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Research Article

Isolation and Characterization of Novel Bioactive Compound 5,7-dihydroxy-2-(4-hydroxyphenyl)-3-{[(3S,4S,5S,6R)-3,4,5-6-Methyltetrahydro-2H-pyran-2-yl] oxy}-5,6,7,8-tetrahydro-4Hchromen-4-one from *Coccinia grandis* (L.)

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ABSTRACT

Coccinia grandis (L) is an important medicinal plant having its great medicinal value in trebles, the compound 5,7-dihydroxy-2-(4-hydroxyphenol)-3-{(3S,4S,5S,6R)-3,4,5-trihydroxy-6methyltetrahydro-2H-pyran-2yl]oxy}-5,6,7,8-tetrahydrd-4Hchlomen-4-one was isolated and it is characterized by ultraviolet, infrared, nuclear magnetic resonance, and mass spectroscopy. Thin-layer chromatography profiling was confirmed a single compound present in the fraction from column chromatography and that's why the characterized bioactive compound enhances to drug delivery.

Key words: Bioactive compound, Characterization, *Coccinia grandis* (L), Isolation, Spectroscopy, Thin-layer chromatography profiling.

1. INTRODUCTION

Humans and natural products have had a special relationship from the beginning of time. Plants have always been important to man, mostly because they provide clothes, food, and shelter from the dawn of human civilization. The earth's richest source of organic compounds is found in plants, which are considered nature's "pharmaceutical industries." Natural vegetation's are a tremendous blessing to the world; some are employed in traditional medicine to treat a wide range of illnesses and disorders [1,2], others are also beneficial as flavoring agents, and still, others serve as food additives and preservatives [3-5]. The primary approach of the chemists of previous centuries has been to focus on these chemicals and their isolation, as well as their chemical and biological features that are useful in the formulation of novel drugs. India has the greatest diversity of plants and animals in the world due to its biodiversity. These days, a lot of research has been done on Indian health-care practices, both conventional and modern [6]. The most fruitful source for the development of materials, especially anticancer and anti-infective drugs, has been natural product sources. The availability of new substances that are easily generated in bacteria has led to an increase in the use of molecular biological techniques in modern times [7]. Attention has been paid to combinatorial chemistry methods for natural product scaffolds that provide screening literacy that closely resembles drug-like molecules. Different screening strategies are being developed to increase the natural products' usability in data mining and drug development campaigns. While the current research on plant species has been unfocused in terms of drug development, natural product chemistry has concentrated on bioactive secondary metabolites that are plants' source chemicals [8]. For example, the bioactive molecule derived from a freshwater living organism is a topic of intense controversy among researchers since it is a challenging region that has not yet been studied (it has been restricted to the terrible environment).

The plant *Coccinia grandis* (L) (Family: *Cucurbitaceae*, Synonyms: *Coccinia indica*.) was chosen in this study, based on evidence in folk medicine in India. *C. grandis* Linn. Voigt, commonly referred to as *C. indica*, is a member of the *Cucurbitaceae* family [9,10]. In traditional medicine, it is widely used to cure a wide range of conditions, including leprosy, jaundice, burns, bronchitis, asthma, earaches, indigestion, eye infections, nausea, bug bites, and fever. The presence of phenols, tannins, saponins, terpenoids, flavonoids, arabinose, xylose, mannose, galactose, glucose, and rhamnose is demonstrated by phytochemical investigations [11]. Research on plant extracts, especially leaf extract, reveals that it has antihyperglycemic, analgesic, anti-inflammatory, antipyretic, antioxidant, antihyperlipidemic, antibacterial, anti-hepatotoxic, and anti-insecticidal properties. It also inhibits the activity of xanthine oxidase [12]. The plant's ability to combat diabetes is one of these that have been well studied. Its potential for cancer treatment is revealed by recent studies on its antioxidant properties [13]. In addition, the plant leaf extract demonstrates a strong chemoprotective effect against cyclophosphamide, a drug that is frequently used to treat autoimmune disorders and cancer [14]. To comprehend the plant's therapeutic qualities, an overview of the numerous researches on it is given.

A perennial herbaceous climber with glabrous stems, tuberous roots, and axillary tendrils, *Coccinia grandis* is dioecious. Simple, alternating leaves are present. The fruit is a smooth, ovoid to ellipsoid, 2.5-6 cm, bright red berry. It is an aggressive, suffocating vine with a vast tuberous root system. *C. grandis* is a highly naturalized species in Hawaii that spreads quickly in disturbed areas up to a height of

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245 meters. In residential neighborhoods and agricultural settings, it typically covers trees, understory plants, fences, power poles, and other man-made structures. When *C. grandis* stems come into contact with soil, they quickly strike roots at the nodes. Birds serve as seed dispersers since many of them may consume the fruit. Asian cuisine makes use of shoot tips and immature fruits; thus, commerce and migration of people have caused long-distance dissemination of the cuttings or seeds for sprouting in new places. There are two identified varieties of *C. grandis*; the latter is utilized in Asian cooking. Tender fruits of one type are bitter, while the other is not bitter at all. Despite the fact that there is no morphological difference between them, both types are invasive and frequently grow close to one another [15, 16].

2. MATERIALS AND METHODS

2.1. Collection, Drying, and Grinding

Plant samples of *C. grandis* (L) grow year round in Maharashtra's northern regions, particularly in the dry winter months. Prof. D.A. Patil of the Botany Department of the Dr. P. R. Ghogray Science College in Deopur Dhule, Maharashtra, India, identified the species as *C. grandis* (L). Following the review of the herbarium sheet and a newly acquired specimen, new plant samples were gathered from several Satpuda region growth sites. To avoid photolysis and thermal degradation, the plant material was cleaned with sterile water to eliminate any related soil debris. It was then shade-dried, chopped into small pieces, and coarsely processed into a powder using a mechanical grinder. After that, the dehydrated powdered samples were stored at room temperature in a sterile, sealed container until further use [17].

2.2. Preparation of Plant Extracts

The plant extracts were made using a serial exhaustive extraction technique, which allowed for the extraction of a wide spectrum of chemicals with polarity. The 500 g of powdered materials were first put in a stoppered container with methanol as the solvent, and they were let to stand at room temperature for 21 days while being constantly stirred until the soluble matter was completely dissolved. Subsequently, the extract was passed through Whatman No. 1 filter paper. After extraction, the leftover marc was allowed to air dry before being extracted once more for a further 21 days using methanol as the solvent. Different solvents were employed for the extraction process; however, for this investigation, methanol extract was chosen since it produced more spots in thin-layer chromatography (TLC) profiling. In a rotary evaporator operating at lower pressure, the extracts were further concentrated by recovering surplus solvents to thick, oily crude. The extracts were kept for later research at 4°C in airtight plastic vials [18].

2.3. Subsequent Isolation of Crude Extract

Using a separating funnel and a solvent extraction procedure, bioactive compounds were extracted. The macerated methanol extract was first separated in ethanol, which has a number of bioactive components. After that, the extract was added to the water and allowed to sit for 15 min. In a separating funnel, more hexane was added to the water-ethanol combination. Hexane and the water-ethanol mixture were held at a constant 1:2 ratio. After shaking, the extract was let to stand for 10 min. After separating the two layers, more hexane was added and the top layer of hexane was removed. This process was repeated 5 or 6 times. To extract the hexane fraction, all of the collected hexane was recovered by rotary evaporation. The process was repeated with a solvent such as ether, chloroform, or ethyl acetate on the residual aqueous layer. Bioassay fractions served as guidance for further procedures [19].

2.4. Column Chromatography

Compounds produced from the above-mentioned extraction method were separated using the column chromatography technique. N-hexane was used to elute the extract from the silica gel (60–120 mesh) on which it had been adsorbed. Ethyl acetate was used to increase the minor polarity. Forty portions were gathered and kept in a borosilicate 500 mL reagent bottle. Using an ethyl acetate and diethyl ethyl ether solvent system, TLC was applied to each fraction. The column was run through the entire 100 mL fraction utilizing a solvent system (9.5:0.5, 9.0:1.0, 8.5:1.5, 4:1). The purity level of the isolated chemical was verified using independent high-performance liquid chromatography analyses of these fractions [20].

3. RESULTS AND DISCUSSION

3.1. TLC Profiling

C. grandis (L) ethyl acetate extract in acetone solvent system: After 10% H_2SO_4 was sprayed, hexane (1:3) with standard revealed five spots at Rf values of 0.41 (orange), 0.35 (blue), 0.33 (pink), 0.32 (blue), and 0.29 (green) [Figure 1a]. *C. grandis* (L) methanol extract when diluted with acetone: When 10% H_2SO_4 was sprayed on hexane (1:3) with standard, eight spots were observed with Rf values ranging from 0.77 (orange); 0.75 (blue); 0.68 (orange); 0.58 (blue); 0.55 (pink); 0.5 (green); 0.27 (blue); 0.18 (pink); and 0.06 (blue) [Figure 1b]. Water extract of *C. grandis* (L) when run in solvent system solvent system was run methanol: chloroform (1:2) elution solvent was run 6 cm and showed the one spot with visible at Rf 0.81 (violet), by spraying with 10% H_2SO_4 [Figure 1].

Methanol extract showed the maximum spot in TLC profiling; methanol crude extract has maximum bioactivity so methanol extract was chosen for the present study.

3.2. Ultraviolet (UV) Analysis of Isolated Bioactive Compound

Using TLC analysis, compound-2 was found from chromatographic fractions with an Rf value of 0.69 cm, which may suggest that there is just one chemical present in this fraction. Figure 2 illustrates the yield of 130 mg from the ethyl acetate (100%) ratio fraction of *C. grandis* (L) leaves. The primary absorption bands at λmax of 252 nm and maximum absorbance of 1.77, as revealed by the UV-visible spectroscopy study for the isolated chemical [Figure 2(B)], also point to the presence of a single compound.

3.3. Fourier-transform infrared spectroscopy Spectrometric Analysis of Isolated Compound

1215.88 cm^{-1} for the aliphatic/aromatic ester; 1377.78 cm^{-1} for the –CH3 group; 1638.52 cm^{-1} for the C=O carbonyl group; 3312.84 cm^{-1} for the –OH group; 1480.72 cm^{-1} for the aromatic group; and 1109.31 cm^{-1} for the C–O–C ether group [Figure 3].

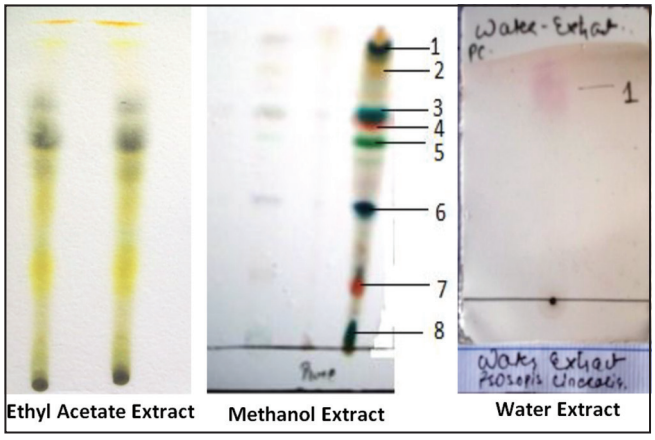
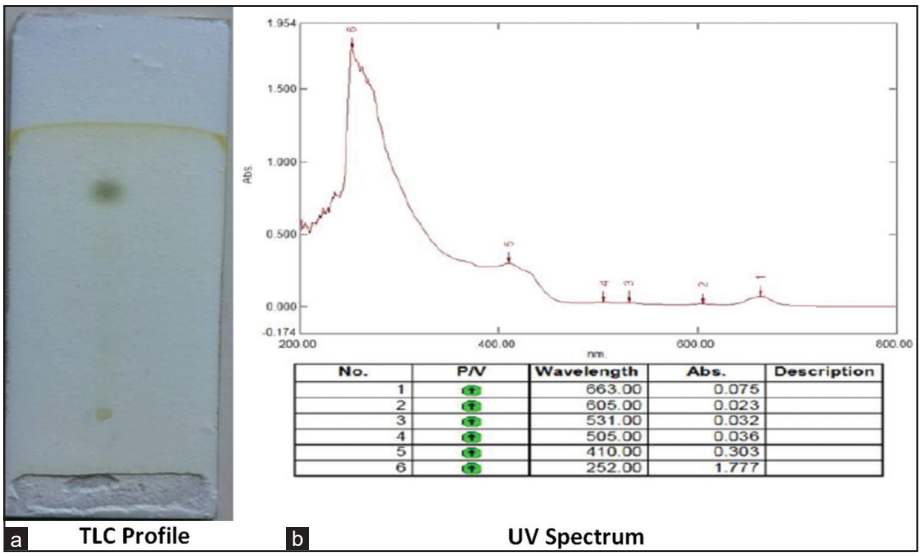


Figure 1: Thin-layer chromatography profiling of crude extract.



No.	P/V	Wavelength	Abs.	Description
1	+	663.00	0.075	
2	+	605.00	0.023	
3	+	531.00	0.032	
4	+	505.00	0.036	
5	+	410.00	0.303	
6	+	252.00	1.777	

Figure 2: (a) Thin-layer chromatography analysis and (b) Ultraviolet spectrum of compound.

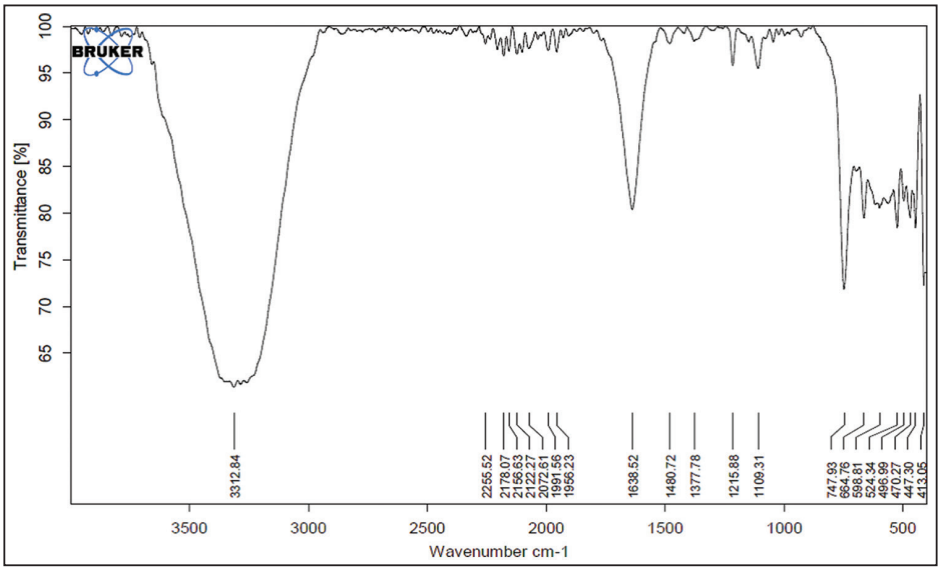


Figure 3: Fourier-transform infrared spectroscopy spectrum of isolated compound.

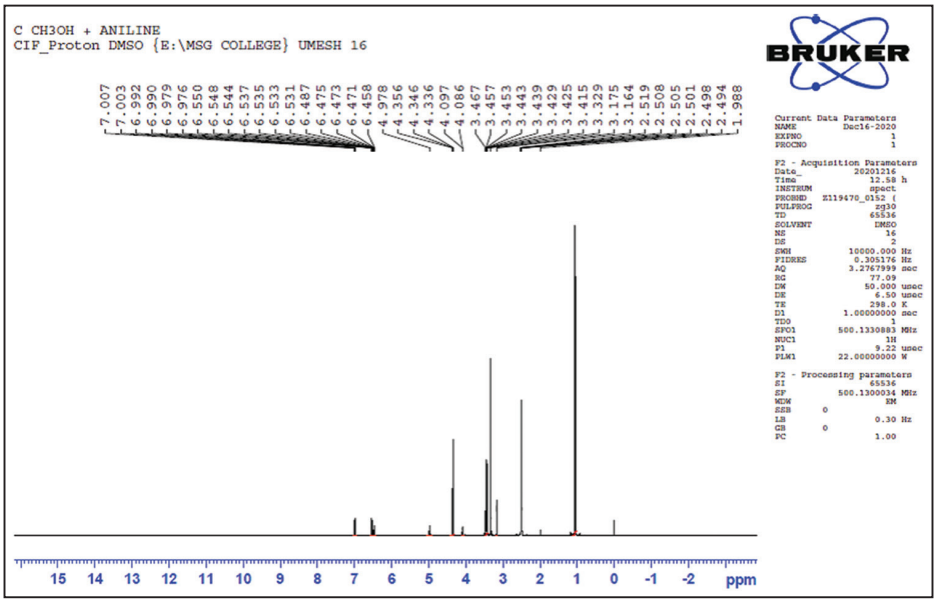


Figure 4: [1]HNMR spectrum of isolated compound.

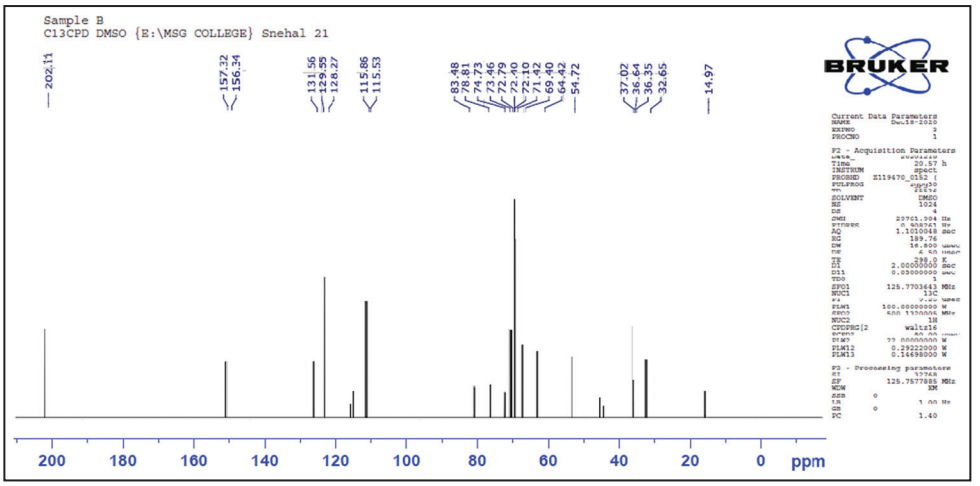


Figure 5: ^{13}CNMR spectrum of isolated compound.

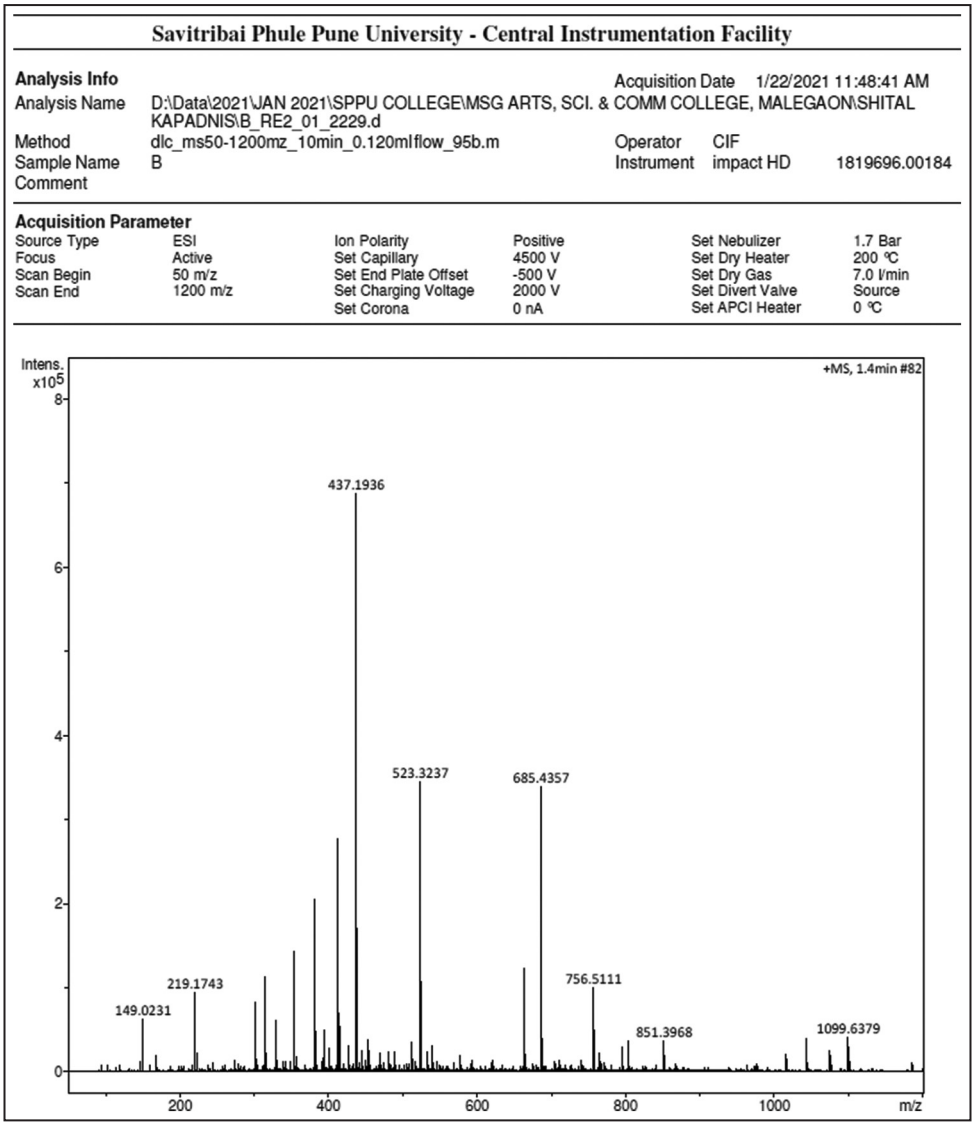


Figure 6: High-resolution mass spectrometry spectrum of isolated compound.

3.4. ^{1}HNMR Spectrometric Analysis of Isolated Compound (500.13 MHz; DMSO d6) δ ppm

δ 1.98 (3H, d,), 2.49–2.50 (2H, dd), 2.51 (2H, d), 3.16 (1H, dd), 3.17 (1H, dd), 3.40 (1H, dd), 3.41–3.46 (1H, tt), 4.08 (1H, dq), 4.33–4.35 (1H, t), 4.97 (1H, d), 6.45–6.99 (2H, dd), 7.0, (2H, dd) [Figure 4].

Signals at δ 1.98 in the 1 H NMR spectrum indicate that a doublet is caused by a 1H present in the cyclohexane ring; signals at δ 2.49–2.50 indicate that 2H protons are responsible for the doublet; signals at δ 2.51 indicate that hydrogen is responsible for the doublet; 1H demonstrates

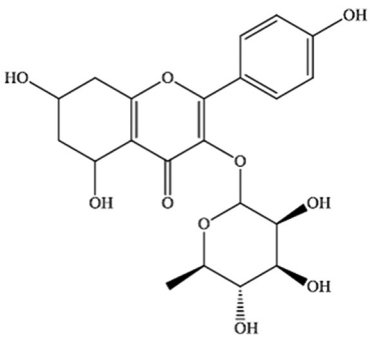


Figure 7: Structure of isolated compound.

the doublet of doublet due to –CH– at δ 3.16 and 3.17; 1H provides the doublet of doublet at δ 3.40 for –CH proton, and at 3.41–3.46 The triplet of triplets in 1H indicates the presence of a proton in the cyclic ring. The doublet of quartate methyl group and hydrogen in 1H is indicated by δ 4.08 in the cyclohexane ring. Due to two oxygen atoms adjacent at δ 4.97, 1H produces a doublet at upfield, and at δ 6.45–7.0, 4H produces a doublet of doublets that are attached to aromatic ring. The drug's hypothesized that structure is depicted in figure, and based on all of the spectrum tests conducted above, the isolated compound was characterized, supporting the preceding findings suggesting that the compound is a steroid.

3.5. ^{13}CNMR Spectrometric Analysis of Isolated Compound (500.13 MHz; DMSO d6) ppm

δ 14.9 (1C, s), 32.5 (1C, s), 36.1 (2C, s), 36.4 (1C, s), 52.5 (1C, s), 63.0 (1C, s), 71.5 (2C, s), 72.7 (1C, s), 73.2 (1C, s), 74.6 (1C, s), 78.8 (1C, s), 83.4 (1C, s), 112.7 (2C, s), 123.0 (2C, s), 156.0 (1C, s), 202.0 (1C, s) [Figure 5].

In the 13CNMR spectrum, the existence of methyl carbon is indicated by the signal at 14.9, the nature of the carbon is C–O–C at 63.0, and the carbon is carbonyl carbon at 63.0. Carbon is shown to be present in the aromatic area by the signal at 115.3–127 and in the cyclic ring by the signal at 83.4.

3.6. High-resolution Mass Spectrometry (HRMS) Spectrometric Analysis of Isolated Compound (Bruker Compass)

The mass-to-atomic number ratio *(M/z) of isolated compound is* 437.19 (M^+) [Figure 6]

From all the above, data were compiled the projected structure of the compound–2 [Figure 7] having a Molecular Formula: $C_{21}H_{24}O_{10}$; Molecular Weight: 436.40 and the molecular weight is nearer to the HRMS data.

Herbal remedies for leprosy, jaundice, burns, bronchitis, asthma, earaches, indigestion, eye infections, nausea, bug bites, and fever include *C. grandis* (L.) [21-25]. Consequently, this plant might be abundant in antimicrobial agents – ESI, QTOF, UHPLC, and MS/MS. Antibacterial and antifungal compounds were identified in *C. grandis* (L.) by the use of molecular networking-guided isolation and dereplication [26,27].

4. CONCLUSION

The present work reports the isolation and identification of bioactive compounds from the leaves of *C. grandis* (L) methanolic extract. These compounds are newly isolated from *C. grandis* (L). Compound 1 was selected for additional *in vitro* and *in silico* research due to its steroid-like characteristics. These organic substances might have a wide range of biological actions that could treat different human illnesses. These bioactive substances may prove to be a valuable resource for developing new drugs in the future and guarantee medicinal necessity.

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6. CONFLICTS OF INTEREST

There are no conflicts of interest in this research work.

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