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Chemoprofiling of *Cucumis pubescens* Willd. fruits

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ABSTRACT:

Cucumis pubescens, a notable therapeutic plant belonging to the Cucurbitaceae family is extensively utilized in South India's habitual medicine. Despite its medicinal importance, the phytochemical content of this plant remains largely unexplored. The objective of the present study was to examine the phytochemical composition of the fruits of *C. pubescens*. Initially, HPLC analysis was employed to separate secondary metabolites, revealing seven major phytochemical fractions. The use of a suitable mobile phase system (Acetic acid: Acetonitrile: Water, 4:2:10) at 280 nm facilitated clear isolation. Subsequent spectral analyses confirmed the presence of bioactive compounds. UV-Vis spectral analysis indicated the abundance of flavonoids and tannins. The presence of functional groups, for instance, C=O (carbonyl), C-C (benzene), and Ar-C-H (aromatic hydrocarbon) were validated through FTIR. Further analysis through GC-MS identified 23 bioactive compounds, with quercetin and kaempferol being the predominant ones, followed by gallic acid and caffeic acid. The pharmacological activity of these compounds underscores the therapeutic potential of *C. pubescens*. In conclusion, this study highlights the rich chemical diversity of *C. pubescens*, suggesting its potential as a valuable medicinal species with pharmaceutical significance.

KEYWORDS: *Cucumis pubescens*, HPLC, Spectral analysis, GC-MS, Bioactive compounds

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INTRODUCTION

Cucurbitaceae, commonly known as Cucurbits, constitute a vital category of vegetable crops, contributing approximately 5.0% to the overall vegetable production (Rajasree *et al.*, 2021). This group stands out as a prominent force in the realm of vegetables, showcasing remarkable adaptability across diverse climates, ranging from dry regions to the moist tropics. Throughout Asia, wide arrays of 23 edible cucurbits are cultivated and widely utilized (Jeffrey, 1990). Within the cucurbitaceae, the genus *Cucumis* emerge as a significant group of plants, actively exploited for a different of occasions in traditional herbal medicine, inclusive of anti-oxidant (Chen *et al.*, 2018; Olaniyan & Afolabi, 2018; Rajasree *et al.*, 2021), anti-inflammatory (Demsie *et al.*, 2019; Ezzat *et al.*, 2019; Wahid *et al.*, 2021; Ani *et al.*, 2022), antitumor (Swaminathan *et al.*, 2015; Tuama & Mohammed, 2019), hepatoprotective (Araya *et al.*, 2019; Ma & Wei, 2021), cardiovascular (Adebayo-Gege *et al.*, 2022; Wahid *et al.*, 2022) and immunoregulatory (Mallek-Ayadi *et al.*, 2017; Yang *et al.*, 2020) activities. The fruits and seeds of *Cucumis* generally contain a various class of secondary metabolites, including cucurbitacins, triterpenes, sterols, flavonoids, phenols and alkaloids (Bisognin, 2002). Among the various *Cucumis* species, *Cucumis pubescens* distinguishes itself as a well-known medicinal plant with high pharmacological activities. In Asian countries, the fruit is traditionally used as a laxative, diuretic, diaphoretic, and to strengthen the heart, brain, and body. It is also known to treat Ophthalmia and urinary discharges (Rajasree *et al.*, 2021).

In recent times, the exploration of phytochemical compounds in numerous plant species has been validated through sophisticated techniques. Among these, chromatography followed by spectrochemical analysis has emerged as highly consistent and successful tools for phytochemical investigations (Patle *et al.*, 2020). HPLC (High-Performance Liquid Chromatography) remains the preferred and supreme analytical separation system. It is broadly employed for both qualitative and quantitative analyses of instinctive products derived from unexploited plant samples or herbal formulations (Steinmann & Ganzera, 2011). Ultraviolet-visible spectroscopy (UV/Vis), strictly associated with photon spectrometry that utilizes light to resolve the presence of functional groups, polynuclear compounds, and the extent of conjugation by assessment with standards in the UV Visible region (Fathima & Johnson, 2018). Fourier transform-infrared (FT-IR) spectrophotometer is employed to illustrate and categorize the functional groups of major compounds (Yano *et al.*, 2003).

Gas chromatography coupled with mass spectroscopy (GC-MS) is a comprehensive analytical method that plays a crucial mission in the analysis of bioactive compounds and taxonomic studies especially in chemotaxonomy (Héthelyi *et al.*, 1987; Ye, 2009). To the best of our knowledge, the phytochemical profiling of *C. pubescens* has not been demonstrated yet, primarily due to the limited availability of reference information in this regard. Therefore, the current investigation is dedicated to exploring the chemical profiling of *C. pubescens* fruit through the aforementioned analytical techniques for the first time.

MATERIALS AND METHODS

Collection of Plant Sample and Identification

The fruits of *Cucumis pubescens* Willd, were collected from in and around Namakkal in Tamil Nadu, India. The plant sample obtained was meticulously identified at the Department of Botany, Arignar Anna Govt. Arts College, Namakkal, Tamil Nadu, India. The identification process followed the guidelines outlined in botanical floras (Gamble, 1935; Mathew, 1983) and the botanical nomenclature adhered to the floras of Tamil Nadu (Nair & Henry, 1983, Hendry *et al.*, 1987). A voucher specimen (BOT-AAGAC–08/2018) has been securely deposited in department herbarium at Arignar Anna Govt. Arts College, Namakkal, Tamil Nadu, India.

Preparation of Plant Extract

The freshly harvested fruits of *C. pubescens* underwent a thorough cleaning process to eliminate grime and superfluous matter. Then they were rinsed with deionized water, and any excess moisture was carefully wiped with blotting paper. Subsequently, the fruits were dehydrated for a period of 20 days, ground into a fine powder, sieved with No. 40 mesh, and then stored in a cool place for future use. A dried powder sample weighing 15 g was combined with 150 mL of ethanol and placed in a shaker (Orbital shaker Neolab, India) for 1 day at 30-32 °C (room temperature) with a rotation speed of 120 rpm. Following agitation, the sample was centrifuged for 20 minutes at 5,000 rpm. Then the supernatant was recovered for further investigations.

HPLC Analysis

An Agilent 1200 series HPLC-DAD (Agilent Technologies, Santa Clara, CA, USA) system was utilized for HPLC analysis. C18 reversed-phase column (4.6×250 mm, 5 µm particle size, Supelco, USA) equipped with a quaternary pump were employed for Chromatographic separations. The gradient elution system was composed of five diverse solvents, encompassing both polar and non-polar characteristics. These solvents, namely acetonitrile, acetic acid, water, methanol, and formic acid were combined in different ratios. The detector (multi-wavelength detector) was set to monitor at 280 nm. The injection volume was ten microliter for the sample solution with a flow rate of 1.0 mL/min, and the column temperature was retained at 35 °C. The method's run time was 10 minutes, and all compounds were effectively separated within this timeframe.

UV/Visible and FT-IR Spectral Studies

The plant sample was subjected individual analysis using UV/Vis spectrophotometer (V-630 UV—Visible spectrometry, JASCO, Shimadzu, Japan). The spectroscopy was outfitted with a quartz cell featuring an optical path of one centimeter. It had a spectral resolution of one nanometer within the wavelength range of 200-400 nm. Fourier transform-infrared (FT-IR) investigation was conducted through an Equinox-55 spectrometer manufactured by Bruker, Milano, Italy. It was outfitted with a horizontal attenuated total reflectance (ATR) tool featuring a Potassium Bromide (KBr) crystal. Subsequently, the homogenized mixture was pressed and inserted into the infrared spectrometer to collect spectrum data. The measurements were performed at 20 °C. The spectra were documented from 4400 to 400 cm^{-1} through a 20-scan process along with a spectral resolution (2 cm^{-1}). Each data point was recorded in three replicates (Griffiths & de Haseth, 1986).

GC-MS Analysis

The natural bioactive compounds in the fruit of *C. pubescens* were identified by using a GC-7890A/MS-5975C system invented by Agilent Technologies, Santa Clara, CA, USA. The instrument was outfitted with an HP-5MS column with of 30 meter in length × 250 micrometer in diameter × 0.25 micrometer in thickness of film. In addition, the analysis was performed using a GC-MS setup with high-energy electrons (70 eV) as an ionization system. Helium (99.995%) with a flow rate of 1 mL/min was applied as a carrier gas. The primary temperature was lay down at 50-150 °C with 3 °C/min ramping rate (Holding time - Approximately 10 minutes). Subsequently, the final temperature was raised to 300 °C (The temperature was increased by 10 °C per minute). For injection, one ml of the prepared 1% extract, diluted with the appropriate solvent (ethanol), was introduced in splitless form. The proportionality of bioactive compounds in *C. pubescens* extract was stated as a percentage in accordance with the peak area observed in the GC-MS chromatogram. To identify the active metabolites, the fragmentation patterns of MS were assembled with those stored in the spectrometry database. This was done utilizing the National Institute of Standards and Technology (NIST) Mass Spectral Library.

RESULTS AND DISCUSSION

Validation through High Performance Liquid Chromatography (HPLC) Analysis

HPLC analysis of the fruit extract of *C. pubescens* is shown in Figure 1. The analysis was performed with eight different ratios to determine the appropriate gradient elution system (Table 1). Out of the eight gradient elution systems tested, the results indicated that the most appropriate solvent system for the separation of compounds was Acetic acid: Acetonitrile: water in the ratio of 4:2:10. A total of seven peaks were obtained at retention times of 0.141, 1.960, 2.298, 2.746, 3.558, 3.698, and 3.876 minutes. These retention times are crucial for identifying

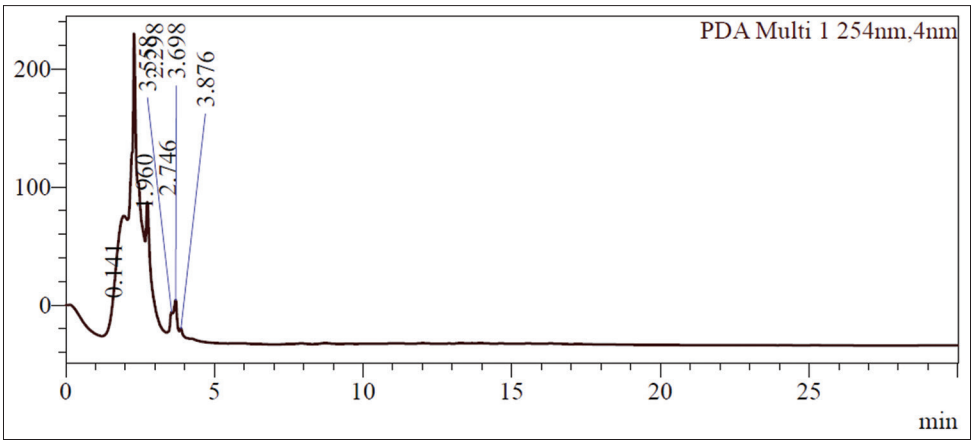

Figure 1: HPLC Chromatogram of *C. pubescens* fruit extract

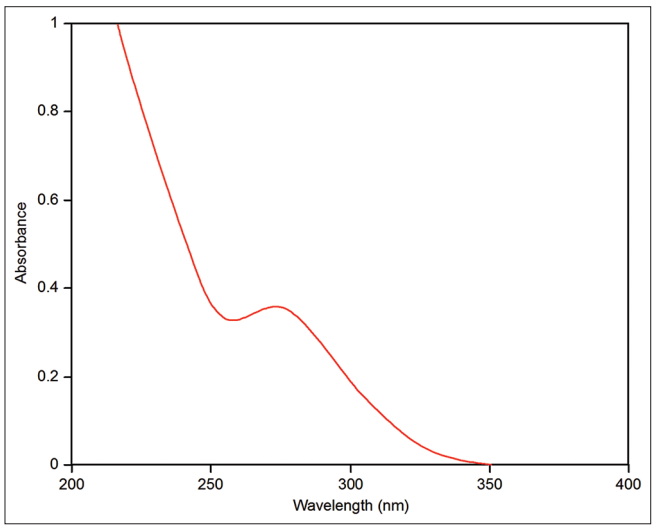

Figure 2: UV/Visible spectra of *C. pubescens* fruit extract

and characterizing each compound present in the extract. The existence of diverse bio active constituents, including phenols, tannins and flavonoids was indicated by these corresponding peaks (Nkwocha *et al.*, 2022). These secondary metabolites have been utilized for medicinal purposes owing to their multiple activities in pharmacology, contributing to the treatment of various illnesses (Prasathkumar *et al.*, 2021; Olasehinde *et al.*, 2022).

The identification of these phytocompounds was accomplished using reference standards. Quercetin, kaempferol and rutin served as the reference standards for flavonoids, while gallic acid and caffeic acid were employed for phenols. For tannins, tannic acid and gallotanins were used as reference standards. The highest peak, representing a specific compound, was notified at a retention time of 2.298 minutes, while another significant peak appeared at 2.746 minutes. These retention times suggest the presence of flavonoids, specifically quercetin and kaempferol, respectively (Figure 1). Both these compounds are extensively familiar with their antioxidant and cytotoxic properties, as well as their role in inducing apoptosis (Lan *et al.*, 2019). These peaks may correspond to the most abundant or prominent compounds in *C. pubescens*. The presence of gallotanins and tannic acid

Table 1: Different solvent system for chromatographic separation of *C. pubescens*

S. No.	Solvent system	Ratio
1	Methanol: Acetonitrile: Water	2:1:10
2	Methanol: Acetic acid: Water	9:2:10
3	Formic acid: Acetic acid: Methanol	1:2:5
4	Formic acid: Acetic acid: Water	1:2:9
5	Acetic acid: Acetonitrile: Water	4:2:10
6	Methanol: Formic acid: Water	5:2:12
7	Methanol: Acetonitrile: Acetic acid	3:1:2
8	Methanol: Acetonitrile: Formic acid	9:2:1

was confirmed with the reference standard at retention times of 0.141 and 1.960 minutes, respectively (Nkwocha *et al.*, 2022). Phenols, including gallic acid and caffeic acid, were obtained at retention times of 3.698 and 3.876 minutes.

UV-Vis and FTIR Analysis

UV/Visible spectroscopy was employed to ascertain the occurrence of aromatic rings with chromophoric groups in plant extracts. This analytical technique is based on measuring the electronic transitions of unpaired electrons, π-bonds, and σ-bonds (Patle *et al.*, 2020). The UV absorption spectra of *C. pubescens* are depicted in Figure 2, revealing a distinct absorption band between 250-280 nm. This observation strongly suggests the existence of tannins and flavonoids in the plant extract (Scano, 2021; Bationo *et al.*, 2022). These findings were assisted by Patle *et al.* (2020) in their study on the determination of phenolics and flavonoids in *Dillenia pentagyna*. The infrared spectrum of *C. pubescens* is illustrated in Figure 3, encompassing a frequency range from 3000 to 600 cm^{-1}. The emergence of FTIR spectra within this limit signifies functional groups. The instance of strong absorption bands between 2300 and 1000 cm^{-1} corresponds to the FTIR region. According to Patle *et al.* (2020), the stretching vibration of C-H typically occurs in the range of 2900 to 2800 cm^{-1}. Specifically, the absorbance peaks at 2931.60 and 2854.45 cm^{-1} indicate the stretching of asymmetric and symmetric compound CH_2. Notably, the prominent bands between 1160 and 1100 cm^{-1} suggest stretching of ester (C-O-C), indicative of a flavonoid compound (Patle *et al.*, 2020).

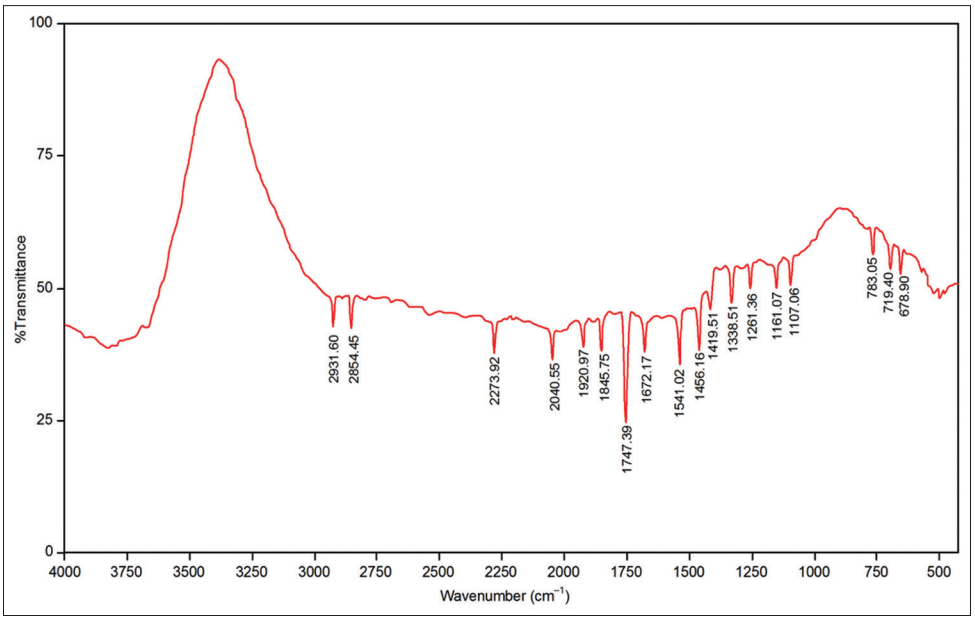

Figure 3: FTIR spectra of *C. pubescens* fruit extract

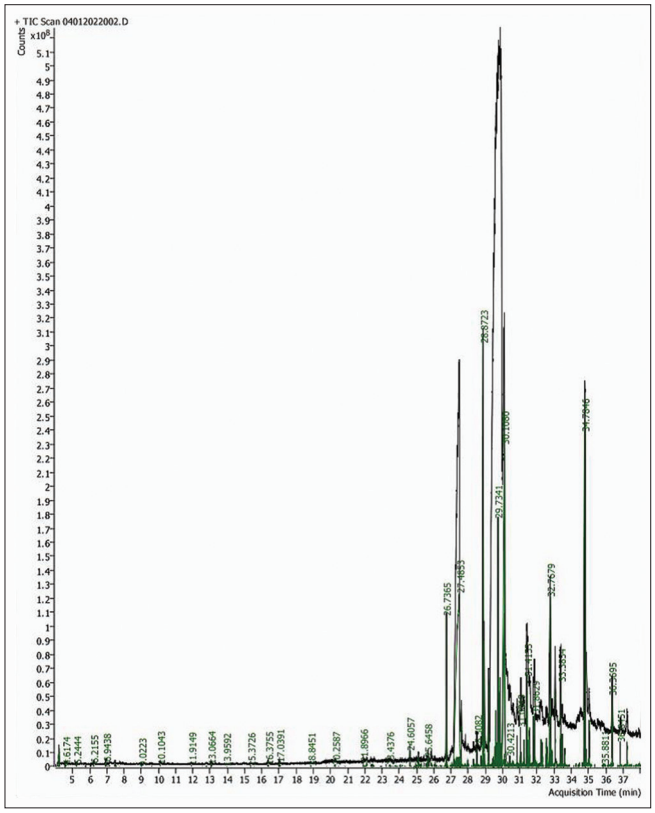

Figure 4: GC-MS analysis of *C.pubescens* fruit extract

Further analysis reveals that the peak area between 1657 to 994 cm^{-1} corresponds mainly to C=O (Carbonyl), C-C (benzene) and (C-O) Carbohydrate groups (Mukrimin *et al.*, 2019). Significant peak at 1747.39 cm^{-1} and 1672.17 cm^{-1} are certified to the stretching of the C=O (Carbonyl) group (Mukrimin *et al.*, 2019; Scano, 2021). Spectral features at 1541.02 cm^{-1}, 1456.16 cm^{-1}, and 1419.51 cm^{-1} are notably connected with the stretching vibration of benzene (C-C) bonds. Additionally, COOH (carboxylic acids) and an O–H stretch exhibits at 1338.51 cm^{-1} and 1261.36 cm^{-1} (Karpagakalyaani *et al.*, 2022). Aromatic hydrocarbons are identified at 783.05 cm^{-1}, 719.40 cm^{-1}, and 678.90 cm^{-1} (Patle *et al.*, 2020). These spectral features provide valuable insights into the diverse chemical components present in the *C. pubescens*.

Identification of Active Compounds through GC-MS Analysis

The GC–MS investigation of *C. pubescens* exposed the presence of 23 bioactive compounds (Figure 4). The retention time, name of the compound, molecular formula, percentage of peak area and the match factor was depicted in table 2. Notably, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one (quercetin) was identified in the highest concentration, constituting 34.8% of the peak area, followed by 3,5,7-trihydroxy-2-(4-hydroxyphenyl)chromen-4-one (kaempferol) with 21.3% of the peak area. Quercetin plays a pivotal role in various pharmacological aspects, including antioxidant and anticancer (Huang *et al.*, 2020), cardiovascular, and anti-immunosuppression treatments (Yang *et al.*, 2020). Kaempferol exhibits diverse pharmacological activities, particularly demonstrating multipotential neuroprotective actions (dos Santos *et al.*, 2021). Following kaempferol, 3,4,5-trihydroxybenzoic acid, commonly known as caffeic acid, was present at 9.3% of the peak area, and gallic acid followed with 6.8% of the peak area. The pharmaceutical applications of these phenolic derivatives in the field of pharmacy have been well-documented recently (Alam *et al.*, 2022; Kaczmarek-Szczepańska *et al.*, 2023). In addition, tannic acid, with a moderate peak area, was also identified in the GC-MS analysis. Hydrolysable tannins like tannic acid have been recommended for their potential antidiarrheal, antidiabetic, antifungal, and cardioprotective activities (Reggi *et al.*, 2020). Furthermore, a few more compounds were predicted, although in trace amounts (Table 2).

Table 2: Major secondary metabolites of *C. pubescens* fruit extract

S. No.	Component RT	IUPAC Name of the compound	Formula	Percentage of peak area	Match Factor
1.	4.2102	Formamide, N, N-dimethyl-	C_3H_7NO	0.75	83.2
2.	4.2165	Ethanol, 2-(2-hydroxyethoxy)-, 1-nitrate	$C_4H_9NO_5$	0.86	81.8
3.	4.6174	Furfural	$C_5H_4O_2$	0.58	87.5
4.	5.2243	3,4,5-trihydroxybenzoic acid	$C_7H_6O_5$	6.81	80.2
5.	6.2155	(E)-3-(3,4-dihydroxyphenyl) prop-2-enoic acid	$C_9H_8O_4$	9.36	92.4
6.	6.2356	Methyl propargyl ether	C_4H_6O	0.93	80.6
7.	6.9438	2-Heptenal, (E)-	$C_7H_{12}O$	0.85	88.9
8.	7.1388	2-(1H-Pyrazol-3-yl) acetaldehyde	$C_5H_6N_2O$	0.72	76.5
9.	7.1612	Ethanol, 2,2'-oxybis-	$C_4H_{10}O_3$	1.03	76.4
10.	7.4578	Pentanoic acid	$C_5H_{10}O_2$	0.90	87.7
11.	10.1043	Thymine	$C_5H_6N_2O_2$	1.01	79.4
12.	11.9149	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6- methyl	$C_6H_8O_4$	0.52	82.8
13.	17.0253	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one	$C_{15}H_{10}O_7$	34.84	76.8
14.	17.0391	3,5,7-trihydroxy-2-(4-hydroxyphenyl) chromen-4-one	$C_{15}H_{10}O_6$	21.37	82.6
15.	23.4376	Cyclooctasiloxane, hexadecamethyl-	$C_{16}H_{48}O_8Si_8$	0.76	77.6
16.	23.7532	2-Butanol, 3-methyl-	$C_5H_{12}O$	0.92	75.7
17.	24.9984	Cyclopentaneacetic acid, ethenyl ester	$C_9H_{14}O_2$	0.76	77.2
18.	25.0718	3,4-Hexanedione, 2,2,5-trimethyl-	$C_9H_{16}O_2$	0.54	76.6
19.	25.5635	[2,3-dihydroxy-5- [[3,4,5,6-tetrakis [[3,4-dihydroxy-5-(3,4,5-trihydroxybenzoyl) oxybenzoyl] oxy] oxan-2-yl] methoxycarbonyl] phenyl] 3,4,5-trihydroxybenzoate	$C_{76}H_{52}O_{46}$	4.39	77.1
20.	28.4193	1H-1,2,4-Triazol-3-amine, 1-methyl-	$C_3H_6N_4$	0.98	76.5
21.	28.8723	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	$C_{19}H_{34}O_2$	0.37	79.8
22.	28.9137	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	0.25	76.7
23.	31.4766	4-Nitrobenzoic acid, 3-pentyl ester	$C_{12}H_{15}NO_4$	0.67	78.0

CONCLUSION

The present phytochemical investigation of *C. pubescens* offers valuable insights into the composition of the ethanolic fruit extract. Through a range of spectral analyses, specific compounds have been identified, each with its distinct pharmacological applications. The present finding indicates that flavonoids, phenols, terpenoids and tannins are the major secondary metabolites present in *C. pubescens*. This exploration represents a promising approach in pharmacological research, with the potential to uncover new drugs for various ailments.

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