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IN-VITRO ANTIOXIDANT, ANTIDIABETIC, AND ANTHELMINTIC ACTIVITY OF LIMONIN AND NOMILIN CONTAINING METHANOL EXTRACTS OF *CITRUS MAXIMA* SEEDS

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ABSTRACT

Objective: The objective of the present work is to study the in-vitro anti-oxidant, antidiabetic, and anthelmintic activities of the methanol extract of *Citrus maxima* (*C. maxima*) seeds. **Methods:** The anti-oxidant activity of the *C. maxima* seed extracts (250, 125, 62.5, 31.25, and 15.63 µg/ml) was studied using 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity and hydrogen peroxide (H_2O_2) scavenging activity against standard ascorbic acid (same concentration). Antidiabetics were studied using the α-amylase enzyme inhibitory study (Starch-Iodine colon assay method). Anthelmintic activity of *C. maxima* seeds (5, 10, and 20 mg/ml in distilled water) was observed against *Pheretima posthuma*, which involved the determination of the time of paralysis (TP) and time of death (TD) of the worms. Albendazole (5, 10, and 20 mg/ml in distilled water) was included as a standard reference. **Results:** Methanol extracts of *C. maxima* seed extracts had DPPH scavenging activity (IC_{50} 367.6301 µg/ml), hydrogen peroxide scavenging activity (IC_{50} 617.289 µg/ml), and were comparable with standard ascorbic acid. *C. maxima* seed extract exhibited significant antihelmintic activity at the highest concentration of 20 mg/ml. The extracts were found not only to paralyze (*Pheretima posthuma*) but also to kill the earthworms (*Pheretima posthuma*). Phytochemicals have been observed from *C. maxima* seed, such as terpenoids, flavonoids, saponins, phenols, tannins, phlobatannines, steroids, anthraquinones, alkaloids, vitamins, amino acids, glycosides, cardiac glycosides, resins, carbohydrates, proteins, fats, and fixed oils. **Conclusion:** Limonin- and nomilin-containing *C. maxima* seeds crude methanolic extract extracts possess antioxidant activities (DPPH and H_2O_2 inhibition activity), antidiabetic activities (α-amylase inhibitory), and anthelmintic activity. *C. maxima* by-product seeds therefore have the potential to be utilized as a complementary therapy. Additionally, more research should be done.

KEYWORDS: *Citrus maxima seeds*, 1,1-diphenyl-2-picryl hydrazyl (DPPH), Ascorbic acid, IC_{50} value, limonin, nomilin.

1. INTRODUCTION

Plants Limonoids, which mostly occur in the tissues of Rutaceae and Meliaceae family plants, are highly oxidized secondary metabolites having polycyclic triterpenoid backbones (Jayaprakasha, Dandekar, Tichy, & Patil, 2011). Limonin is the bitterest limonoid, followed by nomilin. Citrus plants from the Rutaceae family are the primary source of natural liminoids; 21 glucosides, 39 liminoid aglycones, vitamin C, carbohydrates, minerals, flavonoids, and alkaloids (Sun, Chen, Chen, & Chen, 2005). Citrus fruits source of multivitamins, pectins, carotenoids, fatty acids, and especially polyphenols, folate, dietary fiber, and other bioactive components, (Tripoli, Guardia, Giammanco, Majo, & Giammanco, 2007). Furthermore, citrus fruits such as lemons, oranges, grapefruits, and pomelos frequently contain limonoids as secondary metabolites (Richa et al., 2023a). The primary sources of limonoids are citrus peel, pulp, and seeds (Minamisawa, Yoshida, & Uzawa, 2014). Limonoids in citrus fruits exhibit a wide range of biological activities, including antitumor, anti-inflammatory, anti-insect, antineoplastic, and other pharmacological activities (Shi et al., 2020). Citrus peel essential oils are strong free radical scavengers and antifungal agents with anti-pathogenic properties that help relieve sore throats, coughs, earaches, and vomiting (Liu et al., 2022b). Citrus fruit/seeds have been used as a sedative and cardiac tonics (Liu et al., 2022a). Citrus/ Tangerine fruits have antibacterial, insecticidal, and anti-inflammatory qualities due to the presence of flavonoids, coumarins, citric acid, and flavanones in their seeds, peels, and pulp (Sahlan et al., 2018). Like other citrus plants *C. maxima* is rich in vitamin C and belongs to the family Rutaceae (Richa et al., 2023b). The perennial citrus tree *C. maxima* is also referred to as Bhogate, Shaddock, Papanus, Pummelo, and Pomelo. The *C. maxima* plant is native to Asia and is farmed for commercial purposes in numerous Asian nations, including China, Nepal, Thailand, Malaysia, India, Vietnam, Indonesia, Philippines, and Japan (Sapkota, Devkota, & Poudel, 2022). Additionally, pomelos have significant concentrations of polyphenolic compounds, including ferulic acid, hesperidin, naringin, caffeic acid, and p-coumaric acid(Anmol et al., 2021). Citrus fruits' limonin and nomilin can act as antioxidants in the β-carotene bleaching test (Huang et al., 2019); (Sun et al., 2005). Lemonin, nerolol, nerolyl acetate, and geraniol are found in the essential oil extracted from the leaves and immature fruits (Petretto et al., 2023). Fresh leaf oil has fungicidal and antidermatophytic properties. It has been stated that leaves can help with stomach pain, ulcers, swellings, convulsive cough, epilepsy, and chorea (Sapkota, Devkota, & Poudel, 2022). In cases of nervous affection, flowers are said to have sedative properties. The bark and root of *C. maxima* comprise β-sitosterol, acridone alkaloid. Fruit and peel rinds are antiasthmatic, sedative in nervous affliction, brain tonic, and helpful in treating vomiting, abdominal gripping, diarrhea, headaches, and vision problems (Vijaylakshmi & Radha, 2015). According to the author (Sarkar et al., 2022), *C. maxima* seed oil contains fatty acid, saturated fat, monounsaturated fatty acid, polyunsaturated fatty acid, trans-fatty acid, hexadecanoic acid methyl esters, 9-Octadecenoic acid methyl ester. Although *C. maxima* seed is regarded as a byproduct of the fruit, it contains important components that have been shown to have chemopreventive effects, such as limonin and nomilin (Poulose, Harris, & Patil, 2005). Moreover, *C. maxima* (Burma) seed oil has antimicrobial properties in vitro against coughs, dyspepsia, and lumbago. (Singh & Navneet, 2016); With this knowledge, the current study's objective was to assess limonin, nomilin containing *C. maxima* seeds in-vitro Antioxidative, antidiabetic, and anthelmintic activity.

2. MATERIALS AND METHOD

2.1. Reagent and chemicals

Methanol, Chloroform, Concentrated Sulphuric acid (Conc. H_2SO_4), Acetic anhydride, Sodium hydroxide (NaOH), Concentrated Hydrochloric acid (Conc. HCl), Zinc dust, Lead acetate, Ferric chloride, Ammonia solution, Potassium hydroxide (KOH), Mayer's reagent, Wagner's reagent, Hager's reagent, Acetic acid, Acetone, Benedict's reagent, α-

Naphthol, Iodine solution, Fehling's solution, Concentrated Nitric acid (Conc. HNO_3), Copper sulphate, Sodium carbonate (Na_2CO_3), Aluminium chloride ($AlCl_3$) Ethanol, picric acid, Follin-ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Ascorbic acid, hydrogen peroxide, sodium hydrogen phosphate (Na_2HPO_4), 3.476 gm sodium dihydrogen phosphate (NaH_2PO_4), Sodium Phosphate buffer, sodium chloride, α-amylase solution, Starch solution, Iodine solution, and Alben-DS 400 mg were purchased from Labtex Bangladesh, Dhaka1217, Bangladesh..

2.2 Plant Sample Collection

C. maxima fruits were properly cleaned to remove any remaining sand or dust. The seeds were obtained by carefully scraping off the pulp. After that, for 2 weeks, the seeds were dried in the absence of direct sunlight; through shade drying. The dried seeds were further cut into pieces and ground to get powder via a grinder (RRH-500 A, Zhejiang, China). The resultant fine powder was kept in an air-tight container and stored in a cool, dry, and dark place.

2.3 Preparation of methanol extract

700 gm of seeds powder and 2 L of methanol were kept in two already cleaned beakers. The bottles were kept tightly closed for 14 days with occasional shaking. By this time, a major portion of the powder had dissolved in methanol, which was further filtered by using cotton wool (Azad et al., 2022). Additionally, the residual methanol was removed by a rotary evaporator (EV311H, Labtech Inc, China) at 45°C and reduced pressure. The resultant methanol extract was kept in a refrigerator. The % yield of methanol extract was calculated by following equation (Azad and Mohamed, 2023; Shafira et al., 2020; Azad et al., 2019).

$$\%\ Yield = \frac{Weight\ of\ concentrated\ extract}{Weight\ of\ dry\ plant\ powder} \times 100$$

2.4. Qualitative phytochemical screening

For seed extract, the active phytoconstituents were identified using the then-standard methods that are detailed below.

2.4.1. Test for Terpenoids

2.4.1.1. Salkowski's test

One milliliter of chloroform was added to two milligrams of extract. A few drops of concentrated H2SO4 were carefully applied to form a coating. The interface turned reddish-brown when let to stand, and the presence of terpenoids was indicated by the lower layer turning yellow.

2.4.1.2. Liebermann-Burchard's test

After dissolving 2 mg of dry extract in acetic anhydride, heating the mixture to boiling, cooling it down, and then adding 1 ml of concentrated sulfuric acid along the test tube's edges. Triterpenoids are present at the junction where a deep red color has formed.

2.4.2. Test for Flavonoids

2.4.2.1. Alkaline Reagent test

The crude extract was combined with two milliliters of a 2% NaOH solution. When a few drops of diluted acid were added, the bright yellow color that had formed went colorless, signifying the presence of flavonoids.

2.4.2.2. Zinc-Hydrochloric acid reduction test

Two milligrams of the extract were combined with zinc dust, and then dropwise added a small amount of hydrochloric acid. It was then brought to a boil. Flavones, flavonol, flavonones, and flavonoids were denoted by the colors orange to red, red to crimson, crimson to magenta, and crimson to green or blue, respectively.

2.4.2.3. Lead acetate solution test

A yellow precipitate was produced by mixing 2 mg of crude extract with a few drops of lead acetate (10%) solution.

2.4.2.4. Sulphuric acid test

A reddish-orange color was produced by mixing 2 milligrams of an extract with a few drops of strong sulfuric acid, suggesting the presence of flavonoids.

2.4.3. Test for Saponins

In a test tube, the crude extract was combined with 5 milliliters of distilled water and shaken firmly. It was believed that the production of steady foam indicated the presence of saponin.

2.4.4. Test for phenols

In a test tube, 2 mg of extract solution was taken. A small amount of 5% FeCl3 solution (three to four drops) was then added. Phenols are indicated by a greenish-black precipitate (Azad et al., 2012).

2.4.5. Test for Tannins

2.4.5.1. Test for Phlobatannins

1% aqueous hydrochloric acid was added to 2 mg of plant extract, and the mixture was then brought to a boil using a hot plate stirrer. The appearance of a red precipitate indicates a successful outcome.

2.4.6. Test for Steroid

2.4.6.1. Salkowski's test

One milliliter of chloroform was combined with two milligrams of crude extract, and a few drops of concentrated H2SO4 were added sideways. The presence of steroids was identified by a red hue formed in the bottom chloroform layer.

2.4.6.2. Liebermann-Burchard's test

After dissolving 2 mg of dry extract in acetic anhydride, heating the mixture to boiling, cooling it down, and then adding 1 ml of concentrated H2SO4 along the test tube's sides. Steroid presence was identified by the production of a green hue.

2.4.7. Test for Anthraquinones

After boiling 0.5g of the extract in 10 ml of sulfuric acid, it was filtered while still hot. 5 milliliters of chloroform were shaken with the filtrate. One milliliter of diluted ammonia was introduced to a test tube containing the chloroform layer using a pipette. We checked the final solution for color modifications.

2.4.8. Hydroxyanthraquinone test

Anthraquinone was detected by the red color created by 2 mg of an extract and a few drops of 10% aqueous potassium hydroxide.

2.4.9. Test for Alkaloids

2.4.9.1. Mayer's test

After gently heating the crude extract, two milliliters of 1% HCl were added. After that, the mixture was mixed with Mayer's reagent. According to Azad et al. (2012), the turbidity of the ensuing creamy precipitate was interpreted as proof that alkaloids were present Hager examination.

2.4.9.2. Wagner's test

After gently heating the crude extract, two milliliters of 1% HCl were added. After then, the mixture was mixed with Wagner's reagents. The ensuing reddish-brown precipitate's turbidity was seen as proof that alkaloids were present.

2.4.9.3. Hager's test

A few drops of Hager's reagent were added to 2 mg of the extract that had been taken in a test tube. The presence of alkaloids was established by the production of a yellow precipitate.

2.4.10. Test for Glycosides

2.4.10.1. Sodium hydroxide reagent test

Glycoside was present because 2 mg of the extract, 1 milliliter of distilled water, and a few drops of aqueous sodium hydroxide combined to generate a yellow tint.

2.4.10.2. Liebermann's test

Two milliliters each of acetic acid and chloroform were combined with the crude extract. Ice was used to chill the concoction. A precise concentration of H_2SO_4 was introduced. The glycone component of the glycoside, or the steroidal nucleus, is indicated by a color shift from violet to blue to green.

2.4.10.3. Salkowski's test

Chloroform (2 ml) was combined with the crude extract. After that, 2 milliliters of concentrated H_2SO_4 were added and gently shaken. The glycone part of the glycoside, or steroidal ring, was visible as a reddish-brown tint.

2.4.11. Test for Cardiac Glycosides

2.4.11.1. Keller- Killiani test

A solution of 2% $FeCl_3$ was added to 4 milliliters of glacial acetic acid, which was combined with 10 milliliters of the extract aqueous. After that, the mixture was transferred to a second test tube that held one milliliter of concentrated H_2SO_4. The presence of cardiac glycoside was shown by a brown ring at the interphase.

2.4.11.2. Baljet's test

One milliliter of freshly made sodium picrate solution turned yellow to orange when added to one milliliter of aqueous plant extract, suggesting the presence of cardiac glycoside.

2.4.12. Test for Resins

After dissolving 2 mg of the extract in acetone, the mixture was added to distilled water. Resins were indicated by turbidity.

2.4.13. Test for Carbohydrates

2.4.13.1. Benedict's examination

When 2 milliliters of Benedict's reagent were added to the crude extract and heated, a reddish-brown precipitate appeared, indicating the presence of carbohydrates (Azad et al., 2012).

2.4.13.2. Molisch's examination

Add a few drops of 1% α-naphthol and 2-3 ml of concentrated sulfuric acid along the test tube's side to 2 mg of plant extract. The test is validated by the reddish violet or purple ring that forms at the intersection of two liquids (Azad et al., 2012).

2.4.14. Test for Iodine

A solution of iodine (2 ml) was combined with the crude extract. The presence of the carbohydrate was indicated by a dark blue or purple coloring (Azad et al., 2012).

2.4.14.1. Fehling's test (Reducing sugar test)

After dissolving 2 mg of dry extract in 1 ml of distilled water, Fehling's (A+B) solution is added in 1 ml. After a ten-minute water bath, shake and warm. The test is validated by the formation of a brick-red precipitate (Azad et al., 2012).

2.4.15. Test for Proteins

2.4.15.1. Biuret test

Add 5 drops of 1% copper sulfate solution to 2 milligrams of plant extract. After that, add 1 milliliter of 10% NaOH and fully stir. Protein can be detected by the development of purple or violet hue (Azad et al., 2012).

2.5 Antioxidant activity

2.5.1 DPPH test

C. maxima seeds methanol extract was performed *in vitro* 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay according to (Azad et al., 2020). To perform this, 5 mg of extract was dissolved with 10 ml of methanol to prepare the stock solution having a concentration of 500 µg/ml. From this, serial dilutions were carried out to get 5 solutions having concentrations 250, 125, 62.5, 31.25, and 15.63 µg/ml. Ascorbic acid (500, 250, 125, 62.5, 31.25, 15.63 µg/ml) was used as a standard and DPPH was used as a control, which was prepared by using the method of sample preparation without a sample. 4 mg DPPH was dissolved in 100 of methanol to prepare 0.004% (w/v) DPPH solution and was stored at 20^{o}c C until required. 2 ml of extract or standard with varied concentrations was added with 3 ml of DPPH solution which was then shaken well and kept in darkness for 30 minutes at room temperature. Methanol was used as a blank for measuring the absorbance of the investigated samples by using a UV- Vis spectrophotometer. The following equation will be used to calculate the DPPH scavenging activity (%) (Khan et al., 2023).

$$\%\ Inhibition = \frac{(\text{Acontrol} - \text{Asample})}{\text{Acontrol}} \times 100$$

Here, $A_{control}$= Absorbance of Control and A_{sample}= Absorbance of Sample

2.5.2 Hydrogen Peroxide Scavenging Assay

Hydrogen Peroxide scavenging assay of *C.maxima* seeds methanol extract was performed according to (Azad et al., 2020). To perform this, 5 mg of each of the extracts was dissolved in 10 ml of distilled water to prepare a stock solution of concentration 500 µg/ml. The stock solutions were serially diluted to 250, 125, 62.5, 31.25, and 15.63 µg/ml solutions with distilled water. After this, 4 ml of extracts or standard with varied concentrations were added with 0.6 ml of 4 mM H_2O_2 solution prepared in phosphate buffer, followed by incubation for 10 minutes and measuring of absorbance at 230 nm. To prepare 50mM phosphate buffer (pH 7.4), 14.058 gm of sodium hydrogen phosphate (Na_2HPO_4) and 3.476 gm of sodium dihydrogen phosphate (NaH_2PO_4) were added with 1100 ml of distilled water. 100 ml is separated for pH set in the spectrophotometer. 50mM phosphate buffer without hydrogen peroxide was used as a blank for measuring the absorbance of the investigated samples by using a UV-Spectrophotometer. 0.4533 ml (453.3 µl) of 30% (w/v) H_2O_2 solution mixed with 1000 ml phosphate buffer. Ascorbic Acid (500, 250, 125, 62.5, 31.25, 15.63 µg/ml) was used as a positive control, and solvents without extract were used as a negative control or control. The abilities to scavenge hydrogen peroxide were calculated using the following equation.

$$\%\ \text{of scavenging effect (H2O2)} = \frac{(\text{Acontrol} - \text{Asample})}{\text{Acontrol}} \times 100$$

Here, $A_{control}$= Absorbance of Control and A_{sample}= Absorbance of Sample

2.6 Antidiabetic activity α amylase enzyme assay (Starch-Iodine color assay method)]

The antidiabetic potential of *C.maxima* seeds methanol extract was performed by α-amylase enzyme assay according to Nahar et al., (2024). 1 ml Extract solutions having different concentrations were added with 40 µL of phosphate buffer (0.02M, pH 7.0), followed by addition of 60 µL of α-amylase enzyme (10 mg enzyme in 1 ml distilled water) and incubation for 10 minutes at 37°C. After that, 200 µL of 1% starch solution was added and again incubated for 15 minutes at 37°c. Upon completion of the incubation period, 120 µL of 0. 1 N HCI and 600 µL of 1% iodine solution (which included 5 mmol I2 and 5 mmol KI) were added. Acarbose was used as a standard. The UV spectrophotometer was used to detect the absorbance at 620 nm. The following formula was used to calculate the inhibition of enzyme activity (Nahar et al., 2024). In the reaction mixture, starch is generally indicated by a dark blue color; its absence is indicated by a yellow color; and partial degradation of the starch is shown by a brownish color.

$$\%\ Inhibition = 100 - \frac{(OD\ of\ control - OD\ of\ sample)}{OD\ of\ control} \times 100$$

2.7 Anthelminthic activity of *C. maxima* seeds methanol extract

The anthelmintic property of *C. maxima* seeds methanol extract was performed according to Azad et al., (2020). Earthworms, or *Pherithema posthuma*, with an average size of 4–8 cm, were gathered from the soil-soaked sections in order to conduct this test. To get rid of any dirt that had adhered, they were cleaned with tap water. Two formulations containing two different quantities of methanolic seed extract (5, 10, and 20 mg/ml in distilled water) each were made. Albendazole 200 mg tablets were broken with a mortar and pestle, and the corresponding weight was dissolved in 20 ml distilled water to adjust at 20 ml. The test and reference solutions were put in a petri plate with three earthworms within. There were notes on "time for death" and "time for paralysis".

2.8 Statistical analysis

The results are shown as mean ± SEM. ANOVA was used to evaluate the data in one or both ways. Tukey's multiple comparison tests were then run (using IBM SPSS Statistics for Windows, Version 25.0, Armonk, N.Y., USA) to establish significance at $p<0.05$.

3. RESULTS

3.1. Percentage yield and phytochemical profile of methanol extract of *C. maxima* seeds

The percentage yield of *C. maxima* seeds methanol extract was 17.67 % and the phytochemical profile is shown in Table 1.

Table 1: Phytochemical screening of methanolic extract of *C. maxima.*

Secondary Metabolite	Name of the test	Observation	Results
Terpenoids	Salkowski's test	Yellow lower layer	+
	Liebermann- Burchard's test	The deep red color at the junction	++
Flavonoids	Zinc-Hydrochloric acid reduction test	Orange to red Red to crimson Crimson to magenta Crimson to green or blue	++
	Alkaline Reagent test	The intense yellow color turns colorless	+
	Lead acetate solution test	Yellow precipitate	+
	Sulphuric acid test	Reddish or orange color	++
Saponins	Foam test	Stable foam	-
Phenols	Ferric Chloride test	Bluish black color	-
Tannins	Ferric Chloride test	Greenish black Precipitate	++
Phlobatannins	General test	Red precipitate	-
Steroid	Salkowski's test	Red color in the lower chloroform layer	-
	Liebermann-Burchard's test	The green color at junction	-
Anthraquinones	General test	Color change	-
	Hydroxyanthraquinone test	Red color	-
Alkaloids	Mayer's test	Turbidity	++
	Wagner's test	Turbidity of reddish-brown precipitate	++
	Hager's test	Yellow precipitate	+
Glycosides	Sodium hydroxide reagent test	Yellow color	++
Cardiac Glycosides	Keller –Klilliani test	Brown ring between layers	-
Resins	General test	Turbidity	++
Carbohydrates	Molisch's test	Reddish violet/purple ring	-
	Benedict's test	Dark blue or purple	-
	Fehling's test	Brick red precipitate	-
Proteins	Biuret test	Purple/violet	-
	Nitric acid test	Yellow color	-
Fats and Fixed Oils	General test	Clear blue solution	+

(+) = Presence of Phytochemicals and (-) = Absence of Phytochemical.

3.2. Antioxidant test (DPPH Scavenging test and Hydrogen Peroxide Scavenging Assay)

The antioxidative activity of *C. maxima* seeds methanol extract was evaluated using the DPPH free radical scavenging method and hydrogen peroxide scavenging assay and the activities were compared with ascorbic acid, which is a standard antioxidant. The extract elicited a negligibly significant level of scavenging and neutralizing activity in both tests with IC_{50} 367.6301 µg/ml and 617.2897 µg/ml for DPPH and Hydrogen Peroxide tests respectively (table 3.2).

Table 3.2 IC_{50} of *C. maxima* seeds methanol extract and ascorbic acid in DPPH and hydrogen peroxide scavenging assay.

Group	DPPH IC_{50} (µg/ml)	H_2O_2 IC_{50} (µg/ml)
Methanol Extract	367.6301	617.2897
Ascorbic Acid	0.534	1.016

In both of these tests, the extract showed dose-dependent activity. Figures 3.1 and 3.2 illustrate the DPPH and hydrogen peroxide scavenging activity of *C. maxima* seeds methanol extract and ascorbic acid respectively.

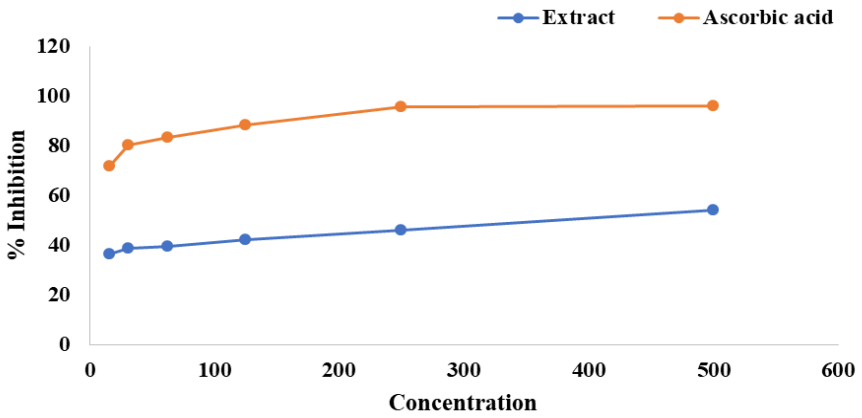

Figure 3.1: DPPH Scavenging activity of *C. maxima* seeds methanol extract and ascorbic acid.

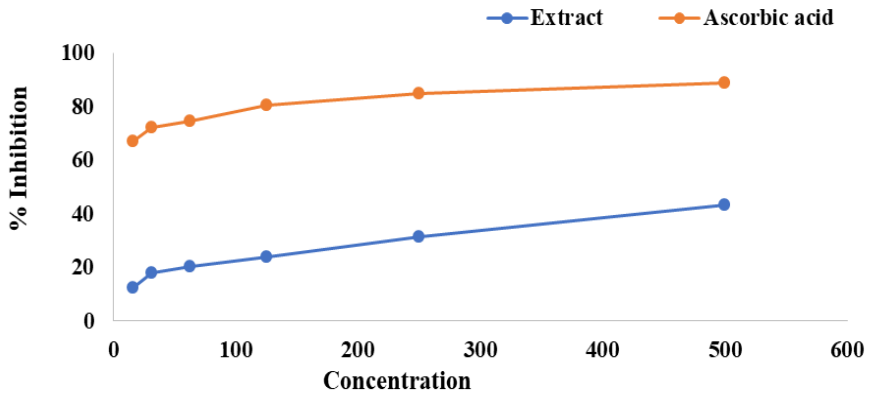

Figure 3.2: Hydrogen peroxide scavenging activity of *C. maxima* seeds methanol extract and ascorbic acid.

3.4. Antidiabetic activity

This study evaluated the α-amylase inhibitory effects of the *C. maxima* seeds methanol extract compared to a reference medication; acarbose via starch iodine colorimetric assay. *C. maxima* seeds methanol extract elicits 53.12%, 75.62%, and 89.64% inhibition at doses 250, 500 and 1000 µg/ml respectively (Table 3.3). At a concentration of 1000 µg/ml, the acarbose exhibited a very high level of inhibition of 98.18%, surpassing that of the CLP extract. Acarbose and *C. maxima* seeds methanol extract had IC_{50} values of 54.36 µg/ml and 135.3814 µg/ml, respectively (Table).

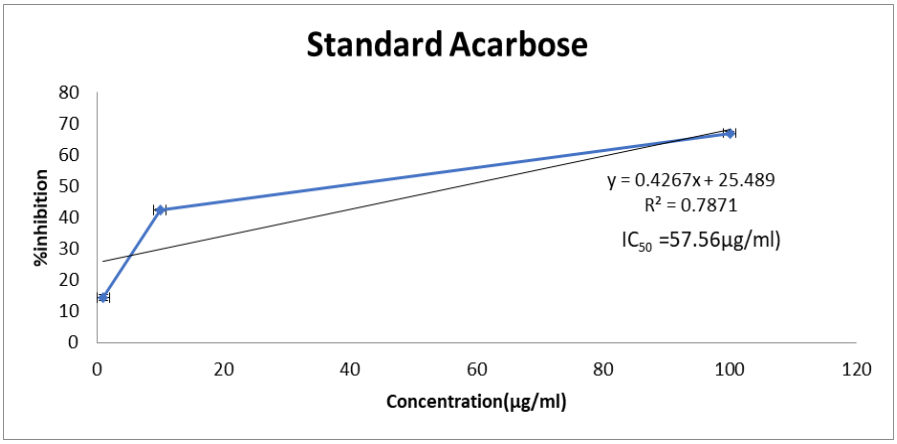

Table 3: Concentrations, % Inhibition, and IC_{50} of *C. maxima* seeds methanol extract.

Samples	Concentration (µg/ml)	% Inhibition (R1)	% Inhibition (R2)	% Inhibition (R3)	Mean	STD	IC_{50}
Extract	250	55.90	50.60	52.84	53.11	2.17	135.38
	500	70.43	70.80	70.62	70.62	0.14	
	1000	90.21	89.06	89.63	89.63	0.46	
Acarbose	1	15.41	13.37	15.11	14.39	1.44	57.56
	10	42.73	42.15	42.79	42.44	0.41	
	100	67.44	66.57	67.51	67.01	0.62	

3.5. Anthelmintic activity

Parameters	Samples and treatment concentrations						
	Std			Extract			Control
	5	10	20	5	10	20	
Time of Paralysis (TP)	103	81	37	79	57	25	221
Time of Death (TD)	283	172	178	239	131	69	493

4. DISCUSSION

Plants naturally contain substances called phytochemicals that have a range of therapeutic uses (Mendoza & Silva, 2018). In addition to their defensive properties (anti-inflammatory, anti-diabetic, anti-aging, anti-microbial, anti-parasitic, anti-depressant, anti-cancer, antioxidant, and wound healing), plants' abundance of different phytochemicals, such as alkaloids, tannins, saponins, flavonoids, phenols, steroids, and carotenoids, is essential for the prevention of disease (Asaduzzaman & Asao, 2018). In this instance, the presence of terpenoids, flavonoids, tannins, alkaloids, and glycosides were demonstrated by the phytochemical screening of the methanol extracts from *C. maxima* seeds. Nevertheless, saponin, phenols, steroids, anthraquinone, cardiac glycosides, carbohydrates, and proteins were absent from the extract.

The capacity to donate hydrogen is what gives DPPH, a free radical with a reputation for being highly stable, its antioxidant effect on DPPH radical scavenging (Stankovic et al., 2011). This assay is typically used to assess the phenolic components in plant extracts' antioxidant qualities (Shalaby, & Shanab, 2013). In this assay, the prevalence of antioxidant components in plant extract is indicated by the change of DPPH solution to diphenylpicryl hydrazine, which is yellow (Rahman et al., 2015).

In the current study to evaluate the antioxidant activity of the investigated extracts by using DPPH assay, the higher the percentage (%) of scavenging effect, the higher the antioxidant potential of the extracts. On the other hand, the lower

the IC_{50} value, the higher the antioxidant activity of the extracts. In the study, the scavenging effect of the reference standard AA was found to be 96.014% at the highest concentration whereas the IC_{50} value was much less (0.534 µg/ml) which showed its highly effective potential as a free radical scavenger. Additionally, the negative control methanol showed no antioxidant activity. However, *C. maxima* seeds methanol extract showed a week but dose-dependent free radical scavenging effect compared to that of the reference standard. Conversely, hydrogen peroxide can enter our bodies by contact with the skin or eyes, or by inhaling vapor. Rapid breakdown of H_2O_2 into oxygen and water can result in the production of hydroxyl radicals (OH*), which can damage DNA in the body and induce lipid peroxidation. Thus, in order to assess plant extracts' potential as antioxidants, their capacity to scavenge hydrogen peroxide was calculated.

In the present study, the higher the percentage (%) of the scavenging effect, the higher the antioxidant potential of the extracts. Conversely, the lower the IC_{50} value, the higher the antioxidant activity of the extracts. In this study, ascorbic acid showed the highest H_2O_2 scavenging effect of 96.014% at the highest concentration investigated. It showed good H_2O_2 scavenging effectiveness even at the lowest concentration investigated, whereas the control didn't show any H_20_2 scavenging effect. Along with this, *C. maxima* seeds methanol extract didn't show effectiveness as an antioxidant at lowest the concentration thereby having the IC_{50} value very high to ascorbic acid.

The alpha-amylase inhibiting action of *C. maxima* seeds methanol extract exhibited weak antidiabetic activity compared to that of the standard acarbose, since, it had an IC50 value much higher than that of the standard. In the case of the anthelmintic activity test, earthworms (Pherithema posthuma) are a great representative of intestinal roundworm parasites, since they share similar anatomical and physiological patterns (Aziz et al., 2014). The outcomes of the anthelmintic activity test revealed that the extract had moderate anthelmintic activity at a higher dose. This activity may be due to the prevalence of phytochemicals such as alkaloids, since, secondary metabolites such as alkaloids and saponins have been reported to have effective anthelmintic activity (Tagoe et al., 2021). The weaker activity of the extract may be due to the presence of only alkaloids, not saponins.

Overall, the outcomes of all in vitro and in vivo tests are aligned with each other. The intensity of the presence of several alkaloids is self-explanatory for the results of other tests. The phytochemical screening showed the presence of flavonoids but the absence of phenolic compounds. This could be the reason for the low antioxidant activity, observed in DPPH and H_2O_2 tests because flavonoids and phenolics have been reported to be associated with antioxidant properties. Furthermore, the weaker antioxidant activity can be associated with milder effects in antidiabetic and anthelmintic tests.

5. Limitations of the study

The main limitation of this study is that this study was performed mostly in vitro. There are substantial differences in in vitro and in vivo test results. Additionally, crude methanol extract was utilized rather than the isolation of bioactive compounds available in that extract. Generally, the crude extract contains a mixture of different types of compounds. Therefore, it is possible that one compound's bioactivity can be antagonized by other compounds available. Hence, compound isolation and molecular docking approaches can allow us to know the real potential.

6. CONCLUSION

To recapitulate, more insightful scientific research is required to generate more reproducible outcomes in the sectors of antioxidant, antidiabetic, and anthelmintic activities. Furthermore, apart from these, some other activities must be evaluated so that the potentiality of the seeds of this medicinal plant can be assessed appropriately.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

All data and materials will be available upon request to the corresponding author.

Competing interests

The authors declare no conflict of interest.

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