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Phytochemical Analysis of *Anacamptis sancta*: Glucomannan, Phenolic Components, and Antioxidant Activity

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Abstract

Background and purpose: *This study investigated the extracts obtained from both the roots and above-ground parts of* Anacamptis sancta. A. sancta*, an orchid species traditionally used for its potential health benefits due to its rich phytochemical composition, was the focus. The primary aim of this research was to analyze the phytochemical components of* A. sancta *extracts and evaluate their antioxidant potential. By conducting comprehensive phytochemical analyses and assessing antioxidant activities, this study seeks to provide a deeper understanding of the medicinal properties of* A. sancta *and contribute to its applications in functional foods and pharmaceuticals.*

Materials and methods: *In this study, extracts derived from both the tubers and above-ground parts of* A. sancta *were investigated. Plant samples were collected from the Ondokuz Mayıs University Botanical Garden. Fourier transform infrared spectroscopy (FTIR) analysis was conducted using a Perkin Elmer Spectrum 400 spectrophotometer. Glucomannan content was measured calorimetrically using the Megazyme K-GLUM 03/20 analysis kit and UV-visible spectrophotometer. Analysis of secondary metabolites in the above-ground parts included determination of total phenolic, flavonoid, flavanol, tannin, and proanthocyanidin contents using Folin-Ciocalteu, $AlCl_3$, and other appropriate methods. Antioxidant capacity was assessed through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, and compound identification in the above-ground parts was performed using gas chromatography/mass spectrometry (GC-MS) analysis.*

Results: *In the tubers, a glucomannan content of 17.93% was detected, and FTIR analyses revealed the characteristic peaks of glucomannan. The extracts demonstrated high antioxidant potential, with total phenolic content measured at 120.61 mg GAE/g extract, total flavonoid content at 295.46 mg QE/g extract, flavanol content at 32.30 mg QE/g extract, total tannin content at 3.05 mg GAE/g extract, and total proanthocyanidin content at 119.43 mg CAE/g extract. The IC_{50} value for DPPH radical scavenging activity was calculated to be 47.79 mg/mL. GC-MS analysis of the above-ground parts of the plant identified the presence of significant bioactive compounds, including hydroxyacetic acid, hydrazide (42.52%), propane, 1,1-dipropoxy- (16.94%), and 1,3-dioxolane-4-methanol, 2-ethyl (12.86%). In summary, this study presents a comprehensive analysis of the phytochemical profile of* A. sancta.

Conclusions: *The findings highlight its significant glucomannan content in tubers and strong antioxidant activity. Additionally, bioactive com-*

pounds were identified in the above-ground parts. This research provides valuable insights into potential medicinal and industrial applications of A. sancta.

INTRODUCTION

Orchidaceae, with 32,913 officially recognized species, constitutes 8.61% of all flowering plants, making it the second richest family among flowering plants *(1, 2)*. Orchids exhibit a global distribution in various forms epiphytic, lithophytic, and terrestrial excluding deserts and glaciers. Terrestrial orchids, typically growing in soil and characterized by fleshy round tubers, have garnered significant scientific interest due to their intricate floral structures, complex pollination mechanisms, and dependence on mycorrhizal symbiosis *(3)*.

Orchids have long been valued not only for their aesthetic appeal but also for their pharmaceutical properties, which include anti-carcinogenic, anti-inflammatory, antirheumatic, antimicrobial, neuroprotective, and antiviral effects *(4)*. They contain a variety of bioactive compounds such as alkaloids, flavonoids, phenanthrenes, terpenoids, and bibenzyl derivatives *(5–7)*. Orchid species have gained widespread recognition in the extensive literature for producing secondary metabolites that are significant in the fields of physiology, ecology, and pharmacology. These metabolites encompass a broad chemical spectrum, including phenolic compounds, terpenes, and alkaloids *(2, 8)*. However, there is relatively limited research on the phytochemistry of terrestrial orchids and their associated biological activity.

The growing interest in the utilization of tubers from certain terrestrial orchids as food additives has further intensified research curiosity. These tubers are traditionally used in beverages and treatments, notably in the production of Salep, a starch-like polysaccharide derived from the dried and ground tubers of certain orchid species *(5)*. The composition of Salep varies significantly depending on the harvesting season and orchid species. According to Fatahi *et al.*, the main components of Salep include glucomannan (7.7-54.6%), starch (5.44-38.7%), and protein (3.11-4.95%) *(3)*. Salep is popular primarily in countries such as Türkiye, Greece, Iran, Iraq, and Albania. Salep is consumed as a remedy for colds and coughs in countries such as Turkey, Greece, Iran, Iraq, and Albania, and is a key ingredient in the famous Turkish ice cream, Maraş *(5, 9, 10)*. However, excessive harvesting poses a serious threat to orchids in the region, increasing the risk of extinction *(11, 12)*.

In our previous study conducted by Deniz *et al. (13)*, the focus was on uncovering the potential for conservation and cultivation of *A. sancta*. Within this framework, successful reintroduction of plants produced from *A. sancta* seeds into their natural habitat has been achieved. Moreover, studies with fungi belonging to the Ceratobasidiaceae, isolated from its roots, have shown their effectiveness in the germination and seedling development processes of *A. sancta*. This study can be considered an important step towards conservation and cultural heritage preservation.

This study aims to determine the glucomannan content and chemical structure of cultured *A. sancta* tubers, as well as to evaluate the potential use of its aerial parts. Fourier transform infrared spectroscopy (FTIR) analysis has been particularly favored for elucidating the molecular structures and chemical bonds in the tubers. Additionally, the antioxidant capacities will be evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay and content analyses including total phenolic content, Total flavonoid content, total flavanols content, total tannin content, and total proanthocyanin content will be conducted using spectrophotometric techniques. Gas chromatography/mass spectrometry (GC-MS) analysis has also provided significant data through content analysis. Analysis of glucomannan, a component of the tubers, was performed using spectrophotometric methods. These analyses have been conducted to reveal both the current chemical profile of the tubers and the potential uses of the plant's above-ground parts.

The findings obtained through these methods will help us understand the phytochemical properties of *A. sancta* and evaluate its potential biological and industrial applications. Particularly, the determination of glucomannan content and antioxidant capacities is crucial for understanding the health benefits and industrial applications of the plant. This study may present new opportunities for the pharmaceutical, cosmetic, or food industries regarding how this plant can be utilized.

Figure 1. *View of A. sancta in the cultural area.*

MATERIALS AND METHODS

Plant material and extract preparation

The material used in this study evaluated both the tubers and above-ground parts of these cultivated orchids. *A. sancta* samples were collected from the cultivation area at Ondokuz Mayıs University Botanical Garden in Samsun. They were identified during the flowering stage by Prof. Dr. Yasemin Özdener Kömpe. Two-year-old tubers were harvested, washed, dried, and had soil residues removed. Additionally, the above-ground parts of the plants (Figure 1) were dried and ground, like the tubers. *A. sancta* plant samples were first pulverized into fine powder. For the maceration method, 0.5 g of powder was extracted with 10 mL of 80% methanol at 35 °C for 24 hours *(14)*.

Preparation of tubers

The preparation process involved boiling the tubers in water for approximately 10-15 minutes. The softened tubers had their outer skin gently peeled, and the boiled tubers were left to dry on filter paper at semi-shaded room temperature. Once the tubers reached a suitable hardness for cutting, they were roughly broken into pieces using a hand grinder and subsequently ground again in ceramic mortars. The ground sample was sifted through a 100-mesh sieve, and 0.1 g of the resulting Salep powder was weighed and prepared for analysis (FTIR and glucomannan content). The obtained tuber powder was stored at 4 °C for further analysis.

FTIR Analysis

For the FTIR studies, powder from Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) analyses were performed using a Spectrum 400 spectrophotometer (Perkin Elmer, Waltham, MA, USA) equipped with a DTGS (Deuterated triglycine sulfate) detector. Typically, scans were obtained with a resolution of 4 cm^{-1} in the range of 4000-400 cm^{-1}. Samples were analysed without any prior processing. Peak frequencies were determined using the Perkin Elmer Spectrum One FTIR software.

Colorimetric determination of glucomannan content

To determine the Glucomannan content, the Megazyme (Wicklow, Ireland) K-GLUM 03/20 analysis kit was utilized. The glucomannan contents of the samples were estimated by measuring the absorbance values of the blind and sample solutions (GM A1, A2, A3) prepared at 340 nm using a UV-visible spectrophotometer. The glucomannan contents of the samples were calculated using the following formula:

$\Delta A_{glucoomannan}$ = (A3-A1) samples – (A3-A1) blank

Total glucomannan content (%) = $\Delta A_{glucomannan} \times 36.8$.

Spectroscopic analysis of secondary metabolites in A. sancta above – ground parts

Total phenolic content

The total phenolic content of the extract was determined using the Folin-Ciocalteu method with some modifications, following the procedure by *(15)*. In this research, 200 µL of the extract (1 mg/mL) was combined with 200 µL of Folin-Ciocalteu reagent that had been diluted in a 1:1 ratio with distilled water. After allowing the reaction mixture to incubate at room temperature for 3 minutes, 1 mL of a 2% Na_2CO_3 solution was introduced. Following a 1-hour incubation in darkness at room temperature, the absorbance was measured at 760 nm using a UV spectrophotometer (Thermo Scientific Varioskan). The total phenolic content was quantified in milligrams per gram of dried extract and expressed as gallic acid equivalent (GAE) (mg GAE/g extract). All measurements were conducted in triplicate.

Total flavonoid content

The total flavonoid content of the extracts was determined using the $AlCl_3$ method with some modifications, following the procedure by *(16)*. In this research, 1 mL of the extracts (at a concentration of 1 mg/mL) was blended with 6.4 mL of distilled water. Following this, 0.3 mL of $NaNO_2$ (5%) was introduced, and the mixture was allowed to stand for 5 minutes. Subsequently, 0.3 mL of $AlCl_3$ (10%) was added, and the solution was left to incubate for 6 minutes. Afterward, 2 mL of NaOH (1M) was incorporated, and the solution was maintained at room temperature for 30 minutes. The absorbance was then recorded at 510 nm using a UV spectrophotometer. The total flavonoid content was quantified in milligrams per gram of dried extract and expressed as quercetin equivalent (QE) (mg QE/g extract). All measurements were conducted in triplicate.

Total flavanols content

The total flavonoids content was determined using the $AlCl_3$ method *(17)*. In summary, 1 mL of the extracts (at a concentration of 1 mg/mL) was combined with 2 mL of $AlCl_3$ (2%). Following this, 3 mL of a 5% CH_3COONa solution was introduced. The mixture was then left at room temperature in a dark environment for 30 minutes. After the incubation period, the sample's absorbance was measured at 415 nm using quercetin as the reference standard. The total flavanols content was quantified as quercetin equivalents (mg QE/g). All measurements were conducted in triplicate.

Total tannin content

The total tannin content was determined using the Folin–Ciocalteu reagent following the method described in *(18)*. A calibration curve with various concentrations

of gallic acid in methanol was prepared. For samples, 100 µL of a 5-fold diluted extract in methanol was mixed with 500 µL of a 10-fold diluted Folin–Ciocalteu reagent in water, followed by the addition of 2 mL of 4% (w/v) aqueous Na_2CO_3 solution. After a 30-minute incubation in the dark at room temperature, absorbance was measured at 760 nm. Total phenolic content was expressed as gallic acid equivalent (GAE) (mg GAE/g extract). All measurements were performed in triplicate.

Total proanthocyanin content

The total proanthocyanidins content was determined using the butanol-acid assay *(19)* with the following steps: 500 µL of diluted phenolic extract (1:5, v/v) was mixed with 3 mL of the n-butanol/HCl reagent (95:5, v/v), followed by the addition of 100 µL of 2% $FeNH_4(SO_4)_2{\cdot}12H_2O$ in 2N HCl. The mixtures were boiled for 60 minutes and then allowed to cool. The absorbance of the solutions was measured at 550 nm. The total proanthocyanidins content was expressed as catechin equivalent (CAE) in milligrams per gram of dried extract (mg CAE/g extract). All measurements were performed in triplicate.

Analytical methods

The phenolic compound content of the samples was determined using the following methods: expressed as milligrams of Gallic Acid Equivalent (GAE) per gram of dry extract. A linear regression equation derived from the Gallic Acid calibration curve ($y=0.134x+0.1059$, $R^2=0.9962$) was used for calculation. Total flavonoid content was calculated using the quercetin calibration curve ($y=0.078x+0.0222$, $R^2=0.9789$), while flavonol content was determined using the quercetin calibration curve ($y=0.0363x+0.1767$, $R^2=0.9831$). Total tannin content was measured using the Gallic Acid calibration curve ($y=0.0103x+0.0848$, $R^2=0.9942$). Lastly, total proanthocyanidin content was determined using the catechin calibration curve ($y=0.0038x+0.3639$, $R^2=0.9978$).

Determination of antioxidant capacity

DPPH (2,2-diphenyl-1-picrylhydrazyl) assay

The free radical scavenging potential of the extracts was assessed using the DPPH assay, with a comparison to the IC_{50} value of the synthetic antioxidant ascorbic acid, with slight modifications based on the method described by *(20)*. In this assay, different concentrations of the extract (1 mL) were combined with a methanol solution containing DPPH radical (0.1 mM) in a test tube. After a 30-minute incubation at room temperature in darkness, the absorbance was recorded at 517 nm using a UV spectrometer (Thermo Scientific Varioskan Flash) with a blank as the reference. Ascorbic acid served as the standard reference compound. The percentage of DPPH radical scavenging activity of the extract was calculated using the following formula:

$$\text{DPPH scavenging activity (\% inhibition)} = [(A_{control} - A_{sample}) / A_{control}] \times 100,$$

where Acontrol is the absorbance of the control and Asample is the absorbance of the reaction mixture with the extract. To determine the concentration of the extract required to cause a 50% decrease in the initial DPPH concentration, a concentration curve of the extract versus percentage inhibition was constructed, and the IC_{50} value was obtained through linear regression analysis. A lower IC_{50} value indicates higher antioxidant activity. All measurements were conducted in triplicate.

Identification of compounds in above – ground parts using GC-MS

The plant sample was ground into a fine powder, and 0.5 g of it was extracted with 10 mL of 80% methanol at 35°C for 24 hours *(14)*. After centrifugation at 3500xg for 10 minutes, the supernatant was collected, diluted 100 times, and placed in 1.5 ml vials for GC-MS analysis. Bioactive compounds in the methanol extracts from *A. sancta* above-ground parts were identified using GCMS-QP2010 Ultra (Shimadzu Europa GmbH, Germany) with an electron ionization system (70 eV ionization energy). Pure helium served as the carrier gas at a flow rate of 0.95 ml/min (99.99% purity). The temperature started at 70 °C, increased at 3°C/min after a 10-minute hold, reached 150 °C, held for 5 minutes, and then programmed to reach 250 °C at a rate of 10°C/min, followed by a 5-minute hold. A diluted microliter of 1% extract in methanol was injected in split mode. The identification of each compound was based on the comparison of its retention index (RI) (calculated using n-alkanes series between C9 and C31) and its mass spectra (MS) spectra with those described in the literature and by computer matching with standard reference databases (NIST).

RESULTS

Glucomannan content and FTIR analysis in tuber

The *ex-vitro* method resulted in a glucomannan content of 17.93% in *A. sancta*, which was produced from seeds to ensure adaptation to the natural environment. Figure 2 presents the FTIR analysis of the tubers. According to the results, peak areas were detected at 3303, 2921, 1630, 1404, 1246, 1155, 1012, 873, and 768 cm^{-1}. The peak at 3303 cm^{-1} represents the characteristic O - H stretching vibration *(21)*. The peak at 2921 cm^{-1} represents $-CH_2-$ stretching *(22)* while the peaks at 1630 and 1404 cm^{-1} represent C=O stretch vibrations of the amide groups in glucomannan proteins *(23–25)*. The presence of β-1,4 glucosidic and β-1,4 mannosidic linkages, which are characteristic of GM, is assigned to the C-O-C stretching observed between 1012 and 1246 cm^{-1} *(23)*. The C-H

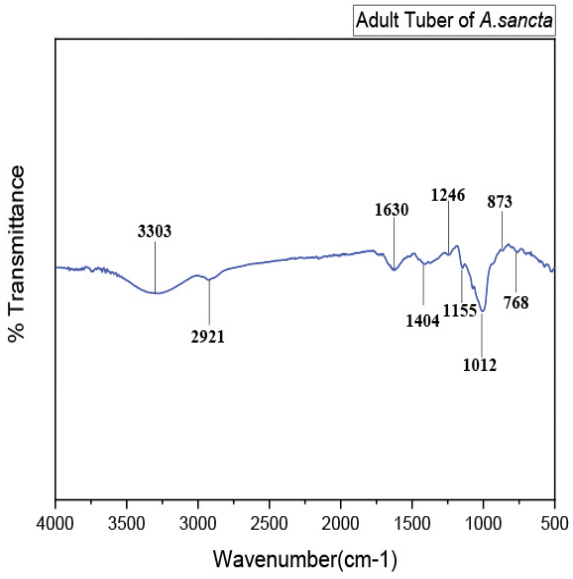

Figure 2. *FTIR peaks of A. sancta adult tuber.*

bending appeared at 873 and 768 cm^{-1}, which correspond to the β-pyranose form of glucose and mannose, respectively *(26, 27)*.

Determination of antioxidant capacity

The changes in the content of various groups of phenolic compounds and the antioxidant activity of *A. sancta* above-ground portions were quantified. The total phenolic content in the extract was determined to be 120.61±2.23 mg GAE/g crude extract. The total flavonoid content resulted in an estimated content of 295.46±51.50 mg QE/g crude extract. Flavonol content yielded an estimated content of 32.30±10.53 mg QE/g crude extract. Total tannin content was estimated at 3.05±0.08 mg GAE/g crude extract. Finally, the total proanthocyanidin content resulted in an estimated content of 119.43±22.84 mg CAE/g crude extract. These findings highlight the richness of phenolic compounds in *A. sancta* above-ground parts portions, as summarized in Table 1. Additionally, Table 1 presents the DPPH radical scavenging

Table 1. *Bioactive components and antioxidant activity of A. sancta above-ground parts*

Plant Name	DPPH (IC_{50} mg/mL)	Total Flavonol Compound (mg QE/g extract)	Total Flavonoid Compound (mg QE/g extract)	Total Phenolic Compound (mg GAE/g extract)	Total Proanthocyanidin content (mg CAE/g extract)	Total Tanen Content (mg GAE/g extract)
A. sancta-above-ground parts	47.79 ± 11.74	32.30 ± 10.53	295.46 ± 51.50	120.61 ± 2.23	119.43 ± 22.84	3.05 ± 0.08

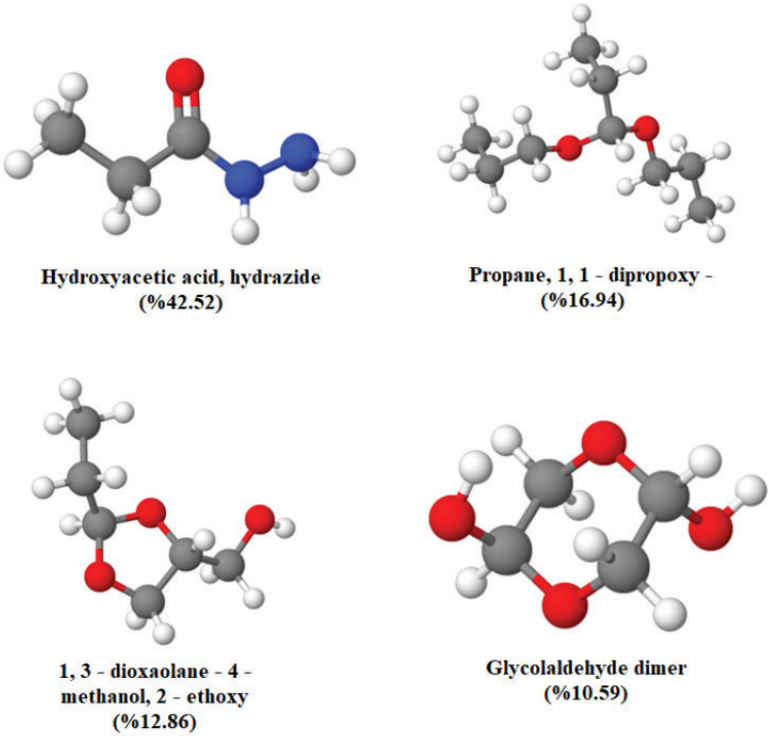

Figure 3. *A. sancta above-ground parts are major substances in GC-MS analysis.*

activity of *A. sancta* above-ground parts (IC_{50}: 47.79 mg/ml) compared to the well-known natural antioxidant, ascorbic acid.

Detection of bioactive compounds from GC-MS

We identified various bioactive contents from the methanol extract of the above- ground parts of *A. sancta*, a terrestrial orchid. The retention time (RT), molecular formula, retention index (RI) and concentration (% area) of nine bioactive phytochemical compounds in the extract are shown in Table 2 based on these results, the main components were identified as hydroxyacetic acid, hydrazide (42.52%), propane, 1,1-dipropoxy- (16.94%), 1,3-dioxolane-4-methanol, 2-ethyl (12.86%), and glycolaldehyde dimer (10.59%) (Figure 3).The minor components are determined as follows: 1-propanol (4.83%), 1,2,3-propanetriol (3.57%), guanosine (3.48%), ethanol,2-ethoxy (3.13%), and 4-ethyloctane (2.08%). The chemical structures of the obtained compounds were drawn using the free online software MolView (https://molview.org/).

DISCUSSION

Although the historical use of terrestrial orchids in Europe and the Mediterranean regions has been documented since antiquity, there exists a dearth biological activity *(8)*. This study aims to determine the diversity of phenolic compounds in the aerial parts of *A. sancta* and evaluate their antioxidant activity. Phenolic compounds are natural antioxidants found in plants and may have various health benefits due to their biological activities. Therefore, understanding the relationship between the phenolic content and antioxidant potential of *A. sancta* is crucial, providing significant insights into the pharmacological and biological potential of the plant. Furthermore, the identification of the biochemical composition of the plant allows us to assess its potential applications in the medical and nutritional fields. The antioxidant properties of phenolic compounds can effectively combat free radicals, offering a range of positive effects such as anti-aging, anti-cancer, and overall health improvement. Hence, determining the phenolic content and antioxidant activity of *A. sancta* is essential for evaluating its potential therapeutic and nutritional value. Moreover, this research contributes to sustainable production practices, ensuring the determination of usage potentials without causing harm to individuals in nature. The findings from this study not only enhance our understanding of the biological properties of the plant but also open avenues for applications in drug development, natural antioxidant sources, and herbal medicine, all while maintaining sustainable production practices that do not harm the environment.

Studies on the total phenolic compound content in species belonging to the *Orchidaceae* have revealed intriguing findings. For instance, previous research has shown that *Dendrobium* flowers exhibit high total phenolic compound content in ethanol and aqueous extracts (9.220 and 10.310 g extract, respectively) *(28)*. Additionally, the above-ground parts of *Dactylorhiza osmanica* were reported to have a TPC value of 20.6 mg GAE/g *(29)*. *D. chuhensis (30)*, along with *D. maculata* and the natural tubers of *Habernaria edgeworthii*, were observed to have high total phenolic compound content, with values of 13.9 mg, 2.76 mg, and 5.28 mg GAE/g DW, respectively *(31, 32)*. The total phenolic content of *Epidendrum secundum* leaves was determined to be 4.03 ± 0.1, while the leaves of *in vitro*-cultivated *E. secundum* seedlings yielded an extract with a total phenolic content of 1.21 ± 0.14 *(6)*. Finally, the flowers and aerial parts of *Anacamptis pyramidalis* were found to have total phenolic contents of 32.82 and 54.16, respectively *(33)*.

A study conducted by Hürkan *et al. (34)* focused on the above-ground parts of *Anacamptis morio*, *A. pyramidalis*, *Neotinea tridentata*, *Ophrys mammosa*, *Ophrys lutea*, and *Ophrys speculum* orchids, revealing the total phenolic

Table 2. *Bioactive compounds detected in A. sancta methanol extract.*

No	RT (min)	Name of the compound	Chemical Class	RI*	Molecular formula	Area %
1	3.056	Hydroxyacetic acid, hydrazide	hydrazide	1106	$C_2H_6N_2O_2$	42.52
2	3.238	Glycolaldehyde	aldehyde	550	$C_4H_8O_4$	10.59
3	3.738	Propane,1,1-dipropoxy	ether	610	$C_9H_{20}O_2$	16.94
4	3.872	1,3-dioxolane-4-methanol,2-ethyl	dioxolane	1042	$C_6H_{12}O_3$	12.86
5	4.020	1-propanol	primary alcohol	520	C_3H_8O	4.83
6	4.275	Ethanol,2-ethoxy	ether	743,5	$C_4H_{10}O_2$	3.13
7	8.810	4-ethyloctane	alkane	951	$C_{10}H_{22}$	2.08
8	17.168	1,2,3-Propanetriol	polyol	2300	$C_3H_8O_3$	3.57
9	32.164	Guanosine	nucleoside	2700	$C_{10}H_{13}N_5O_5$	3.48

**RI: identification by Kovats indices. Retention index relative to C_9–C_{31}.*

and total flavonoid contents of their n-hexane, chloroform, methanol, and water extracts. According to the results, the phenolic content of *A. morio* extracts was found to be 8.33, 20.83, 11.31, and 11.31 mg GAE/g DW, respectively; *A. pyramidalis* had values of 13.10, 26.19, 9.52, and 18.75 mg GAE/g DW; *N. tridentata* exhibited 13.4, 19.64, 4.46, and 6.55 mg GAE/g DW; *O. mammosa* displayed 6.84, 43.45, 37.20, and 10.71 mg GAE/g DW; *O. lutea* demonstrated 14.58, 35.71, 13.10, and 13.99 mg GAE/g DW, and finally, *O. speculum* had values of 21.73, 45.83, 16.37, and 5.36. Furthermore, the total flavonoid contents varied between 0.82 and 8.64 across the studied orchids. Notably, these values were lower when compared to the methanol extracts of *A. sancta (34)*. In another study by Pathak *et al. (35)* the total phenolic content of the endangered *Vanda cristata* orchid's leaves, roots, and stems was investigated through water, methanol, and petroleum ether extracts. The results indicated that the leaf extracts had values of 209.71, 674.49, and 29.02 mg GAE/g DW for water, methanol, and petroleum ether, respectively. The root extracts showed values of 140.79, 623.8, and 89.72, and the stem extracts had values of 165.23, 640.83, and 19.23 mg GAE/g DW for water, methanol, and petroleum ether, respectively. These findings suggested that the extracts from the above-ground parts of *A. sancta* were only higher in petroleum ether extracts, while the other extracts (water and methanol) showed significantly higher values compared to *A. sancta*. In this study, the use of ethanol aims to evaluate the effectiveness of different solvents and contribute new data to the existing literature. The selection of ethanol is aimed at efficiently extracting specific components of interest, using a safe and practical solvent, and obtaining data that can be compared with previous studies in the literature.

In recent years, GC-MS analysis has been widely used for profiling secondary metabolites in plants and other organisms, alongside HPLC and LC-MS. Analysis and extraction of the material play a significant role in the development, modernization, and quality control of herbal formulations. GC-MS is the most used technique for the identification and quantification of phyto-components. Unknown organic compounds in complex mixtures can be identified through interpretation and by matching their spectra with reference spectra. Further research can lead to the isolation of bioactive compounds, enabling their structural elucidation and screening for pharmacological activity, which will aid in the further development of drugs *(35)*. Hydroxyacetic acid hydrazide, one of the main components of *A. sancta* above-ground parts, is an intermediate in synthesis and drug development and is also a highly effective organic compound on its own *(36)*. From the literature, it is well-known that this compound class possesses various biological and pharmacological properties, such as antiviral *(37)*, anti-inflammatory *(38)*, antimicrobial *(39, 40)* and anticancer activities *(41, 42)*. Previous studies have reported the presence of hydroxyacetic acid hydrazide in ethanol and water extracts of *Calendula officinalis* at a percentage of 4.8% *(43)*, 4% in corn stalks *(34)*, approximately 9% in *Polyalthia bullata (45)*. and 0.23% in *Calotropis procera (46)*. In *A. sancta* above-ground parts, it was found at a percentage of 42.2%. In their Fatahi *et al.* 2023 study *(1)* explored the bioactive properties of extracts from both the tubers and leaves of *Dactylorhiza umbrosa*, a terrestrial orchid. *D. umbrosa* and *A. sancta* exhibit noteworthy differences in their phytochemical and biochemical characteristics. The alcohol extraction and GC-MS analysis conducted on *D. umbrosa* revealed a total of 41 different compounds in its leaf and tuber samples. Among these components, significant chemical compounds such as 4-(4-methyl-1-cyclohexen-1-yl) morpholine, 1,2,3-propanetriol, and N-cyclooct-4-enylacetamide were identified. This comparison highlights the chemical diversity of *D. umbrosa* and emphasizes *A. sancta* as a plant with high antioxidant potential. The distinct characteristics of these two plant species may indicate differences in potential medical and industrial applications *(1)*. However, it is crucial to note that both plants possess unique features that require thorough understanding. Additionally, the analysis of *D. umbrosa* tubers revealed a significantly high glucomannan content of 32.97%, whereas *A. sancta* exhibited a lower amount of glucomannan. Furthermore, in a study by Aytar et al. the GC-MS analysis of *A. coriphora* flower parts identified hydroxyacetic acid, hydrazide (50.74%), cytidine (7.63%), and (Z)-7-hexadecenal (5.42%) as the main components. In the seed part, the primary components were identified as 2,2-dimethoxybutane (27.41%), hydroxyacetic acid, hydrazide (12.42%), and 2,5,6-trimethyldecane (7.31%) *(47)*.

Glycolaldehyde dimers (GA) are the smallest reducing sugars that possess both alcohol and aldehyde functionalities. In its pure form, it exists as a crystalline dimeric form of 2,5-dihydroxy-1,4-dioxane at room temperature *(48)* Upon melting, it partially opens the ring and yields a mixture of dimeric and monomeric forms. In the gas phase, GA is found only in the monomeric form *(49)*. Additionally, due to the highly reactive aldehyde functionality, GA is prone to various side reactions, making its handling somewhat challenging *(50)*.

Compared to ethylene and ethylene oxide (EO, retro-aldol-based GA production from carbohydrates aligns better with the 12 principles of green chemistry. Firstly, this pathway fully exploits the functionality of biomass (atom efficiency). Secondly, it will provide a foundation for a safer process as GA is a non-toxic and non-explosive molecule. Studies on GA metabolism is ongoing *(50)*. Previous studies have reported the presence of glycolaldehyde dimers in methanol extracts of *Euphorbia hirta* at a percentage of 41% *(51)*, 5.03% in Pterocarpus santalinus *(52)*). In our study, this ratio was found to be 10.59%. However, no biological activity studies have been con-

ducted on this compound. GA's production via retro-aldol from carbohydrates aligns better with the principles of Green Chemistry. This approach enhances biomass utilization efficiency, thereby increasing atom economy. Moreover, GA's non-toxic and non-explosive nature establishes a solid foundation for safer processing methods. Additionally, the presence of GA at high levels in plant extracts and emerging data indicating its potential therapeutic uses underscore its significant role in biomedical research. Another major component of *A. sancta*, 1,3-dioxolane-4-methanol,2-ethyl, possesses anti-inflammatory and antimicrobial activities *(53)*. Our study provides the initial data on the phytochemical profile of *A. sancta*, which is a terrestrial orchid, and these findings are reported for the first time in the literature.

CONCLUSION

This study documents the successful cultivation of *A. sancta* through sustainable agricultural practices starting from seeds and encompassing the analysis of tubers and above- ground parts. The identification of significant levels of phenolic and flavonoid compounds underscores *A. sancta* robust antioxidant capacity, crucial in combating oxidative stress-related diseases. The determined IC_{50} value of 47.79 for the methanol extract from aboveground parts indicate its potential antioxidant efficacy and suggests potential therapeutic applications. However, while our findings are promising, further research is essential to isolate and characterize individual phytochemicals to elucidate their specific health benefits and pharmacological effects. Future directions should focus on investigating the underlying mechanisms of *A. sancta* antioxidant effects, conducting comprehensive toxicity studies, and exploring synergistic effects with traditional therapies. Additionally, understanding the ecological role of the plant and its interactions within its habitat could provide valuable insights into its broad ecological and medicinal significance. In conclusion, *A. sancta* emerge as a promising candidate for pharmaceutical and dietary supplement development, leveraging its antioxidant properties to contribute significantly to global holistic health approaches and sustainable agricultural practices. Key components such as hydroxyacetic acid, hydrazide, glycolaldehyde, propane-1,1-dipropoxy, and 1,3-dioxolane-4-methanol,2-ethyl further enhance our understanding of its potential.

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