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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:

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 anti-cancer, 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 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

Terminalia arjuna Stem bark was collected from the Vidarbha region of Maharashtra, India (Plate 1). For authentication purposes, the collected plant sample was compressed into a herbarium sheet and authenticated by the expert botanists from the Department of Botany Science, Rashtrasant Tukdoji Maharaj Nagpur University (RTMNU), Nagpur, with given herbarium sheet number 460 (07/06/2024) (Plate 2). The stem bark is then shed, dried, and ground into coarse powder and stored at a clean and dry place before 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 properly stored, clean, and dry stem bark powder of *Terminalia arjuna* was subjected to extraction following the method outlined by **Mandal *et al*., (2013).** Initially, a precisely weighed quantity of the powdered material underwent defatting with petroleum ether using a Jumbo Soxhlet apparatus at approximately 80°C. The defatted residue was subsequently air-dried and extracted with 95% ethanol (comprising 95 parts ethanol and 5 parts distilled water) to obtain the targeted phytochemical constituents. The extraction process continued in the Soxhlet apparatus until the solvent passing through the siphon tube appeared clear, indicating exhaustive extraction (Plate 3). The obtained extract was then concentrated via solvent evaporation using a hot water bath at approximately 80°C. The resulting dark reddish-brown extract was collected in a clean, sterile, pre-weighed Petri dish and stored in an airtight desiccator for further analysis and use (plate 6).

B) Cold maceration extraction

For the preparation of the cold macerated extract, the properly stored, clean, and dry stem bark powder of *Terminalia arjuna* was subjected to extraction following the protocol described by **Eggadi *et al*., (2014)**. A precisely weighed quantity of the plant material was placed in a conical flask containing 95% ethanol (comprising 95 parts ethanol and 5 parts distilled water) and allowed to macerate for five days with at least 2 to 3 mixings per day (Plate 4). After the maceration period, the supernatant was carefully decanted and filtered through Whatman No. 1 filter paper. The obtained filtrate was then concentrated using a rotary evaporator at approximately 45°C to remove the solvent. The resulting bright reddish-brown extract was collected in a clean, sterile, pre-weighed Petri dish and stored in an airtight desiccator for further analysis and application (Plate 7).

The percent extractability of the extracts was determined with the following formula-

$$\% \text{ Extractability} = \frac{\text{Weight of extract (gm)} \quad X\ 100}{\text{Weight of powder used (gm)}}$$

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 *Terminalia 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%

On the other hand, cold extract was prepared by adding coarse stem bark powder in a conical flask containing 95% ethanol, and the mixture was stirred to ensure the proper mixing of plant material and solvent. The plant material was allowed to macerate for five days with at least 2 to 3 stirrings in 24 hours. After the maceration period, the supernatant was carefully decanted and filtered through Whatman No. 1 filter paper. The obtained filtrate was then concentrated using a rotary evaporator at approximately 45°C to remove the solvent. Table 2 shows the physical properties and extractability percentage of the Cold macerated extract.

Plate 1-Photograph showing *Terminalia arjuna* plant

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

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

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 *Terminalia arjuna used* (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 %

Plate 5- Photograph showing Rotary evaporator

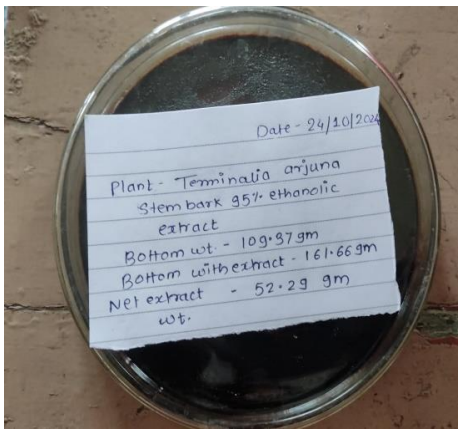

Plate 6- Photograph showing hot continuous extract of *Terminalia arjuna* stem bark

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

Qualitative Phytochemical Analysis of the Extracts:
To evaluate the phytochemical composition, the 95% ethanolic extracts of *Terminalia arjuna* were subjected to a preliminary qualitative phytochemical screening following the methodology described by **Tiwari *et al*., (2011) and Kamalla *et al*., (2024).** A preliminary qualitative phytochemical analysis of 95% ethanolic extracts of stem bark of *Terminalia arjuna prepared* by hot continuous and cold macerated methods was carried out using specific phytochemical tests. Table 3 and Table 4 show the presence and absence of various phytochemicals present in 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	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	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	Purple color formation	Positive
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 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 yellow color at the other layer	Positive
		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	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

The GC-MS analysis of stem bark extracts of *Terminalia arjuna* was conducted using a SHIMADZU GCMS-QP2020 equipped with a quadrupole 2020 MS detector. The capillary column used was GCMS-QP2020 (30 m × 250 µm × 0.25 µm), composed of 5% phenyl methyl siloxane. The initial oven temperature was set at 40ºC for 1 minute, then increased at a rate of 20ºC per minute up to 150ºC, where it was held for 1 minute. Subsequently, the temperature was raised at a rate of 3ºC per minute until it reached 280ºC, where it was maintained for 10 minutes. The injector volume was 4 µL. Carrier gas was used at a constant flow rate with a split ratio of 25:0.

The MS operating conditions were as follows: the source temperature was set at 230ºC (maximum 250ºC), the quadrupole temperature at 150ºC (maximum 200ºC), and a solvent delay time of 3 minutes was applied. The identification of compounds was performed based on their retention time (RT) values and mass spectra, which were compared with those from the NIST search library. Further detailed information on the identified compounds was obtained through additional searches.

Tables 5 and 6 show the phytochemicals found in GC-MS analysis, along with their chemical formula, molecular weight, retention time, peak height, and area percentage (%) for hot continuous extract and cold macerated extract, respectively. Figures 7 and 8 show the chromatograms obtained from GC-MS analysis of hot continuous extract and cold macerated extract, respectively.

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_6Si	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	$C_5H_{12}O_5$	152.15	1130479263	72.40	121778561
4	4.132	Propane, 1-(1-ethoxyethoxy)-	$C_7H_{16}O_2$	132.20	131829311	8.44	55271622
5	4.332	2-Butanol, 3-methyl-, acetate	$C_7H_{14}O_2$	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	$C_3H_5NO_4$	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-	$C_7H_{16}O$	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	$C_6H_{12}O_2$	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-	$C_6H_{10}O_2$	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	$C_3H_8O_3$	92.09	12541904	0.80	5220537
27	14.739	[5-Hydroxymethyl)-1,3-dioxolan-4-yl] methanol	$C_5H_{10}O_4$	134.13	2377096	0.15	1055562
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_6Si	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	$C_7H_{14}0_2$	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	$C_7H_{14}O_2$	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	$C_7H_{16}O$	116.21	1748300	0.11	697065
17	7.036	Acetic acid, butyl ester	$C_6H_{12}O_2$	116.16	6341511	0.41	2598199
18	7.090	2(3H)-Furanone, dihydro-5-methyl-	$C_5H_80_2$	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	$C_7H_{14}O$	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-	$C_6H_{10}O_2$	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)-	$C_6H_{10}O$	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	$C_7H_{16}O_3$	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

RESULTS AND DISCUSSION

The phytochemical analysis reveals distinct variations in the composition of bioactive compounds based on the extraction method employed. Alkaloids, phytosterols, and triterpenoids were absent *T. arjuna* in the extract obtained through the hot continuous extraction method but were detected in the cold macerated extract, suggesting that these compounds may be thermolabile or more efficiently extracted at lower temperatures. Conversely, proteins and amino acids were absent in the preliminary phytochemical analysis of cold-macerated extract but were present in the extract obtained by hot continuous method. This observation indicates that elevated temperatures may facilitate the denaturation of cellular structures, thereby enhancing the release of proteinaceous components. These findings highlight the influence of extraction temperature on the solubility and stability of different classes of phytochemicals, emphasizing the need for method optimization based on targeted bioactive constituents to maximize the yield and preserve the structural integrity of temperature-sensitive compounds **(Aneja *et al*., 2012)**.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis is one of the major phytochemical profiling techniques, which enables the precise identification and

quantitative analysis of the bioactive phytoconstituents present in plant material. GC-MS is a widely used analytical method, which validates the presence of bioactive components within the plant materials, which are potentially responsible for the pharmacological activity of the plants. Gas chromatography can accurately measure even smaller concentrations of phytochemicals present in plant extract. It is a very essential quantification tool in chemistry. It is extensively utilized in qualitative and quantitative analysis of mixtures, purification of compounds, and the 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 presence of Arjunolic acid (2,3,23-trihydroxyolean-12-en-28-oic acid) which is a chiral pentacyclic triterpenoid saponin present naturally in the bark of the plant *Terminalia arjuna* and shown to posses' various biological activities and also possesses ameliorating effects against various drugs and chemical toxicities.

The GC-MS analysis of cold macerated extract of *T. arjuna* bark in present study also demonstrated presence of Arjunolic acid (2,3,23-trihydroxyolean-12-en-28-oic acid) which is reported earlier for demonstration of various pharmacological activities.

SUMMARY AND CONCLUSION

The phytochemical analysis revealed that the hot extract contained carbohydrates, glycosides, phenolic compounds, flavonoids, tannins, proteins, amino acids, fixed oils, and fats, while the cold extract showed the presence of alkaloids, glycosides, phenols, flavonoids, triterpenoids, tannins, carbohydrates, proteins, amino acids, fixed oils, and fats. GC-MS analysis identified 30 compounds in the hot extract and 36 in the cold extract, with the latter showing the presence of key bioactive constituents like Arjunolic acid, flavonoids, phenols, and triterpenoids. These compounds are known for their antioxidant, anti-inflammatory, and cardioprotective properties, suggesting that the rich and diverse phytochemical profile—particularly in the cold extract—may be a major contributing factor to the pharmacological activity of the plant.

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