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Exploration of Bioactive Compounds And Medicinal Properties of Chenopodium Quinoa Willd Seeds

Dr. R. Subitha Shajini[1*], and S.R.Ashika[2]

*[*1] Assistant Professor, Department of Botany,*
Women's Christian College,
Nagercoil - 629001, Tamil Nadu, India.

[2]Post Graduate, Department of Botany,
Women's Christian College, Nagercoil - 629001,
Tamil Nadu, India.
*[*1&2](Affiliated to Manonmaniam Sundaranar University,*
Abishekapatti, Tirunelveli-627012,
Tamil Nadu, India).
Corresponding Author: SUBITHA SHAJINI R
E-mail: sunav03122008@gmail.com
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KEYWORDS

Chenopodium quinoa, phytochemical screening, antioxidant activity, anti-inflammatory activity, GC–MS analysis, nutraceutical potential.

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Abstract

Chenopodium quinoa (quinoa) is a nutrient-rich pseudocereal recognized for its remarkable nutritional value and diverse spectrum of bioactive compounds. The present study aimed to evaluate the phytochemical constituents, antioxidant activity, and anti-inflammatory potential of quinoa seed extracts. Preliminary phytochemical screening was carried out to identify major secondary metabolites, including alkaloids, glycosides, carbohydrates, saponins, betacyanins, proteins, terpenoids, coumarins, oils, and quinones. The antioxidant activity of the extracts was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, whereas the anti-inflammatory activity was evaluated through the inhibition of albumin denaturation method. Furthermore, Gas Chromatography–Mass Spectrometry (GC–MS) analysis was performed to identify the bioactive compounds present in the seed extracts. The findings revealed the presence of several phytochemically active constituents with significant antioxidant and anti-inflammatory properties, suggesting the therapeutic and nutraceutical potential of quinoa seeds. This study provides scientific evidence supporting the utilization of quinoa as a promising natural source of bioactive compounds for pharmaceutical and functional food applications.

Introduction

Medicinal plants have been widely used since ancient times for the prevention and treatment of various diseases due to the presence of bioactive phytochemicals. Natural products derived from plants continue to play a vital role in modern medicine and pharmaceutical research because they are considered safer and more economical than many synthetic drugs (Firenzuoli & Gori, 2007). Plants synthesize a variety of secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids, glycosides, and saponins, which possess significant biological activities including antioxidant, antimicrobial, anti-inflammatory, and anticancer properties (Abdallah, 2011). These phytochemicals contribute greatly to human health and are increasingly being investigated for nutraceutical and therapeutic applications.

Chenopodium quinoa, commonly known as quinoa, is a highly nutritious pseudocereal belonging to the family Amaranthaceae. It originated in the Andean region of South America and has been cultivated for thousands of years as an important food crop (Schmidt et al., 2023). Quinoa has gained worldwide attention because of its exceptional nutritional composition and health-promoting properties. Unlike conventional cereals, quinoa contains higher amounts of proteins, dietary fiber, vitamins, minerals, and essential amino acids (Nisar et al., 2017). It is considered a complete protein source because it contains all essential amino acids required for human nutrition (Chaudhary et al., 2023). In addition, quinoa is naturally gluten-free and therefore suitable for individuals suffering from celiac disease and gluten intolerance. Apart from its nutritional value, quinoa seeds are rich in bioactive compounds such as polyphenols, flavonoids, phenolic acids, saponins, and betalains, which contribute to its medicinal importance (Valencia et al., 2010). These compounds exhibit various pharmacological activities including antioxidant, anti-inflammatory, antimicrobial, and anticancer effects (García & Castillo, 2008). The antioxidant potential of quinoa is particularly important because oxidative stress caused by reactive oxygen species (ROS) is associated with the development of several chronic diseases such as cancer, diabetes, cardiovascular disorders, and neurodegenerative diseases (Agarwal et al., 2012). Natural antioxidants present in plants can neutralize free radicals and protect cells from oxidative damage,

thereby reducing disease risk. Inflammation is another important biological response linked to numerous chronic diseases. Although inflammation is a protective mechanism against infection and tissue injury, prolonged inflammation may lead to disorders such as arthritis, asthma, diabetes, and cancer (Ansari et al., 2017). Conventional anti-inflammatory drugs are effective but may produce adverse side effects when used for long periods. Therefore, the search for natural anti-inflammatory agents from medicinal plants has increased considerably in recent years. Previous studies suggest that quinoa-derived phytochemicals may possess significant anti-inflammatory properties by inhibiting inflammatory mediators and oxidative stress.

Gas Chromatography–Mass Spectrometry (GC–MS) is a widely used analytical technique for identifying volatile and bioactive compounds present in plant extracts. GC–MS analysis provides detailed information regarding the chemical constituents responsible for biological activities and therapeutic properties (Stashenko & Martínez, 2012). Therefore, the present study aims to investigate the phytochemical constituents, antioxidant activity, anti-inflammatory potential, and GC–MS profile of quinoa seed extracts. The findings of this study may provide scientific evidence supporting the medicinal and nutraceutical importance of quinoa seed

Materials and Methods

Plant Material

Chenopodium quinoa is an annual herbaceous pseudocereal cultivated mainly for its edible seeds (Pozo et al., 2023). The plant generally attains a height of 1–3 m and possesses an erect stem that may be branched or unbranched. The leaves are alternate and variable in shape, ranging from triangular to lanceolate forms with serrated margins. The inflorescence consists of dense panicles containing small bisexual or pistillate flowers, predominantly adapted for self-pollination (Jacobsen, 2003). Quinoa seeds are small, spherical grains measuring approximately 2 mm in diameter and occur in different colors including white, red, yellow, purple, brown, and black (Dehghanian et al., 2024). The seeds possess a natural saponin-rich coating responsible for their characteristic bitterness (Aina, 2023).

Collection and Preparation of Plant Material

Quinoa seeds were procured from a local market. The seeds were thoroughly washed with distilled water to remove impurities

and then shade-dried at room temperature. The dried seeds were pulverized using an electric blender to obtain a fine powder. The powdered material was stored in airtight polyethylene containers in a cool, dry, and dark environment until further use.

Preparation of Extracts

The dried quinoa seed powder was subjected to solvent extraction. Briefly, the powdered material was transferred into a conical flask containing the appropriate solvent and agitated thoroughly. The mixture was filtered using Whatman No. 1 filter paper, and the filtrates obtained were collected separately and stored for subsequent phytochemical screening, antioxidant, anti-inflammatory, and GC–MS analyses.

Preliminary Phytochemical Screening

Qualitative phytochemical analyses were performed to identify the presence of various secondary metabolites in the quinoa seed extracts using standard protocols.

Test for Alkaloids

Mayer's test was performed by adding concentrated hydrochloric acid followed by Mayer's reagent to the extract. Formation of a green coloration indicated the presence of alkaloids (Ganesh et al., 2014).

Test for Glycosides

Salkowski's test was carried out by mixing the extract with chloroform and concentrated sulfuric acid. Formation of a reddish-brown color indicated the presence of glycosides (Panchal & Parvez, 2019).

Test for Tannins

Ferric chloride solution was added to the extract. Development of a black coloration confirmed the presence of tannins (Borah & Biswas, 2018).

Test for Carbohydrates

Iodine test was performed by adding iodine solution to the extract. Appearance of dark blue or purple coloration indicated the presence of carbohydrates (Borah & Biswas, 2018).

Test for Saponins

The extract was vigorously shaken with distilled water. Formation of stable foam indicated the presence of saponins (Borah & Biswas, 2018).

Test for Anthocyanins and Betacyanins

The extract was boiled with 1 N sodium hydroxide solution. Bluish-green coloration indicated anthocyanins, whereas yellow coloration confirmed betacyanins (Trease & Evans, 1989).

Test for Steroids

The extract was mixed with chloroform and concentrated sulfuric acid. Formation of a red coloration in the lower layer

indicated the presence of steroids (Borah & Biswas, 2018).

Test for Proteins

Biuret test was conducted by adding copper sulfate and sodium hydroxide to the extract. Appearance of violet coloration confirmed the presence of proteins (Plummer, 1987).

Test for Terpenoids

The extract was mixed with chloroform, evaporated to dryness, and treated with concentrated sulfuric acid. A greyish coloration at the interface indicated terpenoids (Borah & Biswas, 2018).

Test for Sterols

Libermann–Burchard's test was carried out using acetic anhydride and concentrated sulfuric acid. Formation of a brown ring followed by green coloration indicated sterols (Harborne, 1998; Trease & Evans, 1989).

Test for Coumarins

The extract was treated with sodium hydroxide solution. Appearance of yellow coloration confirmed the presence of coumarins (Harborne, 1998).

Test for Anthraquinones

The extract was treated with chloroform and ammonia solution. Development of pink, red, or violet coloration indicated anthraquinones (Borah & Biswas, 2018).

Test for Flavonoids

Alkaline reagent test was performed using sodium hydroxide solution. Formation of intense yellow coloration that disappeared upon acidification indicated flavonoids (Sofowora, 1993).

Test for Oils and Fats

The extract was pressed between filter papers. Appearance of oily stains indicated the presence of oils and fats (Borah & Biswas, 2018).

Test for Quinones

Concentrated sulfuric acid was added to the extract. Formation of red coloration indicated quinones (Babatunde et al., 2019).

Test for Phenols and Phenolic Compounds

Ferric chloride test was employed to detect phenolic compounds. Appearance of blue-green or dark green coloration indicated the presence of phenols and phenolic compounds (Ganesh et al., 2014).

Test for Cardiac Glycosides

The extract was treated with glacial acetic acid, ferric chloride, and concentrated sulfuric acid. Formation of a brown ring at the interface indicated cardiac glycosides (Babatunde et al., 2019).

Antioxidant Assay

DPPH Radical Scavenging Assay

The antioxidant activity of quinoa seed extracts was evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay following the method described by Blois (1958). DPPH solution exhibits a deep violet color in methanol, which changes to pale yellow in the presence of antioxidants. Different concentrations of the extract (6.25, 12.5, 25, 50, and 100 µg/mL) were prepared. To each test tube, 2 mL of DPPH solution and 100 µL of sample extract or standard solution were added. The reaction mixtures were incubated in the dark for 30 min at room temperature. Absorbance was measured at 520 nm using a UV–Visible spectrophotometer.

The percentage inhibition of DPPH radicals was calculated using the following formula:

$$\text{Percentage inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Anti-inflammatory Assay

Inhibition of Albumin Denaturation Assay

The anti-inflammatory activity was assessed using the inhibition of albumin denaturation method according to Mizushima and Kobayashi (1968) with slight modifications. The reaction mixture consisted of equal volumes of quinoa seed extract and 1% aqueous bovine albumin solution. Diclofenac sodium served as the standard drug at concentrations of 6.25, 12.5, 25, 50, and 100 µg/mL.

The pH of the mixture was adjusted using 1 N HCl. The samples were incubated at 37°C for 20 min and subsequently heated at 70°C for 10 min. After cooling to room temperature, absorbance was recorded at 660 nm using a UV–Visible spectrophotometer.

The percentage inhibition of protein denaturation was calculated using the following equation:

$$\text{Percentage inhibition} = \frac{\text{OD of control} - \text{OD of sample}}{\text{OD of control}} \times 100$$

GC–MS Analysis

The phytochemical profile of the quinoa seed extract was analyzed using a GCMS-TQ8040 NX Triple Quadrupole Mass Spectrometer (Shimadzu, Kyoto, Japan) equipped with an InertCap 5MS capillary column (30 m × 0.25 mm, 0.25 µm) (Liu et al., 2024). Helium was used as the carrier gas at a flow rate of 1 mL/min. A sample volume of 2 µL was injected at 280°C. The oven temperature was programmed from 70°C to 300°C at a gradual heating rate. Mass spectrometric detection was carried out using electron ionization at 70 eV with a scan range of m/z 50–500. The ion source temperature

and transfer line temperature were maintained at 230°C and 250°C, respectively (Higuchi et al., 2024). Identification of compounds was achieved by comparing the obtained mass spectra with those available in the National Institute of Standards and Technology (NIST) Mass Spectral Database (Konappa et al., 2020).

Results and Discussion

Chenopodium quinoa is an ancient pseudocereal recognized worldwide for its exceptional nutritional and medicinal value. The seeds are rich in proteins, essential amino acids, carbohydrates, dietary fiber, vitamins, minerals, and bioactive compounds, making quinoa an important functional food crop (Alvarez-Jubete et al., 2009; Vilcacundo & Hernández-Ledesma, 2017). Due to its high nutritional quality and stress tolerance, quinoa has gained global attention as a potential contributor to food security and human health.

Phytochemical Analysis

Qualitative phytochemical screening of aqueous, ethanol and ethyl acetate extracts of quinoa seeds revealed the presence of several important secondary metabolites (Table 1). The identified phytochemicals included alkaloids, glycosides, carbohydrates, saponins, betacyanins, proteins, terpenoids, coumarins, oils, and quinones. However, tannins, flavonoids, steroids, sterols, anthraquinones, phenols, anthocyanins, phenolic compounds, and cardiac glycosides were absent in all extracts. The aqueous extract showed positive results for carbohydrates, saponins, betacyanins, proteins, terpenoids, and coumarins, whereas glycosides were present in both aqueous and ethanol extracts. Alkaloids were detected only in the ethyl acetate extract, while quinones were observed exclusively in the ethanol extract. Oils and fats were strongly present in all extracts. The presence of these phytochemicals indicates the medicinal and nutraceutical importance of quinoa seeds. Saponins are known for their cholesterol-lowering and anticancer properties, while terpenoids and coumarins exhibit antioxidant, antimicrobial, and anti-inflammatory activities (Thakur et al., 2011). The variation in phytochemical composition among different extracts may be attributed to solvent polarity and extraction efficiency. The findings of the present study suggest that quinoa seeds possess significant bioactive potential and may serve as a valuable natural source for the development of functional foods and pharmaceutical products.

Table 1. Phytochemical constituents in the seeds of *Chenopodium quinoa* Willd.

Phytochemical Constituents	Name of the Tests	Aqueous Extract	Ethanol Extract	Ethyl Acetate Extract
Alkaloids	Meyer's test	-	-	+
Glycosides	Salkowski's test	+	+	-
Tannin	Tannin test	-	-	-
Carbohydrates	Iodine test	+	-	-
Saponin	Saponification test	+	-	-
Betacyanin	Betacyanin test	+	-	-
Anthocyanin	Anthocyanin test	-	-	-
Steroid	Steroid test	-	-	-
Protein	Biuret test	+	-	-
Terpenoid	Terpenoid test	+	-	-
Sterols	Libermann–Burchard test	-	-	-
Coumarins	Coumarin test	+	-	-
Anthroquinone	Anthroquinone test	-	-	-
Flavonoids	Alkaline reagent test	-	-	-
Oils	Stain test	+	+	+
Quinone	Quinone test	-	+	-
Phenol	Phenol test	-	-	-
Phenolic compounds	Phenolic compounds test	-	-	-
Cardiac glycosides	Cardiac glycosides test	-	-	-

Antioxidant Assay

The antioxidant activity of *Chenopodium quinoa* seed extracts was evaluated using the DPPH free radical scavenging assay. The assay measures the ability of plant extracts to donate hydrogen or electrons to neutralize free radicals. The results demonstrated a concentration-dependent increase in antioxidant activity in all extracts, with the highest inhibition observed at 100 µg/ml (Table 2). Among the tested extracts, the ethanol extract exhibited the highest antioxidant activity with 12.14% inhibition, followed by the ethyl acetate extract (11.62%) and aqueous extract (6.52%). The IC_{50} values further confirmed the antioxidant potential of the extracts, where lower IC_{50} values indicate stronger free radical scavenging activity.

The ethanol extract showed the lowest IC_{50} value (459.2 µg/ml), indicating greater antioxidant activity compared to ethyl acetate (484.1 µg/ml) and aqueous extracts (896.4 µg/ml).

Table 2. DPPH Radical Scavenging Activity of *Chenopodium quinoa* Seed Extracts

Sample	Concentration	% Inhibition	IC_{50} Value (µg/ml)
Aqueous extract	100 µg/ml	6.52	896.4
Ethanol extract	100 µg/ml	12.14	459.2
Ethyl acetate extract	100 µg/ml	11.62	484.1

The higher antioxidant activity observed in the ethanol extract may be attributed to the presence of bioactive compounds such as phenolics, terpenoids, coumarins, and other antioxidant constituents. Previous studies have reported that quinoa contains important antioxidant molecules including polyphenols, flavonoids, carotenoids, tocopherols, and betalains, which effectively scavenge reactive oxygen species (ROS) (Tang & Tsao, 2017; Pellegrini et al., 2018). Free radicals and ROS are highly reactive molecules capable of damaging cellular biomolecules such as proteins, lipids, and DNA, leading to oxidative stress and several chronic diseases including cancer, cardiovascular disorders, diabetes, and neurodegenerative diseases (Young & Woodside, 2001). Antioxidants protect cells by neutralizing these reactive molecules and reducing oxidative damage. The findings of the present study suggest that quinoa seed extracts possess notable antioxidant potential and may serve as a natural source of antioxidant compounds for nutraceutical and pharmaceutical applications (Table 2 and Fig.5, 6, 7).

Figure. DPPH scavenging activity of *Chenopodium quinoa* Willd. Aqueous extract represented by percentage of inhibition.

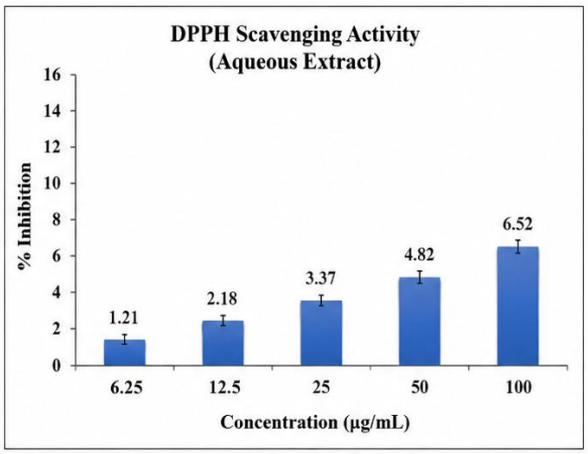


Fig.5. DPPH scavenging activity of *Chenopodium quinoa* Willd. Aqueous extract represented by percentage of inhibition.

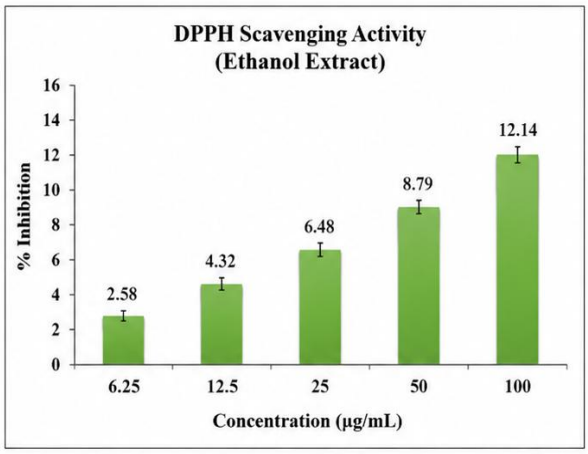


Fig.6. DPPH scavenging activity of *Chenopodium quinoa* Willd. Ethanol extract represented by percentage of inhibition.

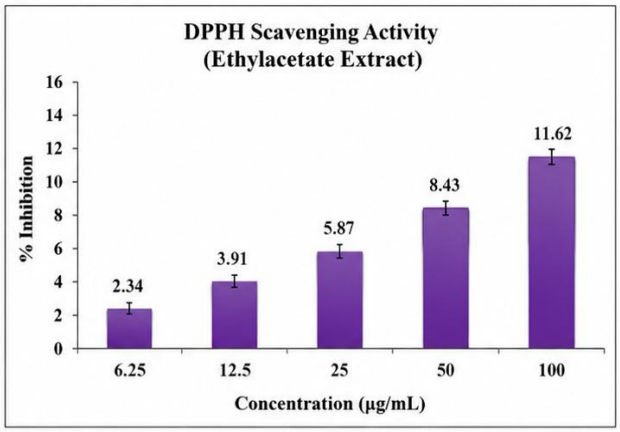


Fig.7. DPPH scavenging activity of *Chenopodium quinoa* Willd. Ethylacetate extract represented by percentage of inhibition.

Anti-inflammatory Assay

The anti-inflammatory activity of *Chenopodium quinoa* seed extracts was evaluated using the inhibition of albumin denaturation assay. Protein denaturation is one of the major causes of inflammation, and substances capable of preventing protein denaturation are considered potential anti-inflammatory agents. The present study demonstrated a concentration-dependent inhibition of albumin denaturation by aqueous, ethanol, and ethyl acetate extracts (Table 3). Among the tested extracts, the ethanol extract exhibited the highest anti-inflammatory activity with 59.25% inhibition at 100 µg/ml, followed by the ethyl acetate extract (49.25%) and aqueous extract (24.07%). The lowest inhibition was observed at 6.25 µg/ml, indicating that anti-inflammatory activity increased with increasing concentration.

Table 3. Inhibition of Albumin Denaturation by *Chenopodium quinoa* Seed Extracts

Sample	Concentration	Absorbance of Control	Absorbance at 660 nm	% Inhibition
Aqueous extract	100 µg/ml	0.071	0.0385	24.07
Ethanol extract	100 µg/ml	0.095	0.015	59.25
Ethyl acetate extract	100 µg/ml	0.095	0.028	49.25

Inflammation is a protective response of the body against tissue injury, infection, and harmful stimuli. However, prolonged inflammation may contribute to several chronic disorders including arthritis, cardiovascular diseases, diabetes, and cancer. During inflammation, reactive oxygen species (ROS) and inflammatory mediators such as histamine, prostaglandins, and cytokines are released, causing oxidative stress and tissue damage (Coussens & Werb, 2002).

The higher anti-inflammatory activity observed in the ethanol extract may be attributed to the presence of bioactive compounds such as terpenoids, coumarins, glycosides, and other phytochemicals detected during phytochemical screening. Previous studies have reported that plant-derived phenolics, flavonoids, and terpenoids possess significant anti-inflammatory properties by stabilizing proteins and inhibiting inflammatory mediators (Govindappa et al., 2011). The results of the present investigation suggest that quinoa seed extracts possess considerable anti-inflammatory potential and may serve as a natural source for the development of therapeutic and nutraceutical products with fewer side effects compared to synthetic anti-inflammatory drugs (Table 3 and Fig.8, 9, 10).

Figure. Inhibition of albumin denaturation activity of *Chenopodium quinoa* Willd. Aqueous extract represented by percentage of inhibition.

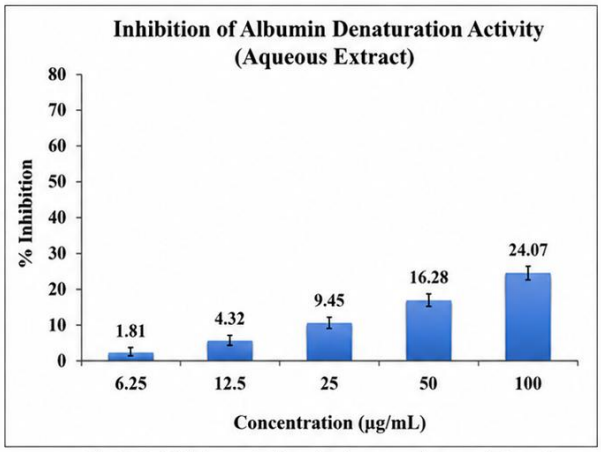


Fig.8. Inhibition of albumin denaturation activity of *Chenopodium quinoa* Willd. Aqueous extract represented by percentage of inhibition.

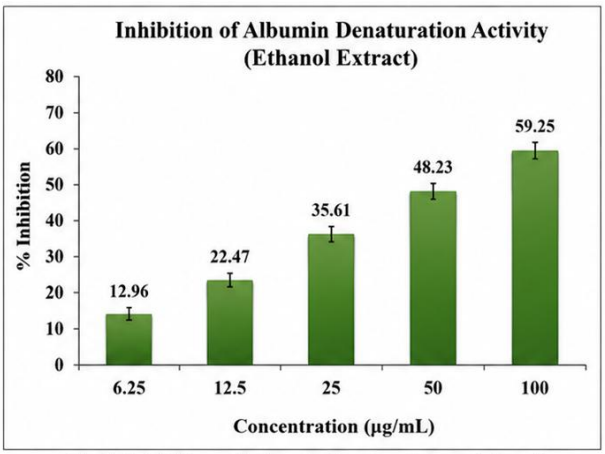


Fig.9. Inhibition of albumin denaturation activity of *Chenopodium quinoa* Willd. Ethanol extract represented by percentage of inhibition.

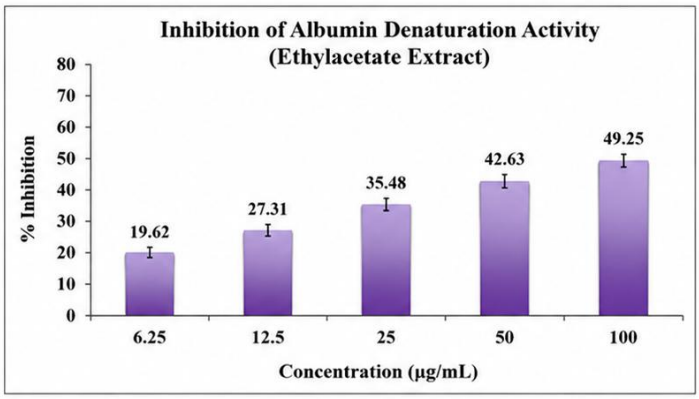


Fig.10. Inhibition of albumin denaturation activity of *Chenopodium quinoa* Willd. Ethylacetate extract represented by percentage of inhibition.

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

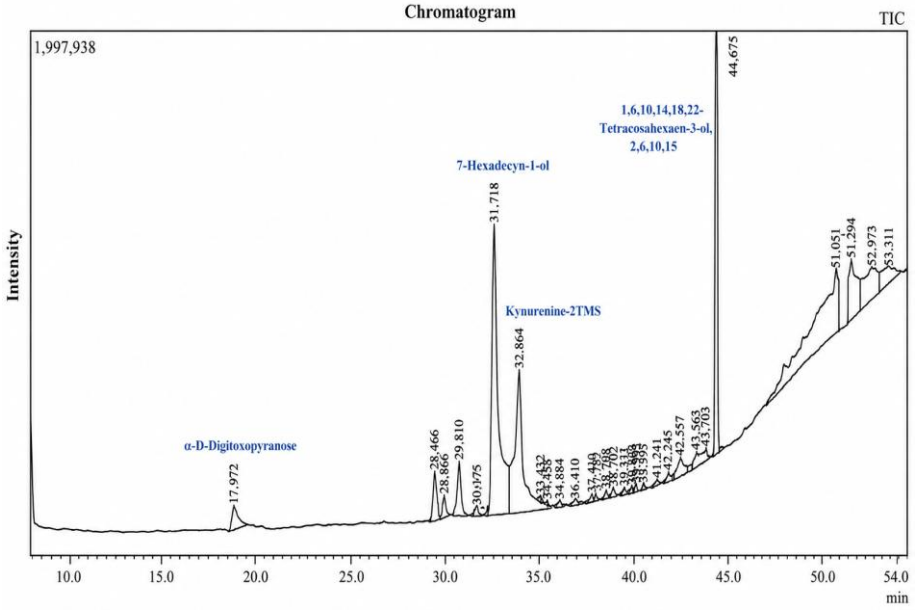
The phytochemical constituents present in the ethanol and ethyl acetate extracts of *Chenopodium quinoa* seeds were analyzed using Gas Chromatography–Mass Spectrometry (GC–MS). The analysis revealed the presence of several bioactive compounds with potential pharmacological significance. In the ethanol extract, the major compounds identified were α-D-Digitoxopyranose (RT: 17.972), 7-Hexadecyn-1-ol (RT: 31.718), Kynurenine-2TMS (RT: 32.684), and 1, 6, 10, 14, 18, 22-Tetracosahexaen-3-ol, 2, 6, 10, 15 (RT: 44.675). Among these, 7-Hexadecyn-1-ol showed the highest peak area (18.87%), indicating its predominance in the extract. Similarly, the ethyl acetate extract revealed the presence of 7-Hexadecyn-1-ol (RT: 31.724), Linoleic acid-TMS (RT: 32.859), Palmitoleic acid-TMS (RT: 39.268), and 5, 9, 13, 17-Tetramethyl-4, 8, 12,16-octodecatetrae (RT: 44.672). The compound 5, 9, 13, 17-

Tetramethyl-4, 8, 12, 16-octodecatetrae exhibited the highest peak area (14.22%).

Table 4. Major Bioactive Compounds Identified by GC–MS.

Compound	Molecular Formula	Retention Time	Peak Area (%)	Biological Importance
α-D-Digitoxopyranose	C6H12O4	17.972	1.63	Energy source and reducing agent
7-Hexadecyn-1-ol	C16H30O	31.718	18.87	Antimicrobial and antifungal activity
Kynurenine-2TMS	C16H28N2O3Si2	32.684	15.62	Immune regulation and neuroprotective role
Linoleic acid-TMS	C21H40O2	32.859	13.13	Anti-inflammatory and antioxidant activity
Palmitoleic acid-TMS	C19H38O2	39.268	0.24	Improves insulin sensitivity
1,6,10,14,18,22-Tetracosahexaen-3-ol	C30H50O	44.675	11.77	Antioxidant and antimicrobial activity
5,9,13,17-Tetramethyl-4,8,12,16-octodecatetrae	C22H36O2	44.672	14.22	Bioactive terpenoid compound

Fig.11. GC-MS chromatogram of *Chenopodium quinoa* Willd. Seeds in Ethanol extract



The presence of 7-Hexadecyn-1-ol in both ethanol and ethyl acetate extracts may be attributed to its amphiphilic nature, containing both hydrophobic and hydrophilic functional groups. This compound is known for its antimicrobial and antifungal activities and may contribute to the medicinal potential of quinoa seeds. Linoleic acid and palmitoleic acid identified in the extracts are biologically important fatty acids reported to possess antioxidant, anti-inflammatory, and cardioprotective properties. Kynurenine derivatives are involved in tryptophan metabolism and are associated with immune regulation and neuroprotection. The GC–MS findings confirm that quinoa seeds contain diverse bioactive compounds with significant therapeutic potential. These compounds may contribute to the antioxidant and anti-inflammatory activities observed in the present study and support the utilization of quinoa as a functional food and nutraceutical resource (Table 4 and Fig. 11).

Conclusion

Chenopodium quinoa Willd. is a nutritionally rich pseudocereal with significant medicinal potential. The present study revealed the presence of important phytochemicals such as alkaloids, glycosides, saponins, terpenoids, proteins, coumarins, quinones, and oils in different solvent extracts of quinoa seeds. The antioxidant and anti-inflammatory assays showed concentration-dependent activity, with the ethanol extract exhibiting the highest activity compared to aqueous and ethyl acetate extracts. GC–MS analysis confirmed the presence of several bioactive compounds associated with antioxidant, anti-inflammatory, and antimicrobial properties. Overall, the findings suggest that quinoa seeds are a valuable source of natural bioactive compounds and may be useful in the development of functional foods, nutraceuticals, and therapeutic products. Further studies are required to isolate active compounds and evaluate their clinical applications.

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