The Journal of Phytopharmacology 2025; 14(5):362-369
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2025; 14(5): 362-369
September- October
Received: 06-09-2025
Accepted: 30-10-2025
Published: 30-11-2025

doi: 10.31254/phyto.2025.14510

Lalit D. Tattu
College of Biosciences and Technology, Pravara Institute of Medical Sciences (Deemed to be University), Ahilyanagar- 413736, Maharashtra, India

Faiyaz K. Shaikh
College of Biosciences and Technology, Pravara Institute of Medical Sciences (Deemed to be University), Ahilyanagar- 413736, Maharashtra, India

Raghavendra L.S. Hallur
College of Biosciences and Technology, Pravara Institute of Medical Sciences (Deemed to be University), Ahilyanagar- 413736, Maharashtra, India

Correspondence:
Dr. Faiyaz K Shaikh
College of Biosciences and Technology, Pravara Institute of Medical Sciences (Deemed to be University), Ahilyanagar- 413736, Maharashtra, India
Email: faiyaz.shaikh@pmtpims.org

Sequential extraction, phytochemical analysis, and enzyme inhibitory activity of polyphenols from black cardamom (*Amomum subulatum* Roxb.) shell and seeds

Lalit D. Tattu, Faiyaz K. Shaikh, Raghavendra L.S. Hallur

ABSTRACT

Background: Black Cardamom (*Amomum subulatum* Roxb.) is traditionally used for its medicinal properties in treating respiratory, digestive, and infectious diseases. However, its bioactive polyphenols and their enzyme inhibitory activities remain underexplored. **Aims and Objectives:** This study aimed to investigate the sequential extraction, phytochemical composition, and enzyme inhibitory activity of polyphenols from the shell and seed of *A. subulatum*. **Materials and Methods:** Sequential extraction was performed using hexane, chloroform, petroleum ether, and water. The phytoconstituents were analyzed using standard qualitative tests. The total phenolic content (TPC) and total flavonoid content (TFC) were determined using spectrophotometric methods. Enzyme inhibition assays were conducted against alpha-amylase and trypsin, followed by gas chromatography-mass spectrometry (GC-MS) analysis to identify bioactive compounds. **Results:** Phytochemical analysis showed the presence of phenols, saponins, terpenoids, flavonoids and alkaloids in both extracts. TPC and TFC results indicated that the seed extract had higher phenolic (141.22 ± 2.52 mg GAE/g) and flavonoid content (1.98 ± 0.42 mg RE/g) compared to the shell. Enzyme inhibition assays demonstrated 100% inhibition of alpha-amylase by hexane, chloroform, and petroleum ether extracts at a 1:3 enzyme-to-extract ratio, while aqueous extracts showed no inhibition. The dot blot assay confirmed pronounced trypsin inhibition in seed extracts obtained using hexane and chloroform. GC-MS analysis identified 80 compounds in the shell, including desogestrel (13.08%) and benzofuran (5.08%), and 67 compounds in the seed, including oleoyl chloride (10.60%) and pentadecanoic acid (10.64%). **Conclusion:** Sequential extraction of *A. subulatum* demonstrated significant alpha-amylase and trypsin inhibitory activity, especially in non-polar extracts. The bioactive compounds identified suggest the plant's potential in managing hyperglycemia and inflammatory conditions.

Keywords: *Amomum subulatum* Roxb, Polyphenols, Enzyme inhibition, Alpha-amylase, Trypsin, GC-MS analysis.

INTRODUCTION

The quest for natural bioactive compounds with potential therapeutic applications has intensified in recent years, driven by the need to find safer and more effective alternatives to synthetic drugs. Among such promising natural sources are polyphenols, a diverse group of secondary metabolites widely distributed in the plant kingdom [1]. Polyphenols are renowned for their significant antioxidant, anti-inflammatory, anticancer, and antidiabetic properties [2]. They have been extensively studied for their health benefits, particularly their ability to modulate various biological pathways and inhibit key enzymes associated with chronic diseases [3-5]. Black cardamom (*Amomum subulatum* Roxb.), a member of the Zingiberaceae family, is a spice commonly used in the Indian subcontinent for its distinct smoky flavor and medicinal properties. Traditionally, black cardamom has been utilized for treating respiratory disorders, digestive issues, and infections [6]. Despite its well-documented medicinal uses, the polyphenolic content of black cardamom and its potential as a source of enzyme inhibitors have not been thoroughly explored.

Proteases and amylases are critical enzymes involved in numerous physiological and pathological processes [7,8]. Proteases like trypsin are crucial for various biological processes, including digestion, immune system function, and cell signaling. However, their overexpression is implicated in various diseases, including cancer and inflammatory disorders [9]. Alpha-amylase are crucial for carbohydrate digestion but are also associated with hyperglycemia and diabetes when dysregulated [10,11]. Inhibiting these enzymes using natural polyphenols could offer a novel approach to managing conditions like cancer and diabetes [12,13].

MATERIAL AND METHODS

Sample Collection

Black cardamom (*A. subulatum*) sample was collected from the masala market in Loni Tal. Rahata Dist. Ahmednagar (MS), India (Latitude: 18.1820N, Longitude: 74.364550E). The samples were authenticated by Dr. A.S. Dr. Wabale, Vice Principal and faculty member in the Department of Botany at Padmashri Vikhe Patil College of Arts, Science, and Commerce, Loni.

Sequential Extraction of Polyphenols

The sequential extraction was performed using hexane, chloroform, petroleum ether, and water. The sample (seed: 20 g; shell: 10 g) was sequentially extracted with each solvent. After each extraction, the residue was dried and weighed before proceeding to the next solvent. Hexane Extraction: Ground sample (seed: 20 g; shell: 10 g) was mixed with hexane (ratio 4:1) and incubated overnight. Extracts were centrifuged at 10,000 rpm, and the supernatant was transferred to petri dishes for evaporation. The residue was dried, weighed, and then subjected to re-extraction using chloroform. Chloroform and Petroleum Ether Extractions: The procedure was the same as for hexane. After evaporation, residues were collected in centrifuge tubes using 1.5 mL DMSO. Aqueous Extraction: The mixture of solvent and sample was prepared in a ratio of 10:1 (w/v) and incubated overnight. Extracts were filtered and collected in centrifuge tubes. Non-aqueous extracts were diluted with 3 mL of 100% methanol [14,15].

Qualitative of Polyphenols

Phytochemicals (phenols and tannins, flavonoids, saponins, alkaloids, and terpenoids) were tested using methods described by [16].

Quantitative Analysis of Polyphenols

The Total Phenol Content (TPC) was measured using the Folin-Ciocalteu method [17]. Samples (1 mL, 100 µg/mL w/v in H_2O) were mixed with 0.5 mL of Folin-Ciocalteu reagent (diluted 1:3 with distilled water) and 2 mL (20%) sodium carbonate. Absorbance was recorded at 650 nm with a UV-visible spectrophotometer. TPC was expressed as milligrams of gallic acid equivalent (GAE) per gram of extract [18]. The Total Flavonoid Content (TFC) was determined using a colorimetric assay [19]. Samples (2 mL) were mixed with 0.1 mL $AlCl_3$ and 0.1 mL potassium acetate, incubated for 30 minutes at room temperature, and absorbance was measured at 415 nm. Results were expressed as mg rutin equivalent (RE)/g extract.

Amylase Inhibitory Activity by starch agar plate

Starch agar plates were prepared with 1.5 g agar and 0.1 g starch. Wells were made and filled with mixtures of enzyme and extracts at different ratios (3:1, 1:1, 1:3 v/v). The plates were incubated overnight at 37°C and subsequently stained with an iodine-potassium iodide solution. Zones of hydrolysis were compared to control wells.

Amylase Inhibition by Solution Assays

Salivary amylase (40 µL) was preincubated with different volumes of extracts and 10 mM sodium phosphate buffer, pH 6.9. Starch (1 mL, 1%) was added and incubated at 37 °C for 15 minutes. One milliliter of DNSA reagent was added to the mixture, which was then incubated at 85 °C for 15 minutes and allowed to cool. Absorbance was measured at 540 nm.

Protease Inhibition by Solution Assays

Trypsin (100 µL) was preincubated with different volumes of extracts and 0.1 M Tris HCl buffer, pH 7.8. One milliliter of 1% casein solution was added and the mixture was incubated at 37 °C for 30 minutes. Reaction was terminated with 1 mL of 5% TCA, centrifuged, and supernatant was mixed with 2.5 mL of 0.5 M Na_2CO_3 and 0.5 mL Folin-Ciocalteu reagent. Absorbance was measured at 660 nm [20].

Protease Inhibitory Activity by Dot-Blot Assay

Different concentrations of trypsin and extracts (Black cardamom) were prepared (1:3, 1:1, 3:1 v/v). Mixtures were applied to X-ray film, incubated at 37 °C for 15 minutes, washed, and dried. Patterns of gelatin hydrolysis indicated inhibitor effectiveness [21].

GC-MS Analysis of Phytochemicals

For sample preparation, 70% methanol was added to the sample (5 g seed, 5 g shell) at a ratio of 1:5, incubated overnight, centrifuged at 10,000 rpm, and supernatant was collected in centrifuge tubes with 1.5 mL DMSO. GC-MS Analysis was conducted on a Shimadzu GCMS-TQ8040 with RESTEK Rxi-5ms column. Injection temperature: 280 °C, carrier gas: helium, flow: 1.2 mL/min. Column temperature: 40 °C to 270 °C. Ion source temperature: 220 °C. Mass range: m/z 45-550. Compound identification was performed using the NIST (National Institute of Standards and Technology) library.

Statistical Analysis

All experiments were performed in triplicate. Microsoft Excel was used to compute the mean values and standard deviations.

RESULTS

The phytoconstituent analysis of *A. subulatum* seed and seed shell extracts was performed using different solvents: hexane, chloroform, petroleum ether, and aqueous extracts. The presence of phenolic compounds, flavonoids, saponins, terpenoids, and alkaloids was determined for both the seed and seed shell.

Phytoconstituents Analysis of Seed Extracts

As shown in Table 1, the analysis of *A. subulatum* seed extracts revealed the presence of phenols and tannins, flavonoids, saponins, terpenoids, and alkaloids in varying amounts across the different solvent extracts. Both the hexane and chloroform extracts showed positive results for phenols, tannins, flavonoids, saponins, terpenoids, and alkaloids, with saponins being present in all solvent types. In contrast, petroleum ether extracts showed a negative result for phenols, tannins, flavonoids, terpenoids, and alkaloids, while saponins were the only phytoconstituent detected. Aqueous extracts showed a limited presence of terpenoids and saponins, while phenols, tannins, flavonoids, and alkaloids were absent.

Phytoconstituents Analysis of Seed Shell Extracts

Table 2 summarizes the phytochemical screening of the seed shell extracts. The hexane and chloroform extracts revealed the presence of phenols, tannins, flavonoids, saponins, terpenoids, and alkaloids, with terpenoids and alkaloids being prevalent in all solvent types. Petroleum ether extracts showed a slightly broader spectrum of phytoconstituents, including flavonoids, saponins, terpenoids, and alkaloids, while phenols and tannins were absent. Aqueous extracts, however, were found to contain saponins and terpenoids but were negative for phenols, tannins, flavonoids, and alkaloids.

Total Phenolic Content (TPC) Analysis

The TPC of *A. subulatum* seed and seed shell extracts was quantified. Based on the standard regression line for gallic acid, with a coefficient of determination $R^2 = 0.9957$, the concentration of phenolics in the shell extract was determined to be 90.81 ± 0 mg/g gallic acid equivalent (GAE). In comparison, the phenolic content in the seed extract was significantly higher, measured at 141.225 ± 2.52 mg/g GAE (Figure 1). These results indicate that the seed of *A. subulatum*

contains a greater amount of phenolic compounds than the seed shell, highlighting its potential as a rich source of antioxidants. The strong linearity of the regression line emphasises the reliability and accuracy of the phenolic quantification method used in this study.

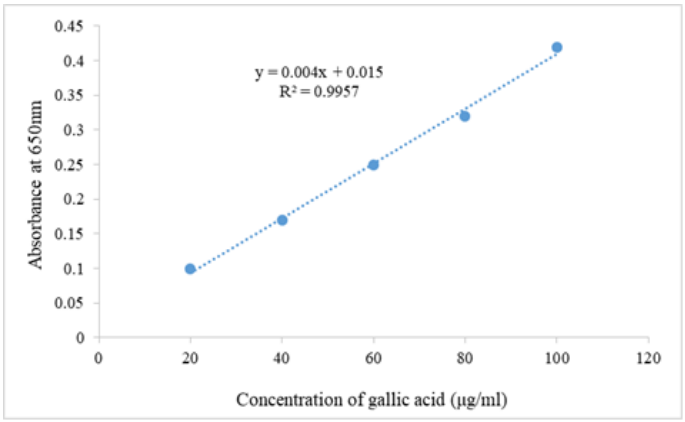

Figure 1: Regression line of gallic acid for estimation of TPC

Total Flavonoid Content (TFC) Analysis

The TFC of the *A. subulatum* seed and seed shell extracts was determined using the standard regression line for rutin, with a coefficient of determination R^2=0.9988, the concentration of flavonoids in the shell extract was measured at 1.01254 ± 0.42 mg/g rutin equivalent (RE). In contrast, the flavonoid content in the seed extract was found to be higher, at 1.983 ± 0 mg/g RE. These results demonstrate that the seed extract of *A. subulatum* contains nearly double the amount of flavonoids compared to the seed shell, suggesting its superior potential as a source of bioactive flavonoids.

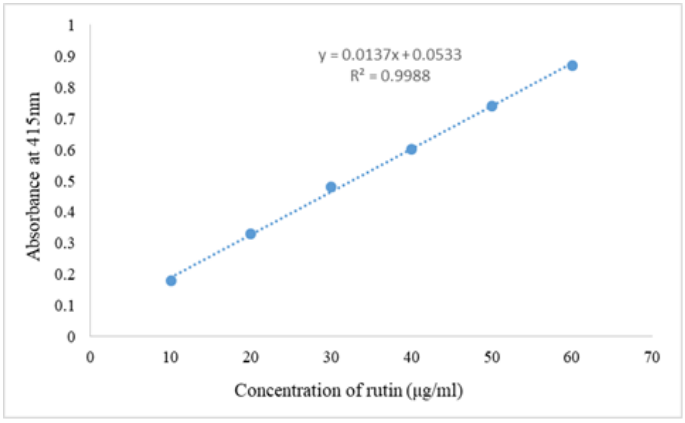

Figure 2: Regression line of rutin for estimation of TFC

Amylase Inhibition Activity

The starch agar plate method was used to detect the amylase inhibitor activity in sequentially extracted samples of *A. subulatum*. The extracts were tested at various enzyme-to- extract ratios (E: I), including 1:1, 3:1, and 1:3 (v/v). It was observed that the hexane shell extract (HCE), chloroform shell extract (CCE), petroleum ether shell extract (PCE), and petroleum ether seed extract (PSE) demonstrated complete inhibition of alpha-amylase activity at a 1:3 (E: I) ratio. Conversely, the water shell extract (WCE) and water seed extract (WSE) exhibited no inhibition of alpha-amylase, as shown in Figures 3 and 4.

Figure 3: Detection of amylase inhibition by starch agar plate method (A-Enzyme, B- (1:1, E: I), C-(3:1, E: I) D- (1:3, E: I), O- Buffer, a) Hexane Shell Extract (HCE); b) Hexane Seed Extract (HSE); c) Chloroform Shell Extract (CCE); d) Chloroform See Extract (CSE).

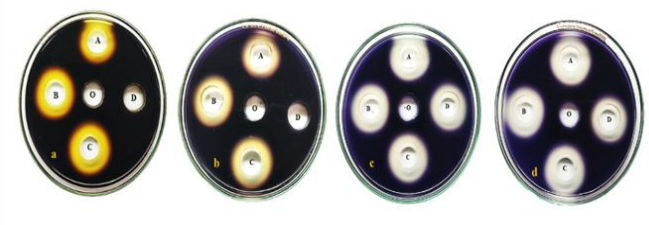

Figure 4: Detection of amylase inhibition by starch agar plate method (A-Enzyme, B- (1:1, E: I), C-(3:1, E: I) D- (1:3, E: I), O- Buffer, a) Petroleum Ether Shell Extract (PCE); b) Petroleum Ether Seed Extract; c) Water Shell Extract (WCE); d) Water Seed Extract (WSE).

The percentage inhibition of alpha-amylase by each extract was calculated based on the diameter of the starch hydrolysis zones surrounding the wells in the agar plates. The HCE, CCE, PCE, and PSE extracts all showed 100% inhibition of alpha-amylase, whereas the WSE showed 0% inhibition (Figure 5). Additionally, a standard solution assay confirmed that the inhibition of alpha-amylase was concentration-dependent, with higher concentrations of extract leading to greater enzyme inhibition.

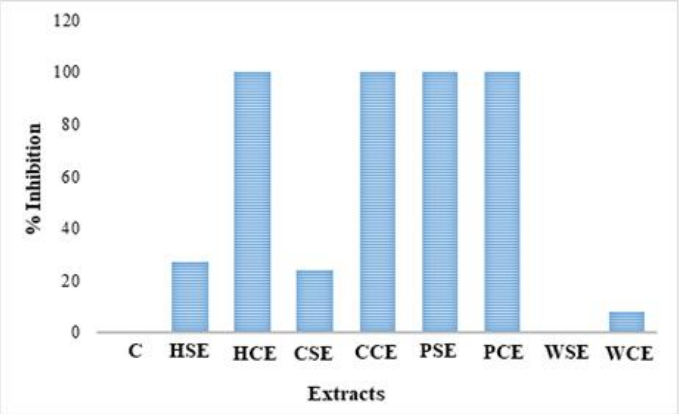

Figure 5: Percent (%) inhibition of alpha amylase with various solvent extracts

Protease Inhibitor Activity against Trypsin

The potency of protease inhibitors (PIs) against trypsin in *A. subulatum* extracts was evaluated using the dot blot assay with X-ray film. The assay revealed that both HSE and CCE were the most effective trypsin inhibitors. In addition, trypsin inhibition was observed with all other extracts, including HCE, CSE, PCE, PSE, WCE, and WSE, at enzyme-to-inhibitor ratios (E: I) of 1:1 and 1:3 (Figure 6).

The results indicate that the inhibition of trypsin is concentration-dependent, as evident from varying degrees of inhibition across different ratios of enzyme to extract. The extracts HSE and CCE exhibited the strongest inhibitory activity, suggesting their high potential as sources of protease inhibitors.

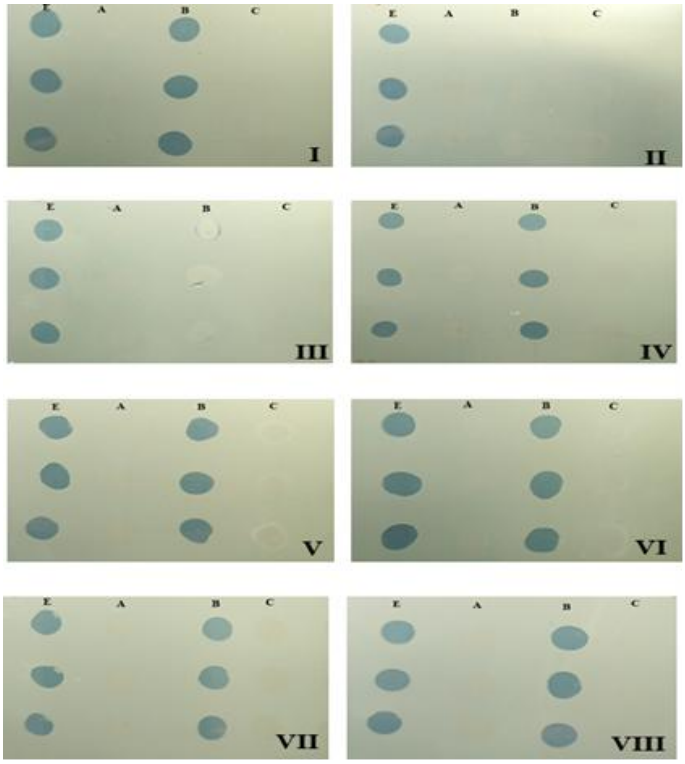

Figure 6: Detection of protease inhibitory (Trypsin) activity by dot blot assay (E- enzyme, A-(1:1, E: I), B-(3:1, E: I) C- (1:3, E: I)), a) HCE; b) HSE; c) CCE; d) CSE; e) PCE; f) PSE; g) WCE; h) WSE.

GC-MS Analysis of Bioactive Phytochemicals

The GC-MS analysis of the methanolic extract from *A. subulatum* Roxb. shell identified 80 distinct bioactive compounds. Figure 7 displays the chromatogram.

The most abundant compound was desogestrel, constituting peak area 13.08%, followed by two structurally similar compounds showing hypoglycemic activity with molecular weights of 410, accounting for peak area 17.34% and 8.48%, respectively (Table 3). Significant bioactive compounds detected include benzofuran (5.08%), phytol (peak area 2.23%), and 9,12,15-octadecatrienoic acid (peak area 4.16%), all known for their antioxidant, antimicrobial, and anticancer activities. Other noteworthy compounds include n-hexadecanoic acid (peak area 4.63%) and retinal (peak area 0.29%), both contributing to antioxidant and antibacterial properties. In addition, compounds such as eugenol, γ-muurolene, and isospathulenol exhibited a range of pharmacological activities.

GC-MS analysis of the methanolic seed extract of *Amomum subulatum* identified 67 distinct bioactive compounds. The corresponding chromatogram is shown in Figure 8.

Among these, Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl (Peak 18, Area% 5.78) and Oleoyl chloride (Peak 66, Area% 10.60) exhibited strong antimicrobial activity, respectively (Table 4). Additionally, Pentadecanoic acid (Peak 48, Area% 10.64) was identified as having anti-cancer property. The extract also contained antioxidants such as 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6 (Peak 4, Area% 3.48), while compounds like α-Terpineol (Peak 5, Area% 0.86) and Furaneol (Peak 2, Area% 0.29) showed potential anticancer effects. Notably, Palmitoleic acid (Peak 46, Area% 1.12) and Hydroxydehydrostevic acid (Peak 49, Area% 4.75) were linked to antitumor and antimicrobial activities, respectively.

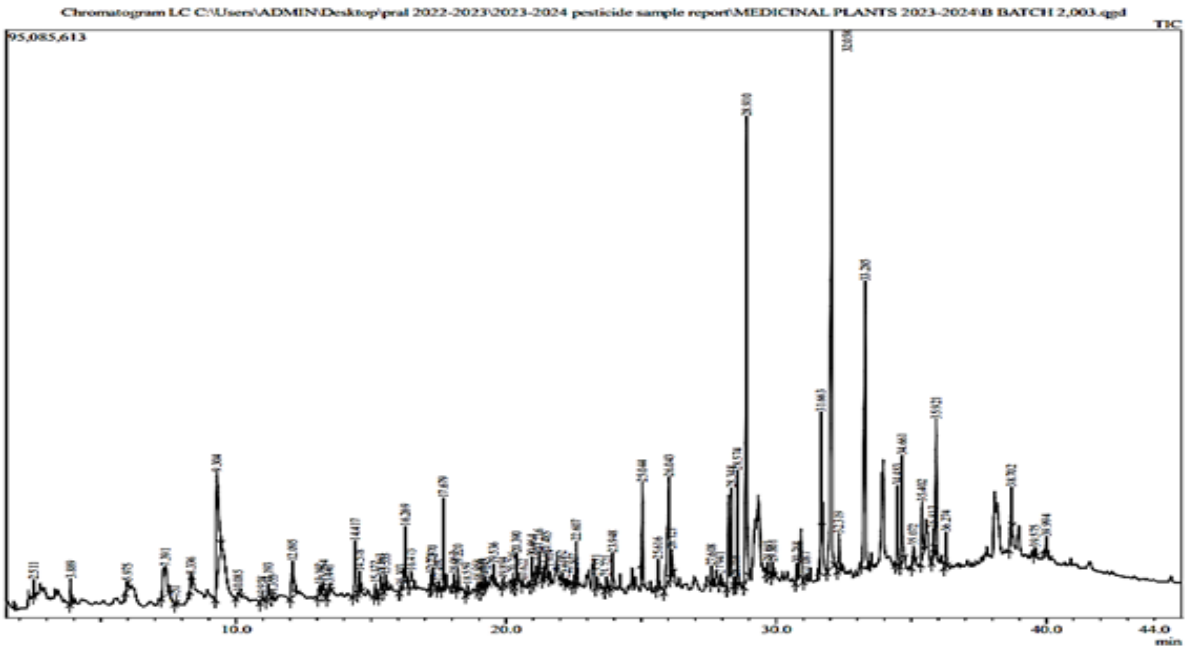

Figure 7: GC-MS chromatogram of methanolic shell extract of *A. subulatum* roxb

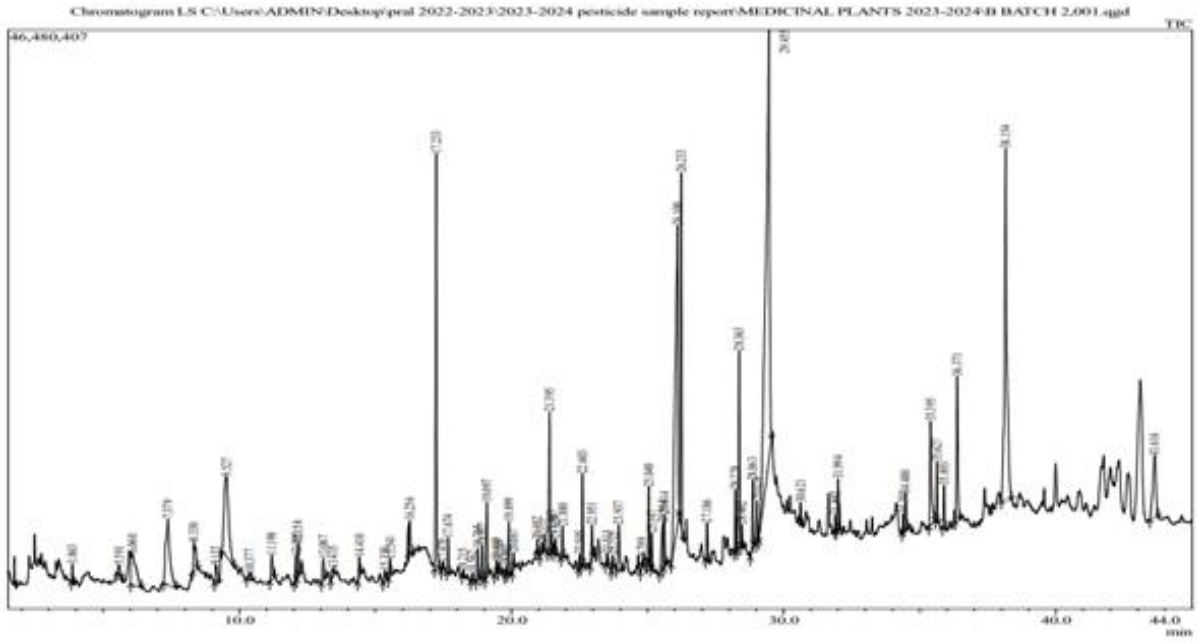

Figure 8: GC-MS chromatogram of methanolic seed extract of *A. subulatum* roxb

Table 1: Phytoconstituents analysis of *A. subulatum* seed extracts

S. No.	Phytoconstituents	Hexane	Chloroform	Petroleum ether	Aqueous
1.	Phenol & Tannins	+	+	-	-
2.	Flavonoids	+	+	-	-
3.	Saponins	+	+	+	+
4.	Terpenoids	+	+	-	+
5.	Alkaloids	+	+	-	-

Table 2: Phytoconstituents analysis of *A. subulatum* seed shell extracts

S. No.	Phytoconstituents	Hexane	Chloroform	Petroleum ether	Aqueous
1.	Phenol & Tannins	+	+	-	-
2.	Flavonoids	+	+	+	-
3.	Saponin	+	-	+	+
4.	Terpenoids	+	+	+	+
5.	Alkaloids	+	+	+	-

Table 3: Major Phytoconstituents of methanolic shell extract of *A. subulatum* roxb

Peak	Compound Name	Area %	Biological Activities
1	2-Furanmethanol	0.23	Antimicrobial, antioxidant [33]
5 & 6	L-.alpha.-Terpineol, .alpha.-Terpineol	0.01, 0.18	Antimicrobial, anti-inflammatory, antioxidant [34]
7	Benzofuran, 2,3-dihydro-	5.08	Antimicrobial, anti-inflammatory [35]
12	Phenol, 2,6-dimethoxy-	1.61	Antioxidant, antimicrobial [36]
17	Eugenol	0.63	Antimicrobial, anti-inflammatory, analgesic [37]
21	gamma.-Muurolene	0.12	Antimicrobial, anti-inflammatory [38]
61	Phytol	2.23	Antioxidant, anti-inflammatory, anticancer [39]
62	Desogestrel	13.08	Contraceptive [38]
59	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	4.16	Anti-inflammatory, cardiovascular protective [39]
53 & 55	Hexadecanoic acid, methyl ester, n-Hexadecanoic acid	1.58, 4.63	Antioxidant, anti-inflammatory [40]
66	Retinal	0.29	Essential for vision, skin health [41]
76	Villosin	3.05	Potential anticancer, antimicrobial [42]
78	Verrucarol	1.16	Antifungal, anticancer [43]

Table 4: Major Phytoconstituents of methanolic seed extract of *A. subulatum* roxb

Peak	Name	Area%	Pharmacological Activities
1	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-on	0.19	Antimicrobial [44]
2	Furaneol	0.29	Anticancer, Antimicrobial [45]
4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6	3.48	Antioxidant, Antimicrobial [46]
5	α-Terpineol	0.86	Anticancer [31]
7	4-Hepten-3-one, 4-methyl-	4.58	Antioxidant, Antibacterial [47]
18	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl	5.78	Antimicrobial [48]
46	Palmitoleic acid	1.12	Antitumor [49]
48	Pentadecanoic acid	10.64	Anti-cancer effects [30]
49	Hydroxydehydrostevic acid	4.75	Antimicrobial
66	Oleoyl chloride	10.60	Antimicrobial [50]

DISCUSSION

Phytochemical screening revealed a rich diversity of bioactive compounds in *A. subulatum* seed and seed shell extracts, including phenolic compounds, flavonoids, saponins, terpenoids, and alkaloids, underscoring the plant's therapeutic potential. The consistent presence of these compounds in hexane and chloroform extracts aligns with previous findings [22], which emphasize the effectiveness of organic solvents for extracting both polar and non-polar phytochemicals. Saponins were notably present across all extracts, reflecting their significant bioactivity, including antimicrobial and anticancer properties [23]. Conversely, the absence of phenolic compounds in petroleum ether extracts highlights the importance of solvent selection in phytochemical analysis. The seed shell extracts showed similar profiles, indicating that both parts of the plant contribute to its overall bioactivity. Aqueous extracts predominantly contained saponins and terpenoids, consistent with findings [24] regarding the solubility of saponins in various solvents. The presence of terpenoids, recognized for their antimicrobial and anti-inflammatory effects [27], and alkaloids, associated with analgesic properties, further supports the pharmacological relevance of *A. subulatum*. The phytochemical analysis confirms the presence of several bioactive compounds, validating traditional uses of the plant and providing a scientific foundation for future pharmacological studies.

The quantification of TPC and TFC in *A. subulatum* extracts revealed significant differences between the seed and seed shell. The TPC analysis indicated that the seed extract contained a notably higher concentration of phenolic compounds compared to the seed shell. This finding supports previous studies that emphasize the antioxidant potential of phenolic compounds, which have been linked to various health benefits, including anti-inflammatory and anti-cancer properties [2]. The TFC results further corroborated the superior bioactive profile of the seed, with almost double that of the seed shell. This highlights the seeds' potential as a valuable source of flavonoids, which are recognized for their role in promoting cardiovascular health and mitigating oxidative stress [28].

The amylase inhibition activity of *A. subulatum* extracts was evaluated using the starch agar plate method, revealing complete inhibition of alpha-amylase by hexane, chloroform, and petroleum ether extracts at a 1:3 enzyme-to- extract (E) ratio. Specifically, HCE, CCE, PCE, and PSE exhibited 100% inhibition, while the water extracts showed no activity. This highlights the solvent extraction efficiency, with non-polar solvents yielding extracts rich in bioactive compounds that effectively inhibit enzyme activity. These findings align with previous research indicating that non-polar phytochemicals are often more effective against carbohydrate-hydrolyzing enzymes, suggesting potential applications in managing carbohydrate metabolism and glycemic response. Similarly, the protease inhibitory activity against trypsin demonstrated that both HSE and CCE were particularly effective, showing significant inhibition across various enzyme-to- extract ratios. The concentration-dependent inhibition observed across all extracts further supports the potential of *A. subulatum* as a source of protease inhibitors, which could have implications in therapeutic applications for conditions associated with excessive protease activity, such as inflammation and cancer.

GC-MS analysis identified 80 compounds in the seed shell and 67 in the seed extracts. Notably, desogestrel was the most abundant in the shell extract, accounting for 13.08% of the peak area, followed by other compounds exhibiting hypoglycemic activity. This finding is consistent with previous studies that highlighted the hypoglycemic potential of similar compounds in medicinal plants, suggesting possible therapeutic applications in managing diabetes. Among the significant compounds, benzofuran (5.08%), phytol (2.23%), and 9,12,15-octadecatrienoic acid (4.16%) were noted for their antioxidant, antimicrobial, and anticancer properties. These results corroborate earlier reports indicating that phytol and octadecatrienoic acid possess potent antioxidant capabilities, which could contribute to the overall health benefits of A. subulatum extracts [29]. The polyphenols in black cardamom likely block enzymes by sticking to their active sites and disrupting their structure12. Non-polar extracts (like hexane) showed stronger inhibition because their fat-soluble compounds bind more effectively to enzymes than water-soluble compounds [13].

In the seed extract, the identification of oleoyl chloride (10.60%) and pentadecanoic acid (10.64%) highlights the anticancer potential of *A. subulatum*, aligning with findings30. Additionally, the presence of compounds such as α-terpineol and 4H-pyran-4-one suggests promising anticancer and antioxidant activities, respectively, which further emphasizes the pharmacological relevance of these extracts [31,32]. Although this study recognised promising bioactive compounds in *A. subulatum*, limitations include the exclusive use of *in vitro* assays requiring future in vivo validation, potential variability in extraction yields due to seasonal/geographical factors, and unexamined synergistic effects among compounds.

CONCLUSION

The phytochemical analysis of *Amomum subulatum* extracts, including both seed and seed shell, reveals a significant presence of bioactive compounds, including phenolics, flavonoids, saponins, terpenoids, and alkaloids, which underscores the plant's therapeutic potential. The seeds exhibited a notably higher concentration of TPC and TFC compared to the seed shells, supporting their recognized role as a valuable source of antioxidants and bioactive agents. The extracts demonstrated effective amylase and protease inhibition, particularly with hexane and chloroform extracts, indicating their potential applications in managing carbohydrate metabolism and conditions associated with excessive protease activity. The results from the GC-MS analysis further confirmed the presence of diverse bioactive compounds, with the seed shell revealing 80 distinct compounds and

the seeds containing 67. Significant compounds included desogestrel, oleoyl chloride, and pentadecanoic acid, all recognized for their pharmacological properties. Additional research is needed to investigate the specific mechanisms of action and potential health benefits associated with these bioactive compounds.

Conflict of interest

The authors declared no conflict of interest.

Financial Support

None declared.

ORCID ID

Lalit D. Tattu: https://orcid.org/0009-0001-6891-2837

Faiyaz K. Shaikh: https://orcid.org/0000-0002-5735-4761

Raghavendra L.S. Hallur: https://orcid.org/0000-0003-4300-109X

REFERENCES

1. Messaoudi A, Dekmouche M, Rahmani Z, Bensaci C. Comparative analysis of total phenolics, flavonoid content and antioxidant profile of date palm (*Phoenix dactylifera* L.) with different watering water from Oued Souf in Algeria. Asian J Res Chem. 2020;13(1):28-32.
2. Singh A, Kumar R. An overview on ethnopharmacological, phytochemical, and clinical significance of selected dietary polyphenols. Asian J Res Chem. 2023;16(1):8-2.
3. Li AN, Li S, Zhang YJ, Xu XR, Chen YM, Li HB. Resources and biological activities of natural polyphenols. Nutrients. 2014;6(12):6020-6047.
4. Zhou Y, Zheng J, Li Y, et al. Natural polyphenols for prevention and treatment of cancer. Nutrients. 2016;8(8):515.
5. Berra D, Dia O, Laouini SE, Menaceur S. The impact of pH value in the phenolic content and antioxidant potential of medicinal plant extract. Asian J Res Chem. 2022;15(5):331-336.
6. Choudhary S, Madhusudan S, Walia B, Chaudhary G. Black cardamom (*Amomum subulatum*): a review. Int J Curr Res. 2021;13(04):17027-17032.
7. Chandrashekharaiah KS. Studies on variation and stability of storage proteins and chymotrypsin inhibitors of an underutilized legume, Mucuna during germination. Asian J Res Chem. 2012;5(11):1388-1391.
8. Pai V, Savadi RV, Bhandarkar A. In-vitro alpha-amylase inhibition action of isolated phytoconstituent in *Zanthoxylum rhetsa* (Roxb) bark. Res J Pharm Tech. 2011;4(7):1147-1150.
9. Amin MA, Hashem AM, Ashour AE, Hammad S. Protease inhibitors: recent advances in anticancer drug discovery. J Enzyme Inhib Med Chem. 2012;27(6):823-841.
10. Sathish M, Nandhini V, Suresh R. In vitro alpha amylase and alpha glucosidase enzyme inhibition of leaf extracts of *Jatropha glandulifera* Roxb. Res J Pharm Tech. 2022;15(6):2493-2497.
11. Akinfemiwa O, Zubair M, Muniraj T. Amylase. In: StatPearls. StatPearls Publishing; 2024. Accessed [May 2025]. https://www.ncbi.nlm.nih.gov/books/NBK557738/
12. Djohan, Sihombing B, Ferdinand S. Exploring nanoherbal tomato: assessing its potential as an α-glucosidase and α-amylase inhibitor, antioxidant and metabolite profiling using LC-HRMS. Res J Pharm Tech. 2024;17(10):4953-4960.
13. Alzeina O, soubh L. Evaluation of polyphenols' inhibitory activity of α-amylase in cinnamon. Res J Sci Tech. 2019;11(3):174-178.
14. Laware SL. Sequential extraction of plant metabolites. Int J Curr Microbiol Appl Sci. 2015;4(2):33-38.
15. Joseph R, Azeez S, Bhageerathy C. Anticancer effect of sequential extracts from *Curcuma caesia* rhizomes on human cancer cell lines and characterization of selected polyphenols in active extracts by LC-MS/MS. Asian J Pharm Res. 2023;13(4):219-226.
16. Yadav RNS, Agarwala M. Phytochemical analysis of some medicinal plants. J Phytol. 2011;3(12):10-14.
17. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods Enzymol. 1999;299:152-178.
18. Patidar P, Dashora K. Development of UV spectroscopy fingerprinting method of total polyphenols as tannic acid in Manibhadra yoga. Asian J Pharm Anal. 2014;4(1):28-30.
19. Bao J, Cai Y, Sun M, Wang G, Corke H. Anthocyanins, flavonols, and free radical scavenging activity of Chinese bayberry (*Myrica rubra*) extracts and their color properties and stability. J Agric Food Chem. 2005;53(6):2327-2332.
20. Bhandari L, Rajbhandari M. Isolation of quercetin from flower petals, estimation of total phenolic, total flavonoid and antioxidant activity of the different parts of *Rhododendron arboreum* Smith. Sci World. 2015;12(12):34-40.
21. Moein S, Pimoradloo E, Moein M, Vessal M. Evaluation of antioxidant potentials and α-amylase inhibition of different fractions of Labiatae plants extracts: as a model of antidiabetic compounds properties. Biomed Res Int. 2017;2017:7319507.
22. Thete RS, Roushani S, Shaikh FK, Kulkarni J, Raghavendra HL. Isolation and characterization of protease inhibitors with antimicrobial activity from sirisa (*Albizia lebbeck*) seed protein extract. Biosci Biotechnol Res Asia. 2023;20(1):85-95.
23. Shaikh FK, Bradosty SW, Hamad SW, Shinde AA. *In vitro* screening of seed extracts of medicinal plants for protease inhibitory activity. Cihan Univ-Erbil Sci J. 2019;3(1):61-65.
24. Bhat AS, Ghosh S. Phytochemical analysis and antioxidant activity of *Amomum subulatum* Roxb. Int J Pharm Sci Res. 2019;10(12):5234-5240.
25. Oleszek W. Saponins as bioactive compounds in human health. Food Res Int. 2017;100(Pt 1):182-192.
26. Timilsena YP, Phosanam A, Stockmann R. Perspectives on saponins: food functionality and applications. Int J Mol Sci. 2023;24(17):13538. doi:10.3390/ijms241713538.
27. Mansoor T, Ahmad M. Terpenoids: pharmacological effects and therapeutic applications. Front Pharmacol. 2018;9:1183.
28. Ciumărnean L, Milaciu MV, Runcan O. The effects of flavonoids in cardiovascular diseases. Molecules. 2020;25(18):4320.
29. Youssef AMM, Maaty DAM, Al-Saraireh YM. Phytochemical analysis and profiling of antioxidants and anticancer compounds from *Tephrosia purpurea* (L.) subsp. apollinea family Fabaceae. Molecules. 2023;28(9):3939.
30. To NB, Nguyen YT, Moon JY, Ediriweera MK, Cho SK. Pentadecanoic acid, an odd-chain fatty acid, suppresses the stemness of MCF-7/SC human breast cancer stem-like cells through JAK2/STAT3 signaling. Nutrients. 2020;12(6):1663.
31. Hassan SB, Gali-Muhtasib H, Göransson H, Larsson R. Alpha terpineol: a potential anticancer agent which acts through suppressing NF-κB signalling. Anticancer Res. 2010;30(6):1911-1919.
32. Čechovská L, Cejpek K, Konečný M, Velíšek J. On the role of 2,3-dihydro-3,5-dihydroxy-6-methyl-(4H)-pyran-4-one in antioxidant capacity of prunes. Eur Food Res Technol. 2011;233(3):367-376.

33. Photolo MM, Mavumengwana V, Sitole L, Tlou MG. Antimicrobial and antioxidant properties of a bacterial endophyte, Methylobacterium radiotolerans MAMP 4754, isolated from *Combretum erythrophyllum* seeds. Int J Microbiol. 2020;2020:9483670.
34. Masyita A, Mustika Sari R, Dwi Astuti A. Terpenes and terpenoids as main bioactive compounds of essential oils, their roles in human health and potential application as natural food preservatives. Food Chem X. 2022;13:100217.
35. Khodarahmi G, Asadi P, Hassanzadeh F, Khodarahmi E. Benzofuran as a promising scaffold for the synthesis of antimicrobial and antibreast cancer agents: a review. J Res Med Sci. 2015;20(11):1094-1104.
36. Kim KW, Park JB, Kim H. Antioxidant and antimicrobial activities of phenol 2,6-dimethoxy. Phytother Res. 2003;17(8):1000-1003.
37. Pramod K, Ansari SH, Ali J. Eugenol: a natural compound with versatile pharmacological actions. Nat Prod Commun. 2010;5(12):1999-2006.
38. Furtado NA, de Sousa JP, Neto RM, Alves TM. Biological activities of gamma-muurolene. Phytomedicine. 2013;20(10):930-934.
39. Corson SL. Contraceptive efficacy of a monophasic oral contraceptive containing desogestrel. Am J Obstet Gynecol. 1993;168(3 Pt 2):1017-1020.
40. Muhammad SA, Muhammad S, Waqar A, Masood P, Raghav Y. A chlorinated monoterpene ketone, acylated β-sitosterol glycoside from *Mentha longifolia* (Lamiaceae). Phytochemistry. 2002;59(8):889-895.
41. Aparna V, Dileep KV, Mandal PK, Karthe P, Sadasivan C, Haridas M. Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. Chem Biol Drug Des. 2012;80(3):434-439.
42. Sajovic J, Meglič A, Glavač D, Markelj Š, Hawlina M, Fakin A. The role of vitamin A in retinal diseases. Int J Mol Sci. 2022;23(3):1014.
43. Barel S, Yagen B, Bialer M. Pharmacokinetics of the trichothecene mycotoxin verrucarol in dogs. J Pharm Sci. 1990;79(6):548-551.
44. Rajasekar G, Ebenezar E, Thiruvudainambi S, Vanniarajan C, Vellaikumar MSS. Exploitation and identification of antifungal compounds of botanicals through gas chromatography-mass spectrometry (GC-MS) against Bipolaris oryzae in rice. J Entomol Zool Stud. 2020;8(3):1509-1515.
45. Dembitsky VM, Poovarodom S, Leontowicz H. The multiple nutrition properties of some exotic fruits: biological activity and active metabolites. Food Res Int. 2011;44(7):1671-1701.
46. Čechovská L, Cejpek K, Konečný M, Velíšek J. On the role of 2,3-dihydro-3,5-dihydroxy-6-methyl-(4H)-pyran-4-one in antioxidant capacity of prunes. Eur Food Res Technol. 2011;233(3):367-376.
47. Ololade ZS, Olatunde OZ, Oyelese OJ, Olaoye OO, Odewande RA. Total phenolic content, free radical inhibition, antioxidant and antibacterial potentials of the medicinal organic compounds in the fruit of *Terminalia catappa* Linn. Nat Sci. 2014;12(2):46-50.
48. Iwu MM, Ezeugwu CO, Okunji CO, Sanson DR, Tempesta MS. Antimicrobial activity and terpenoids of the essential oil of *Hyptis suaveolens*. Int J Crude Drug Res. 1990;28(1):73-76.
49. Harada H, Yamashita U, Kurihara H, Fukushi E, Kawabata J, Kamei Y. Antitumor activity of palmitic acid found as a selective cytotoxic substance in a marine red alga. Anticancer Res. 2002;22(5):2587-2590.
50. Evran S, Yasa I, Telefoncu A. Modification of lysozyme with oleoyl chloride for broadening the antimicrobial specificity. Prep Biochem Biotechnol. 2010;40(4):316-325.

HOW TO CITE THIS ARTICLE

Tattu LD, Shaikh FK, Hallur RLS. Sequential extraction, phytochemical analysis, and enzyme inhibitory activity of polyphenols from black cardamom (*Amomum subulatum* Roxb.) shell and seeds. J Phytopharmacol 2025; 14(5):362-369. doi: 10.31254/phyto.2025.14510