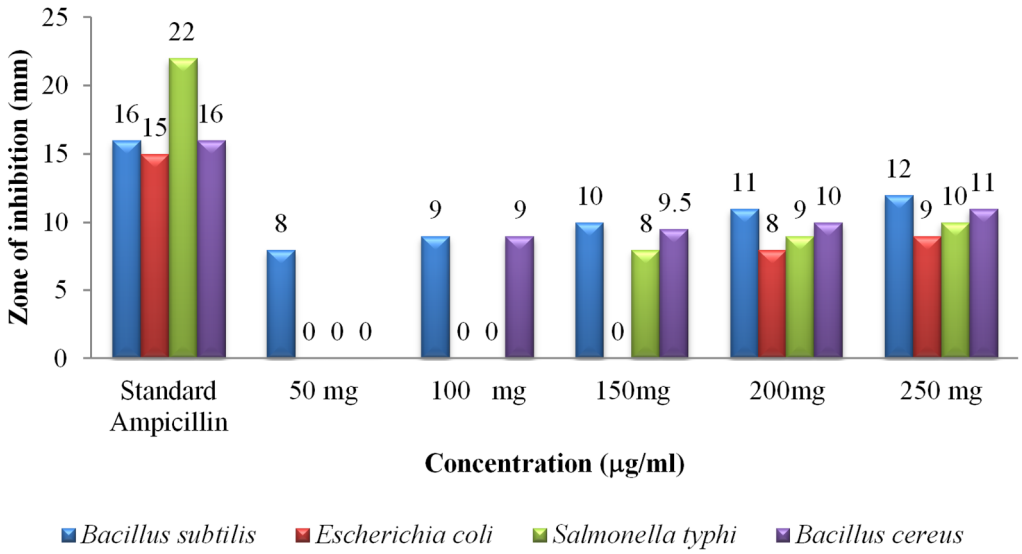

Figure 3. Antibacterial activities of *C. grandis* leaf extract and the standard.

8 to 10 mm against *S. typhi* and 9 to 11 mm against *B. cereus.* Leaf extract showed the highest antibacterial activity against *B. subtilis* (12 mm) at a concentration of 250 µg/ml, and less inhibition zone (8 mm) was revealed against *E. coli* (200 µg/ml), *S. typhi* (150 µg/ml) and 9 mm against *B. cereus* at 100 µg/ml, respectively.

DISCUSSION

Phytochemical profile

Plants produce numerous metabolites that have various biological and pharmacological effects on living beings (Wink, 2015). In this study, alkaloids, glycosides, steroids, tannins, terpenoids, quinines, phenol, phlobatanins and protein detected from *C. grandis* leaf extract are of great importance in drug research. Plant metabolites have been used for generations to treat a wide range of illnesses (Arif et al., 2009). These have notable antioxidant, anti-inflammatory, anti-carcinogenic, anti-bacterial, anti-diabetic, anti-malarial, anti-cholinesterase, and anti-leprosy properties. The findings of this study are completely consistent with Khatun et al. (2012).

Antioxidant activity

Antioxidants are essential because they can shield the organism from harm brought on by oxidative stress brought on by free radicals (Kahkonen et al., 1999). The leaf extract has potent antioxidant activities that are comparable to those of a typical antioxidant. This investigation was in line with the finding of Pratoomsoot et al. (2020).

It could be brought on by the phenolic compounds present in it (Ahmed et al., 2014). Previous research has found a connection between the number of total phenols and flavonoids in plant extracts and their antioxidant activity (Aryal et al., 2019; Bisognin et al., 2019). These findings support the notion that phenolic compounds may be associated with a strong scavenging activity of the plant.

Anti-inflammatory activity

Denaturation of proteins triggers inflammatory reactions. The effectiveness of the leaf extract in this instance provided evidence that it has anti-inflammatory effects (Mohamed et al., 2011). According to a notion, the steroids and alkaloids in it may have the ability to have anti-inflammatory effects (Shukla et al., 2010). Plant chemicals that can prevent or reduce protein denaturation could be used to make anti-inflammatory medications (Osman et al., 2016). The findings of Sakharkar et al. and Albrahim et al. were confirmed through the results of this experiment (Sakharkar and Chauhan, 2017; Albrahim et al., 2020).

Cytotoxic activity

Brine shrimp lethality is used to measure data on cytotoxicity, antibacterial activity, pesticidal effects, and other pharmacologic effects (Meyer et al., 1982). The leaf extract in this bioassay exhibited cytotoxic activity with LC_{50} value of 41.49 µg/ml. The alkaloids, terpenoids, steroids, and tannins that are present therein may be the

cause of this (Yang et al., 2007; Mazid et al., 2008). The current investigation supports the findings of Alamgir et al. (2014). Phenolics cause apoptosis while alkaloids prevent the NF-B pathway from being activated in cytotoxic processes. Both stop the cell cycle at this stage (Habli et al., 2017). The United States National Cancer Institute states that plant extracts with LC_{50} values greater than 20 µg/ml are safe, whereas those with LC_{50} values less than 20 µg/ml are cytotoxic (John, 2001).

This plant is considered safe for use in local medicines because the plant extract showed a value of LC_{50} >20 µg/ml. These results are consistent with past studies that indicated phenolic compounds, alkaloids, and steroids contribute to the cytotoxic effects of plant extracts (Tonisi et al., 2020).

Antibacterial activity

Leaf extracts of *C. grandis* at each dose inhibited *B. subtilis*, 2 doses (200 to 250) inhibited *E coli*; 3 doses (150 to 250) inhibited *S. typhi* and 4 doses (100-250) inhibited *B. cereus* and exerted inhibition zone. The range of zone of inhibition recorded was 8 to 12 against *B. subtilis*; 8 to 9 against E. coli; 8 to 10 against *S. typhi* and 9 to 11 against *B. cereus*. Leaf extract showed the highest antibacterial activity against *B. subtilis* (12 mm) at 250 µg/ml concentration and less inhibition zone 8 mm revealed against *E. coli* (200 µg/ml), *S. typhii* (150 µg/ml) and *B. cereus* as 9 mm at 100 µg/ml respectively.

The tannins, saponins, alkaloids, and terpenoids included in the methanolic extract may be responsible for its antibacterial activity (Mujeeb et al., 2014). Tannins prevent the development of bacteria by forming complexes with enzymes and precipitating proline-rich proteins in membranes (Othman et al., 2019). Terpenoids reduce microbial growth by weakening their membranes (Ja'afreh et al., 2019). While steroids prevent it by forcing the contents of liposomes containing phosphatidyl ethanolamine to seep out (Epand et al., 2007).

CONCLUSION

C. grandis is a widely consumed wild leafy vegetable in Bangladesh with a long history of use in traditional medicine. The bioprospecting of this plant leaf is of great importance as it has the potential to contribute to the development of functional food and healthcare products. The results showed that the leaf extracts were rich in compounds such as alkaloids, glycocides, steroids, tannins, phenols, terpenoids, quinines, and phlobatanins, as well as proteins. Furthermore, the extracts displayed encouraging nutraceutical properties including antioxidant, anti-inflammatory, cytotoxic, and antibacterial properties. Further research is needed to fully understand and identify its naturally occurring bioactive components.

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