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PHYTOCHEMICAL PROFILING AND BIOACTIVE POTENTIAL OF HOT AND COLD BARK EXTRACTS OF *TERMINALIA ARJUNA*: A COMPARATIVE QUALITATIVE AND QUANTITATIVE ANALYSIS

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ABSTRACT

The present study aimed to evaluate the phytochemical composition of a medicinal plant using both hot continuous extraction and cold maceration techniques, and to correlate these findings with its pharmacological potential. Preliminary phytochemical screening revealed the presence of carbohydrates, glycosides, phenolic compounds, flavonoids, tannins, proteins, amino acids, fixed oils, and fats in both extracts, with alkaloids and triterpenoids detected specifically in the cold extract. Detailed profiling through Gas Chromatography–Mass Spectrometry (GC-MS) identified 30 bioactive compounds in the hot extract and 36 in the cold extract. Notably, the cold macerated extract contained Arjunolic acid, a triterpenoid known for its potent antioxidant and cardioprotective properties. Despite a lower extraction yield, the cold extract demonstrated a broader phytochemical spectrum. The presence of bioactive constituents such as flavonoids, phenols, tannins, and arjunolic acid suggests that the pharmacological activities of the plant may be attributed to its rich and diverse phytochemical composition. These findings scientifically support and validate the traditional therapeutic claims made in Ayurveda regarding the medicinal value of this plant.

KEYWORDS: Notably, the cold macerated extract contained Arjunolic acid, a triterpenoid known for its potent antioxidant and cardioprotective properties.

INTRODUCTION

India is blessed with a rich and diverse range of flora, which has been integral to traditional medicine systems like Ayurveda, Unani, and Siddha for treating various ailments. Among these medicinal plants is Terminalia arjuna Wight & Arn., commonly known as arjuna, a deciduous and evergreen tree belonging to the Combretaceae family. This tree, which can grow to heights of 60-90 feet, is native to India and also found in Burma, Sri Lanka, and Mauritius. The bark of this plant is smooth and pinkish-grey, flaking off in large, curved, flat pieces. Both the bark and leaves of Terminalia *arjuna* are widely used in traditional medicine. The bark is employed to treat conditions such as angina, and liver disorders and is an expectorant, purgative, laxative, and a remedy for leucoderma, anemia, hyperhidrosis, asthma, tumors, and cardiovascular and renal disorders. (Amalraj et al., 2012).

Traditionally utilized in Indian medicine for various ailments, *T. arjuna* has garnered attention for its diverse pharmacological effects. The bark of the tree contains approximately 38 phytochemicals, including triterpenoids, tannins, flavonoids, and glycosides, which

contribute to its therapeutic efficacy. These compounds exhibit a range of biological activities such as anticancer, antimicrobial, antiviral, anti-inflammatory, antioxidant, hepatoprotective, anti-allergic, anti-diabetic, and wound healing effects. Notably, the plant has demonstrated significant cardioprotective properties, aiding in the management of coronary artery disease, heart failure, and hypertension. *T. arjuna* has several industrial applications mainly of bark, including its use in the pharmaceutical and cosmetic industries due to its bioactive compounds (Kumar *et al.*, 2022).

Despite of the presence of all of the phytoconstituents in the plant, it is essential to select a particular method of extraction for bringing out the optimum pharmacological or any other desirable potential from certain plant material. There are various methods of extraction, including maceration. Soxhlet extraction. and supercritical fluid extraction, having their applications and efficiencies in extracting bioactive compounds. It is importance of selecting appropriate extraction methods to optimize the yield and quality of phytochemicals, considering factors such as solvent type, temperature, and extraction time. environmental and economic aspects

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of these techniques should also be taken into consideration (**Bitwill** *et al.*, 2023).

The current study focuses on comparative phytochemical analysis of stem bark extracts of *Terminalia arjuna* prepared by both the hot continuous method and cold maceration method. Quantitative phytochemical analysis is done by using Gas chromatography and mass spectrometry (GC-MS) for screening the phytoconstituents present in given extracts.

MATERIALS AND METHOD

1. Collection of plant material

The stem bark of *Terminalia arjuna* was collected from the Vidarbha region of Maharashtra, India (Plate 2). For authentication, the plant sample was pressed into a herbarium sheet and verified by expert botanists from the Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University (RTMNU), Nagpur. The specimen was assigned the herbarium sheet number 460, dated 07/06/2024 (Plate 2). Following authentication, the stem bark was shade-dried, ground into a coarse powder, and stored in a clean, dry place until further processing.

2. Preparations of extracts

Two different extraction methods were employed in the current study to prepare 95% ethanolic extracts from the stem bark of *Terminalia arjuna*: hot continuous

extraction using a Soxhlet apparatus and cold maceration method.

A) Hot continuous extraction using Soxhlet's apparatus.

The clean, dry stem bark powder of *Terminalia arjuna* was extracted using two methods.

A) Soxhlet Extraction

Following Mandal et al. (2013), a measured quantity of the powder was defatted with petroleum ether in a Jumbo Soxhlet apparatus at ~80°C. The defatted material was air-dried and extracted with 95% ethanol (95:5 ethanol:distilled water) until the siphon tube solvent appeared clear (Plate 3). The extract was concentrated using a hot water bath (~80°C) and stored in a sterile, pre-weighed Petri dish in an airtight desiccator (Plate 6).

B) Cold Maceration

As per Eggadi et al. (2014), the powder was macerated in 95% ethanol for five days with intermittent shaking (2–3 times daily) (Plate 4). The supernatant was filtered (Whatman No. 1) and concentrated using a rotary evaporator at ~45°C. The final extract was collected and stored similarly (Plate 7).

Extract Yield (%) was calculated using the formula: % Extractability = Weight of extract (gm) X 100 Weight of powder used (gm)

Extraction details

 Table 1: shows the physical properties and extractability percentage of hot extract.

 Table 1: Extractability percentage and physical properties of stem bark extract of *Terminalia arjuna prepared* using hot continuous extraction method.

Sr. no	Content	Extract the characters of the hot continuous extract
1	Solvent used	95% ethanol
2	Quantity of solvent used (ml)	2000 ml
3	Quantity of stem bark powder of <i>Terminalia</i> arjuna used (grams)	250 grams
4	Quantity of prepared extract (grams)	52.29 grams
5	Color of extract	Dark reddish-brown
6	Consistency	Semi-solid
7	Extractability	20.91%

Cold extraction was carried out by mixing coarse *Terminalia arjuna* stem bark powder with 95% ethanol in a conical flask. The mixture was stirred thoroughly to ensure uniform contact between the solvent and plant material, then left to macerate for five days with 2–3 stirrings per day. After maceration, the supernatant was decanted and filtered using Whatman No. 1 filter paper. The filtrate was concentrated at ~45°C using a rotary evaporator to remove the solvent. The extract was collected in a clean, pre-weighed Petri dish and stored in an airtight desiccator. Table 2 summarizes the physical characteristics and extractability percentage of the cold macerated extract.



Plate 1-Photograph showing *Terminalia arjuna* plant.



Plate 3: Photograph showing Soxhlet's apparatus for hot continuous extraction.



Plate 2: Photograph showing authenticated herbarium sheet of *Terminalia arjuna*.



Plate 4: Photograph showing cold macerated extraction.

Table 2: Extractability percentage and physical properties of stem bark extract of *Terminalia arjuna* prepared using cold maceration method.

Sr.no	Content	Extract characters of cold macerated extract.
1	Solvent used	95% ethanol
2	Quantity of solvent used (ml)	2000 ml
3	Quantity of stem bark powder of <i>Terminalia arjuna used</i> (grams)	250 grams
4	Quantity of prepared extract (grams)	27.10
5	Color of extract	Bright reddish-brown
6	Consistency	Semi-solid
7	Extractability	10.84 %

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Plate 5- Photograph showing Rotary evaporator.



Plate 6- Photograph showing hot continuous extract of *Terminalia arjuna* stem bark.

Date - 30/12/2024
Plant: Terminavia arjuna Stembark 95% ethanolic extract: Bottom wit- 109.99m Bottom with 137.0 gm extract met extract wt 27.10 gm
[Cold macerated extract]

Plate 7- Photograph showing cold Macerated extract of *Terminalia arjuna* stem bark.

Qualitative Phytochemical Analysis of the Extracts

To assess the phytochemical profile, the 95% ethanolic extracts of *Terminalia arjuna* (hot and cold methods) were subjected to preliminary qualitative screening, following Tiwari *et al.* (2011) and Kamalla *et al.* (2024).

Specific standard tests were used to identify major phytochemical groups. The results are summarized in Tables 3 and 4 for the hot continuous and cold macerated extracts, respectively.

 Table 3. - Qualitative phytochemical analysis of stem bark of *Terminalia arjuna* prepared by the hot continuous extraction method.

Sr. no	Active principle	Test performed	Observation	Results
		Mayer's test	No white or creamy precipitate was formed	Negative
1	Alkaloids	Wagner's test No Reddish-brown precipitate was formed		Negative
		Hager's test	The absence of observable yellow precipitate	Negative
		Drangendroff's test	No Yellow precipitate formation	Negative
		Fehling's test Occurrence of red precipitate P		Positive
2	Carbohydrates	Barfoed's test	Formation of red precipitate	Positive

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		Benedict's test	The presence of white precipitate	Positive
		Borntrager's test	Formation of pink color	Positive
3	Glycosides	Keller-Killiani test	Reddish brown color formation at the interface of two liquids	Positive
		Legal's test	Formation of pink color	Positive
4	Saponins	Foam test	Mild foam developed	Positive
5	Proteins and amino acids	Biuret test	Formation of pink color at the methanolic layer	Positive
		Xanthoprotein test	White, yellow precipitate formation	Positive
		Ninhydrin test	rin test Purple color formation	
6	Phytosterols	Salkowski's test	No formation of red color in the chloroform layer, and a greenish yellow color at the other layer	Negative
		Lead acetate test	Formation of bulky white precipitate	Positive
7	Phenolic compounds and tannins	Ferric chloride test	Formation of dark green color	Positive
		Alkaline reagent test	Formation of yellow fluorescence	Positive
8	Fixed oils and fats	Spot test	The presence of oil stains on the paper	Positive
		Saponification test	Partial neutralization of alkali	Positive
9	Resins	Test for resins	No appearance of turbidity	Negative
10	Flavonoids	Shinoda test	Mild formation of a dark pink color precipitate	Positive
11	Triterpenoids	Liebermann Burchard's test	No Formation of the brown ring at the interface	Negative
12	Gum and mucilage	Test for gum and mucilage	No cloudy precipitate was formed	Negative

Table 4: Qualitative phytochemical analysis of stem bark of Terminalia arjuna prepared	d by the cold maceration
method.	

Sr. no	Active principle	Test performed	Observation	Results
		Mayer's test	A white or creamy precipitate was formed	Positive
1	Alkaloids	Wagner's test	A reddish-brown precipitate was formed	Positive
		Hager's test	The Presence of observable yellow precipitate	Positive
		Drangendroff's test	Yellow precipitate formation	Positive
		Fehling's test	Occurrence of red precipitate	Positive
2	Carbohydrates	Barfoed's test	Formation of red precipitate	Positive
		Benedict's test	The presence of white precipitate	Positive
		Borntrager's test	Formation of pink color	Positive
3	Glycosides	Keller-Killiani test	Reddish brown color formation at the interface of two liquids	Positive
		Legal's test	Formation of pink color	Positive
4	Saponins	Foam test	Foam developed	Positive
5	Proteins and amino acids	Biuret test	No formation of pink color at the methanolic layer	Negative
		Xanthoprotein test	No white, yellow precipitate formation	Negative
		Ninhydrin test	No purple color formation	Negative
6	Phytosterols	Salkowski's test	Formation of red color in the chloroform layer and a greenish	Positive

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			yellow color at the other layer	
		Lead acetate test	Formation of bulky white precipitate	Positive
7	Phenolic compounds and tannins	Ferric chloride test	Formation of dark green color	Positive
		Alkaline reagent test	nt test Formation of yellow fluorescence	
8	Fixed oils and fats	Spot test	The presence of oil stains on the paper	Positive
		Saponification test	Partial neutralization of alkali	Positive
9	Resins	Test for resins	No appearance of turbidity	Negative
10	Flavonoids	Shinoda test	Formation of a dark pink color precipitate	Positive
11	Triterpenoids	Liebermann Burchard's test	Formation of the brown ring at the interface	Positive
12	Gum and mucilage	Test for gum and mucilage	No cloudy precipitate was formed	Negative

Quantitative phytochemical analysis using GC-MS GC-MS analysis of *Terminalia arjuna* stem bark extracts were carried out using a SHIMADZU GCMS-QP2020 system with a 30 m \times 250 μ m \times 0.25 μ m capillary column (5% phenyl methyl siloxane). The temperature was programmed from 40°C to 280°C with controlled ramping, and 4 μ L of sample was injected at a split ratio

of 25:0. MS conditions included a source temperature of 230°C and a quadrupole temperature of 150°C, with a 3minute solvent delay. Compounds were identified based on retention times and mass spectra using the NIST library. Tables 5 and 6, and Figures 7 and 8, present the GC-MS results.

 Table 5 - Quantitative phytochemical analysis of stem bark extract of *Terminalia arjuna* prepared by the hot continuous extraction using Soxhlet's apparatus.

Sr.No.	R.Time	Compound identified	Formula	Mol. Wt.	Area	Area%	Height
1	3.371	Silane, methyl	CH ₆ Si	46.15	13962398	0.89	6619710
2	3.780	2,3-Butanediol	$C_4H_{10}O_2$	90.12	35011274	2.24	12190419
3	4.083	Pentane-1,2,3,4,5-pentaol	$C_{5}H_{12}O_{5}$	152.15	1130479263	72.40	121778561
4	4.132	Propane, 1-(1-ethoxyethoxy)-	$C_7 H_{16} O_2$	132.20	131829311	8.44	55271622
5	4.332	2-Butanol, 3-methyl-, acetate	$C_7 H_{14} O_2$	130.18	1143628	0.07	673088
6	4.872	Butane, 1,1-diethoxy-	$C_8H_{18}O_2$	146.23	5229098	0.33	2017035
7	5.428	1,2,3-Butanetriol	$C_4H_{10}O_3$	106.12	1170513	0.07	510420
8	5.758	2-Butanone, 4-hydroxy-3-methyl-	$C_5H_{10}O_2$	102.13	24424643	1.56	11327714
9	6.125	Dimethyl sulfone	$C_2H_6O_2S$	94.13	4756609	0.30	1982333
10	6.175	(3-Methyl-oxiran-2-yl)-methanol	$C_4H_8O_2$	88.11	2973911	0.19	1433445
11	6.255	3-Nitropropanoic acid	C ₃ H ₅ NO ₄	119.08	1300595	0.08	495238
12	6.440	Ethanol, 2,2-diethoxy-	$C_6H_{14}O_3$	134.17	3112022	0.20	1257132
13	6.741	(3-Methyl-oxiran-2-yl)-methanol	$C_4H_8O_2$	88.11	1634974	0.10	944010
14	6.893	1-Pentanol, 2,3-dimethyl-	C ₇ H ₁₆ O	116.20	1728401	0.11	689984
15	7.036	Acetic acid, methyl ester	$C_3H_6O_2$	74.08	6046881	0.39	2531685
16	7.088	2(3H)-Furanone, dihydro-5- methyl-	$C_5H_8O_2$	100.12	3096682	0.20	1288942
17	8.236	(3-Methyl-oxiran-2-yl)-methanol	$C_4H_8O_2$	88.11	29873382	1.91	14047619
18	8.357	Acetic acid, butyl ester	$C_{6}H_{12}O_{2}$	116.16	2839559	0.18	1303708
19	8.409	1-Pentanethiol	$C_5H_{12}S$	104.22	11641988	0.75	4803033
20	8.809	Heptanal	$C_7H_{14}O$	114.19	2095740	0.13	494835
21	9.095	Butane, 1,1-diethoxy-3-methyl-	$C_6H_{20}O_2$	160.25	1433913	0.09	677677
22	9.370	2H-Pyran-2-one, tetrahydro-6- methyl-	$C_{6}H_{10}O_{2}$	114.14	4739165	0.30	2053469
23	9.679	Glyceraldehyde	$C_3H_6O_3$	90.08	4211077	0.27	1090912
24	10.479	Propane, 1,1,3-triethoxy-	$C_9H_{20}O_3$	176.25	2762279	0.18	1266924
25	10.752	1-Cyclohexyl-2-nitro-3- (tetrahydropyran-2-yloxy	C ₁₁ H ₁₉ NO ₄	229.27	1255266	0.08	462665
26	11.408	Glycerine	C ₃ H ₈ O ₃	92.09	12541904	0.80	5220537
27	14.739	[5-Hydroxymethyl)-1,3-dioxolan-	$C_5H_{10}O_4$	134.13	2377096	0.15	1055562

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		4-yl] methanol					
28	19.610	Octadecane	C ₁₈ H ₃₈	254.50	1278135	0.08	593513
29	24.520	octadecanoic acid	$C_{18}H_{36}O_2$	284.48	1770199	0.11	702003
30	28.936	Eicosane	$C_{20}H_{42}$	282.55	1097560	0.07	422206

Table 6: Quantitative phytochemical analysis of stem bark extract of *Terminalia arjuna* prepared by the cold maceration method.

S.No.	R.Time	Compound identified	Formula	Mol. Wt.	Area	Area%	Height
1	3.369	Silane, methyl	CH ₆ Si	46.15	16461218	1.06	7446996
2	3.605	2,3-Butanediol	$C_4H_{10}O_2$	90.12	24730892	1.60	11944501
3	3.777	2,3-Butanediol	$C_4H_{10}O_2$	90.12	34686203	2.24	12134635
4	4.083	Pentane-1,2,3,4,5-pentaol	$C_5H_{10}O_5$	152.15	1097012050	70.96	104663072
5	4.134	2,3,23-trihydroxyolean- 12-en-28-oic acid	C ₃₀ H ₃₈ O ₅	488.70	133560242	8.64	57743133
6	4.873	Butane, 1,1-diethoxy-	$C_8H_{18}O_2$	146.23	5012171	0.32	1990083
7	5.124	Butane, 1-(1- ethoxyethoxy)-	$C_8H_{18}O_3$	162.23	32044719	2.07	17256505
8	5.286	1-Butanol, 3-methyl-, acetate	$C_7 H_{14} 0_2$	130.19	8011993	0.52	4174971
9	5.760	2-Butanone, 4-hydroxy-3- methyl-	$C_5H_{10}O_2$	102.13	24194733	1.57	11309232
10	5.823	Pentanoic acid, ethyl ester	$C_7 H_{14} O_2$	130.19	2368772	0.15	1136965
11	6.063	5H-1,4-Dioxepin, 2,3- dihydro-	$C_5H_8O_2$	100.12	1481006	0.10	407783
12	6.127	Dimethyl sulfone	$C_2H_6O_2S$	94.13	4013815	0.26	1955242
13	6.175	(3-Methyl-oxiran-2-yl)- methanol	$C_4H_8O_2$	88.11	4686831	0.30	2150329
14	6.441	Ethanol, 2,2-diethoxy-	$C_6H_{14}O_3$	134.17	2944774	0.19	1153680
15	6.793	Formic acid, ethenyl ester	$C_3H_4O_2$	72.06	1329273	0.09	520806
16	6.894	1-Pentanol, 2,3-dimethyl	C ₇ H ₁₆ O	116.21	1748300	0.11	697065
17	7.036	Acetic acid, butyl ester	$C_{6}H_{12}O_{2}$	116.16	6341511	0.41	2598199
18	7.090	2(3H)-Furanone, dihydro- 5-methyl-	$C_{5}H_{8}O_{2}$	100.12	2617003	0.17	1148144
19	7.276	Hexanal	$C_6H_{12}O$	100.16	1035851	0.07	414156
20	8.238	(3-Methyl-oxiran-2-yl)- methanol			39440148	2.55	18934889
21	8.360	Acetic acid, methyl ester	$C_3H_6O_2$	74.08	2659508	0.17	1194983
22	8.412	1-Pentanethiol	$C_5H_{12}S$	104.21	11391641	0.74	4465487
23	8.809	Heptanal	C ₇ H ₁₄ O	114.19	1465376	0.09	366241
24	9.096	Butane, 1,1-diethoxy-3- methyl-	$C_9H_{20}O_2$	160.25	1282160	0.08	674948
25	9.371	2(3H)-Furanone, dihydro- 4,5-dimethyl-	$C_{6}H_{10}O_{2}$	114.14	4133765	0.27	1781873
26	9.684	2-Butene ozonide	$C_4H_8O_3$	104.10	2070425	0.13	979475
27	9.716	2-Hexenal, (E)-	$C_{6}H_{10}O$	98.14	1519354	0.10	803814
28	10.481	Propane, 1,1,3-triethoxy-	$C_9H_{20}O_3$	176.25	2247999	0.15	957443
29	10.755	1-Cyclohexyl-2-nitro-3- (tetrahydropyran-2-yloxy) propan-1-o	$C_{14}H_{23}NO_5$	285.34	980157	0.06	407791
30	11.220	Propane, 1,1-diethoxy-2- methyl-	$C_7H_{16}O_2$	132.20	2362008	0.15	1053432
31	11.411	Glyceraldehyde	$C_3H_6O_3$	90.08	10385314	0.67	4564349
32	14.741	3,3-Diethoxy-1-propanol	C ₇ H ₁₆ O ₃	148.20	1933358	0.13	857031
33	14.801	[5-Hydroxymethyl)-1,3- dioxolan-4-yl] methanol	$C_5H_{10}O_4$	134.13	10345646	0.67	4003878
34	19.612	octadecanoic acid	$C_{18}H_{36}O_2$	284.48	1233653	0.08	473345
35	24.518	Heneicosane	C ₂₁ H ₄₄	296.58	1781263	0.12	647734
36	28.943	Eicosane	$C_{20}H_{42}$	282.55	1134783	0.07	448323

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RESULTS AND DISCUSSION

Phytochemical analysis revealed notable differences in compound composition based on the extraction method. Alkaloids, phytosterols, and triterpenoids were absent in the hot continuous extract but present in the cold macerated extract, indicating their thermolabile nature or better solubility at lower temperatures. In contrast, proteins and amino acids were detected only in the hot extract, suggesting that heat aids in their release by breaking cellular structures. These results underscore the impact of extraction temperature on phytochemical stability and highlight the importance of selecting appropriate methods to optimize the recovery of specific bioactive compounds. (Aneja *et al.*, 2012).

Gas Chromatography-Mass Spectrometry (GC-MS) is a key analytical technique for precise identification and quantification of bioactive phytoconstituents in plant materials. It is widely used to validate the presence of compounds responsible for pharmacological activity, even at low concentrations. GC-MS is essential in both qualitative and quantitative analysis, compound purification, and determination of thermochemical properties.(**Al-Rubaye** *et al.*, **2017**).

Gas Chromatography – Mass Spectrometry (GC-MS) analysis of *Terminalia arjuna* stem, bark extracts identified a diverse array of phytoconstituents, including triterpenoids, glycoside derivatives, aglycones, and polyphenolic compounds. This indicates that the plant has a significant phytochemical profile for various phytochemical activities (Uthirapathy *et al.*, 2019).

Elsherbiny *et al.*, (2016), reported Arjunolic acid (2,3,23-trihydroxyolean-12-en-28-oic acid) is a chiral pentacyclic triterpenoid saponin naturally found in the bark of *Terminalia arjuna*, known for its broad pharmacological properties and protective effects against drug- and chemical-induced toxicities. In the present study, GC-MS analysis of the cold macerated extract confirmed the presence of Arjunolic acid, supporting previous reports of its bioactive potential.

SUMMARY AND CONCLUSION

Phytochemical screening showed that the hot extract contained carbohydrates, glycosides, phenolics, flavonoids, tannins, proteins, amino acids, fixed oils, and fats. The cold extract included these along with alkaloids and triterpenoids. GC-MS analysis identified 30 compounds in the hot extract and 36 in the cold extract, including key bioactives like Arjunolic acid, flavonoids, phenols, and triterpenoids. These are known for antioxidant, anti-inflammatory, and cardioprotective effects, indicating that the diverse phytochemical profile—especially in the cold extract—may contribute significantly to the plant's pharmacological potential.

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