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TERMINALIA ARJUNA: POSSIBLE TREATMENT FOR SICKLE CELL ANAEMIA & POTENT BIOFUEL A REVIEW ARTICLE

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Keywords:

Terminalia arjuna, Leaf galls, Sickle cell anaemia, *Triozafletcheri*, HMF biosynthesis, Galled leaves

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ABSTRACT: The review "*Terminalia arjuna*: The possible treatment for sickle cell anaemia & potent biofuel" explores various studies on the *Terminalia arjuna* plant, emphasising its potential therapeutic applications for sickle cell anaemia (SCA) and various cardiovascular disorders. Key findings highlight the anti-sickling properties of 5-Hydroxymethylfurfural, a primary bioactive compound found in leaf galls formed in response to insect attacks from *Trioza fletcheri*. Research indicates that extracts from *Terminalia arjuna* have significant anti-sickling effects; however, further clinical studies and safety evaluations are needed to confirm their therapeutic efficacy and standardisation. Additionally, the review discusses the traditional use of Arjuna bark decoction in the Indian subcontinent for managing angina, hypertension, and heart failure. Recent findings reveal its anti-ischemic and antioxidant properties due to beneficial phytoconstituents like triterpenoids and flavonoids. While promising for ischemic cardiomyopathy, the long-term safety of *Terminalia arjuna* requires further investigation, particularly in the context of coronary prevention.

INTRODUCTION: *Terminalia arjuna*, commonly known as Arjuna, is a medicinal plant renowned for its extensive health benefits. Traditional medicine utilises its bark, leaves, and fruits to address various ailments, particularly cardiovascular issues. Healers in India often employ powdered bark as a remedy for conditions like heart failure, cardiomyopathy, and atherosclerosis. The stem bark is abundant in glycosides, flavonoids, and tannins, with flavonoids recognised for their antioxidant, anti-inflammatory, and lipid-lowering effects.

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In addition to its cardiac applications, Arjuna is beneficial for treating anaemia, ulcers, and liver diseases. Research has highlighted its promising properties, including antimicrobial, antitumor, antioxidant, and anti-HIV effects. However, Arjuna faces threats from the hemipteran insect *Trioza fletcheri* minor, which induces gall formation on its leaves, offering the insects food and protection. This gall induction triggers significant changes in the plant, leading to an increase in bioactive compounds that serve as a defence against stressors, including herbivores and pathogens.

In a study using gas chromatography-mass spectrometry (GC-MS), methanolic extracts of both healthy leaves and leaf galls were analysed. The leaf extract contained 21 compounds, while the gall extract revealed 57, with 5-Hydroxymethyl Furfural (HMF) being the dominant compound in the galls. HMF, formed from carbohydrates under

specific conditions, shows promise as an anti-sickling agent for treating severe sickle cell disease.

Results & Interpretation:

GC-MS Analysis: Gas chromatography-mass spectrometry (GC-MS) is an analytical method that combines the features of gas chromatography and mass spectrometry to identify different substances within a test sample. MS is a wide-ranging analytical technique that identifies the charged species according to their mass-to-charge ratio (M/Z). GC-MS is one of the best techniques to identify the constituents of volatile compounds. The GC-MS analysis of *T. arjuna* normal leaf showed the presence of twenty-one compounds, and the galled leaf showed the presence of fifty-seven compounds.

The identification of the phytochemical compounds was confirmed based on their peak areas, retention times, and molecular formulas. The active principles, along with their retention times (RT), area percentages, and names of the compounds present in the methanolic extracts of healthy and galled leaves of *Terminalia arjuna*, are presented in the **Fig. 1** and **2**, and **Table 1** and **2**, respectively.

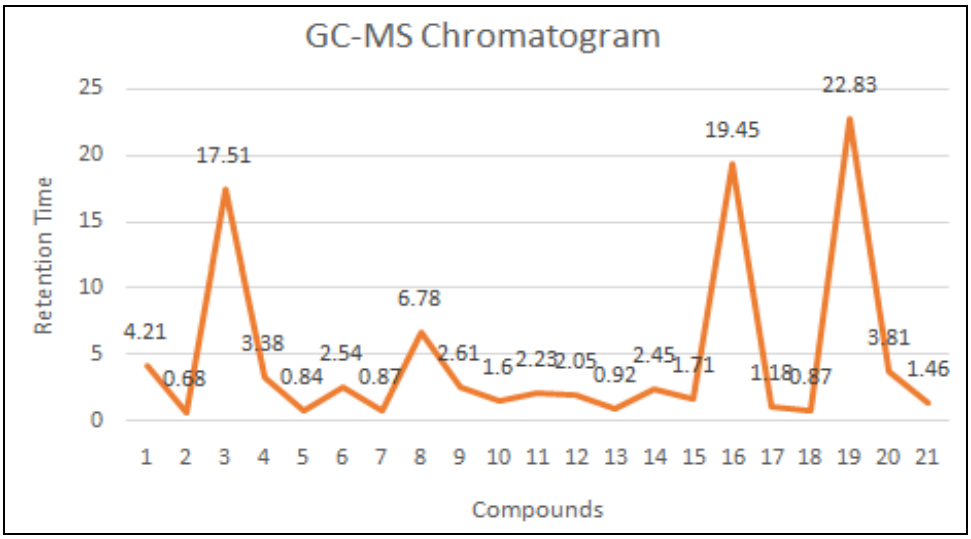

FIG. 1: SHOWS THE GC-MS CHROMATOGRAM OF THE METHANOLIC EXTRACT OF THE NORMAL HEALTHY LEAVES OF *TERMINALIA ARJUNA*

TABLE 1: LIST OF BIOACTIVE COMPOUNDS IDENTIFIED FROM THE METHANOLIC EXTRACT OF THE NORMAL LEAF OF *TERMINALIA ARJUNA* USING GC-MS ANALYSIS

Peak	Retention Time(min)	Area%	Name of the Compound
1	6.165	4.21	(3S)-(-)-3-Acetamidopyrrolidine
2	8.225	0.68	.beta.-D-Glucopyranose, 1,6-anhydro-
3	9.548	17.51	3,6-DIMETHYL-3-OCTENE-2,7-DIONE
4	10.158	3.38	1,5,5-Trimethyl-6-[3-acetoxybutyl]-3,6-epidioxycyclohexene
5	10.275	0.84	cis-Z-.alpha.-Bisabolene epoxide
6	10.416	2.54	1,2,3-Propanetricarboxylic Acid, 2-Hydroxy-,
7	10.867	0.87	3-BUTEN-2-OL, 4-(2,6,6-Trimethyl-1-Cyclohexen-
8	11.121	6.78	Tetradecanoic Acid
9	11.227	2.61	2(4h)-Benzofuranone, 5,6,7,7a-Tetrahydro-6-H
10	12.248	1.60	2,6,10-Trimethyl,14-Ethylene-14-Pentadecne
11	12.625	2.23	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
12	12.948	2.05	Cyclopropanenonaoic acid, 2-[(2-butylcyclopropyl)methyl]-
13	13.204	0.92	Pentadecanoic acid, 14-methyl-, methyl ester
14	13.393	2.45	n-Hexadecanoic acid
15	13.854	1.71	9,12,15-Octadecatrienoic acid, methyl ester
16	14.276	19.45	Octadecanoic Acid
17	15.579	1.18	9,12,15-Octadecatrienoic acid
18	15.766	0.87	Octadecanoic acid
19	15.999	22.83	Di-n-octyl phthalate
20	16.149	3.81	Methyl ester
21	20.221	1.46	Methyl Ester

TABLE 2: ACTIVITY OF A FEW PHYTOCHEMICALS IDENTIFIED IN THE METHANOLIC LEAF EXTRACT OF *T. ARJUNA*

S. no.	Name of the Compound	Molecular Formula	Molecular Weight	Biological Activity
1.	beta-D-Glucopyranose, 1,6-anhydro-	$C_6H_{10}O_5$	162	Used as a chemical tracer for biomass burning in atmospheric chemistry studies, esp. airborne particulate matter, and as a marker for coal combustion.
2.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	Antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, flavour, hemolytic, 5-alpha-reductase inhibitor.
3.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	Antimicrobial, anti-inflammatory.
4.	Octadecanoic acid	$C_{18}H_{36}O_2$	284	Anti-inflammatory and antiarthritic.
5.	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	390	Used as a Plasticiser.
6.	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278	Anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, and antihistaminic.
7.	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270	Antioxidant.

DISCUSSION: The gas chromatography-mass spectrometry (GC-MS) analysis of the methanolic extract derived from the leaves of *Terminalia arjuna* revealed the presence of 21 distinct bioactive compounds, as illustrated in **Fig. 1**. In contrast, the methanolic extract obtained from the galls of *Terminalia arjuna* exhibited a more complex profile, displaying 57 peaks and thus indicating the presence of 57 phytochemicals, as shown in **Fig. 2.** The analysis reveals that the methanolic extract of the normal leaf contains a smaller variety of compounds compared to the leaf gall extract. Among the compounds identified in the leaf gall, the most predominant was 5-(hydroxymethyl)-2-furancarboxaldehyde, which accounted for 46.14% of the total peak area. This compound, widely recognised as 5-Hydroxymethylfurfural, is notable for its diverse applications, particularly as a potent anti-sickling agent in the treatment of sickle cell disease.

TABLE 3: LIST OF BIO-ACTIVE COMPOUNDS IDENTIFIED FROM THE METHANOLIC EXTRACT OF THE GALLED LEAF OF *TERMINALIA ARJUNA* USING GC-MS ANALYSIS

Peak	Retention time (min)	Area %	Name of the compound
1	6.030	46.14	2-Furancarboxaldehyde, 5-(hydroxymethyl)-
2	7.199	1.20	1-[N]N-Methylpiperazine]ethanol
3	8.247	0.67	1-Isopropoxy-2,2,3-trimethylaziridine (sin)
4	9.106	0.57	2-Cyclohexen-1-one, 2-hydroxy-3-methyl-6-(1-methyl)-
5	9.903	0.65	-
6	10.012	0.38	N,N-Bis(2-Hydroxyethyl) Dodecanamide
7	10.419	0.07	1,2-Benzenedicarboxylic Acid, Diethyl Ester
8	11.120	0.11	1,2,3-Propanetricarboxylic Acid, 2-Hydroxy-,
9	11.254	0.09	Tricyclo[5.1.0.0(2,4)]Octane-5-Carboxylic Acid, 3,3,8,8-Tetram
10	11.750	0.44	1-Oxetan-2-One, 4,4-Diethyl-3-Methylene-
11	12.253	1.10	Tetradecanoic Acid
12	12.448	0.23	1-Heptadecene
13	12.678	0.29	2,3-Dioxabicyclo[2.2.2]Oct-5-Ene, 1-Methyl-4-(1-Methylethyl)
14	12.814	0.08	2-Cyclohexen-1-One, 4-Hydroxy-3,5,5-Trimethyl-4-(3-Oxo-1-Bu
15	12.948	0.61	2,6,10-Trimethyl,14-Ethylene-14-Pentadecne
16	13.202	0.17	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol
17	13.269	0.09	Pentadecanoic Acid
18	13.393	0.52	3,7,11,15-Tetramethyl-2-Hexadecen-1-Ol
19	13.731	0.08	6,6,7-Trimethyl-Octane-2,5-Dione
20	13.845	0.76	Hexadecanoic Acid, Methyl Ester
21	14.118	0.46	9-Hexadecenoic Acid
22	14.347	13.15	N-Hexadecanoic Acid
23	14.478	0.29	1-Nonadecene
24	14.821	0.07	Eicosanoic Acid, Methyl Ester

25	15.231	0.23	Heptadecanoic acid
26	15.394	0.15	1-Hexadecanol
27	15.499	1.06	9,12-Octadecadienoic acid, methyl ester, (E,E)
28	15.678	0.11	Phytol
29	15.759	0.49	Octadecanoic acid, methyl ester
30	16.018	14.73	Octadecenoic acid, (Z)-
31	16.203	7.02	Octadecanoic acid
32	17.211	0.09	2,5-Methano-1h-Inden-7(4h)-One, Hexahydro-
33	17.316	0.15	1-Octadecanethiol
34	17.551	0.23	Eicosanoic Acid, Methyl Ester[1]
35[2]	17.973	1.05	Eicosanoic Acid
36	19.067	0.08	Nonadecanoic Acid
37	19.354	0.16	Octadecyl Trifluoroacetate
38	19.806	0.31	Docosanoic Acid, Methyl Ester
39	20.219	0.85	Di-N-Octyl Phthalate
40	20.428	0.37	Docosanoic Acid
41	22.990	0.09	Tetracosanoic Acid, Methyl Ester
42	23.426	0.05	1-Hexadecanol, 2-methyl-

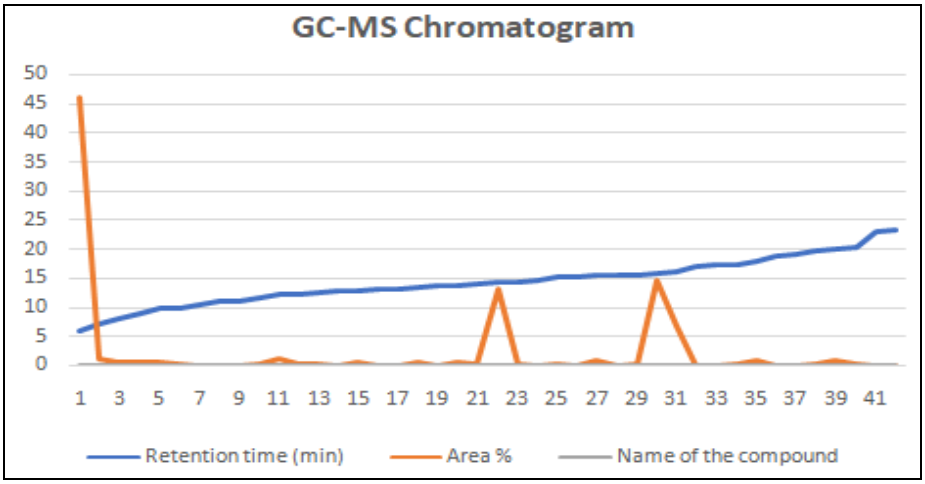

FIG. 2: SHOWS THE GC-MS CHROMATOGRAM OF THE METHANOLIC EXTRACT FROM THE GALLED LEAVES OF *TERMINALIA ARJUNA*

5-HMF has undergone preclinical testing as an effective anti-sickling agent for the treatment of sickle cell disease, a severe and potentially deadly condition. Research has shown that 5-HMF increases the oxygen affinity of sickle red blood cells and inhibits hypoxia-induced sickling in a concentration-dependent manner. Additionally, the presence of hydroxycarbamide enhances this effect.

Notably, 5-HMF is the only anti-sickling agent currently undergoing clinical trials that directly modifies the structure of haemoglobin (Hb). Also known as 5-Hydroxy Methyl Furfural (Aes-103), this aldehyde therapeutic agent forms a reversible Schiff base linkage primarily with the N-terminal amino group of alpha-globin, resulting in a dose-dependent increase in oxygen affinity. Overall, 5-HMF has been shown to increase the oxygen affinity and delay the sickling time of HbS (sickle haemoglobin), making it an ideal candidate for anti-sickling therapy. 5-HMF is a promising anti-sickling agent with no known side effects. Studies show it does not adversely affect red blood cells (RBCs), with no signs of haemolysis or oxidation when sickled haemoglobin is incubated with it. *In-vitro*, 5-HMF inhibits haemolysis under shear stress and does not bind to plasma or tissue proteins like serum albumin or immunoglobulins.

Clinical trials demonstrated that 5-HMF is well tolerated by healthy volunteers, rapidly absorbed, and preferentially taken up by RBCs over plasma. It prevents dehydration of sickled RBCs during deoxygenation and inhibits cation pathways that contribute to dehydration, such as the deoxygenation-induced conductance and the Gardos channel. Research by Fens *et al*. indicates that 5-HMF enhances the generation of nitric oxide

(NO) in RBCs, which promotes vasodilation and improves blood flow, potentially reducing haemoglobin polymerisation. Additionally, 5-HMF exhibits protective effects against oxidative stress by scavenging free radicals and enhancing antioxidant defences. Notably, bioactive compounds in the methanolic leaf and gall extracts of *T. arjuna* warrant further exploration for their medicinal applications.

TABLE 4: ACTIVITY OF A FEW PHYTOCHEMICALS IDENTIFIED IN THE METHANOLIC EXTRACT OF GALLED LEAVES OF *T. ARJUNA*

S. no.	Name of the compound	Molecular Formula	Molecular Weight	Biological Activity
1.	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126	Anti-sickling agent to treat sickle cell anaemia.
2.	6-Octadecenoic acid, (Z)-	$C_{18}H_{34}O_2$	282	Used in cosmetic formulations as, anti-ageing agent.
3.	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	Antioxidant, hypocholesterolemic, nematicide, pesticide, antiandrogenic
4.	Octadecanoic acid	$C_{18}H_{36}O_2$	284	Anti-inflammatory and antiarthritic
5.	Phytol	$C_{20}H_{40}O$	128	Antinociceptive, antioxidant, antimicrobial, anti-inflammatory, antiasthmatic, anticancer and anti-allergic activity
6.	Eicosanoic acid	$C_{20}H_{40}O_2$	312	Used to treat skin inflammation and reparation.
7.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	Antimicrobial, anti-inflammatory
8.	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	390	Used as a Plasticiser.
9.	17-Pentatriacontene	$C_{35}H_{70}$	491	Anti-septic property
10.	Beta-sitosterol	$C_{29}H_{50}O_2$	456	Hypocholesterolemic also relieve symptoms of benign prostatic hyperplasia.
11.	dl-alpha-Tocopherol	$C_{31}H_{52}O_3$	472	dl-alpha-Tocopherol is a synthetic form of vitamin E, a fat-soluble vitamin with potent antioxidant properties.
12.	Stigmasterol	$C_{29}H_{48}O$	412	Anti-angiogenic and cancer effects.
13.	beta-Tocopherol	$C_{28}H_{48}O_2$	416	Antioxidant activity

Effects on Cardiac Haemodynamics, Coronary Flow, and Blood Pressure: Arjuna's bark stem exhibits diuretic, inotropic, and chronotropic properties. Research using Langendorff's rabbit heart preparation has shown that its aqueous extract increases coronary flow and enhances cardiac muscle contraction in frogs and isolated perfused rabbit hearts, also inducing bradycardia. The inotropic effect is believed to arise from the plant's high calcium ion concentration.

Studies indicate that both aqueous and alcoholic extracts of Arjuna bark lower blood pressure in dogs in a dose-dependent manner. Singh *et al.* [1] reported that a 70% alcoholic aqueous extract reduced heart rate and blood pressure, though the mechanism remains unclear. Takahashi *et al.* [5] found that a fraction of tannin-related compounds from the extract caused hypotension not influenced by propranolol but affected by atropine, suggesting cholinergic mechanisms may be involved.

Moreover, another study indicated that the 70% alcoholic extract produced dose-dependent hypotension through adrenergic beta-2 receptor agonism or direct action on heart muscle, while muscarinic or histaminergic mechanisms were deemed unlikely. The mode of administration and selective omission of hydrophobic components might be essential for the efficacy and safety of Arjuna bark in cardiac therapy.

TABLE 5: MAJOR CHEMICAL CONSTITUENTS OF ARJUNA

Part of a plant	Major chemical constituents	Specific Compounds / Details
Stem bark	Triterpenoids	Arjunin, arjunic acid, arjunolic acid, arjungenin, terminic acid, arjunglucosides IV and V, arjunasides A-E, 2-alpha, 3-beta-dihydroxyurs-12,18-dien-28-oic acid 28-O-beta-d-glucopyranosyl ester
	Glycosides	Arjunetin, arjunoside I, arjunoside II, arjunapthaholoside, terminoside A
	Flavonoids	Arjunolone, arjunone, baicalein, luteolin, gallic acid, ethyl gallate, quercetin, kempferol, pelargonidin, oligomeric proanthocyanidins
	Tannins	Pyrocatechols, punicallin, punicalagin, terchebulin, terflavin C,

		castalagin, casuariin, casuarinin
	β-sitosterol	
	Minerals/trace elements	Calcium, aluminium, magnesium, silica, zinc, copper
	Triterpenoids	Arjunic acid, arjunolic acid, oleanolic acid, terminic acid
Roots	Glycosides	Arjunoside I, arjunoside II, arjunoside III, arjunoside IV, 2(□),19(□) dihydroxy-3-oxo-olean-12-en 28-oic acid 28-O-(β)-D-glucopyranoside
	B-sitosterol	
	Flavonoids	
	Alkaloids	
	Tannins	
Leaves	Steroids	
	Phenolic compounds	
	Oxalic acid	
	Inorganic acid	
Fruits	Glycosides	
	Flavonoids	Luteolin
Seeds	Cardenolide	14,16-dianhydrogitoxigenin-3-beta-d-xylopyranosyl (1-->2)-O-beta-d-galactopyranoside

Antioxidant and Cardioprotective Effects: Research indicates that dried, powdered bark of Arjuna enhances the natural antioxidant compounds in a rat's heart, helping to prevent oxidative stress due to ischemia and reperfusion injury. Studies have shown that alcoholic extracts of Arjuna in rabbits boost the production of specific heat shock proteins, further enhancing antioxidant defences against oxidative stress. The active compounds in Arjuna bark protect against oxidative stress induced by carbon tetrachloride and sodium fluoride due to their antioxidant properties.

Tests measuring antioxidant capacity indicate that ethanol extracts improve the heart's ability to combat oxidative stress. Recent findings highlight that methanol extracts contain high levels of phenolic and flavonoid compounds, correlating strongly with total antioxidant capacity. Both alcoholic and aqueous extracts from Arjuna bark have reduced reactive oxygen species production in human monocytic cells by enhancing catalase and glutathione peroxidase activities, maintaining cellular reducing power. In cases of isoprenaline-induced myocardial ischemia, Arjuna has shown prostaglandin E2-like activity, promoting coronary vasodilation and hypotension while preventing oxidative stress increases. Arjunolic acid prevents decreases in several antioxidant levels and demonstrates protective effects against doxorubicin-induced DNA damage. Additionally, Arjuna has mitigated cardiac dysfunction and myocardial injury in rats with congestive heart failure, showing cardioprotective action comparable to fluvastatin. Recent findings confirm that triterpenoids from Arjuna extract, particularly arjunolic acid, enhance the endogenous antioxidant defence system, offering significant prophylactic and therapeutic benefits for heart protection.

Hypolipidemic and Antiatherogenic Activity: Prior animal studies indicate that arjuna bark powder and extract can significantly reduce total cholesterol (TC) and triglyceride (TG) levels. Among various solvent fractions tested in hyperlipidemic rat models, the ethanolic fraction showed the most significant lipid-lowering effect. Both the solvent ether and ethanolic fractions effectively decreased plasma lipid levels in Triton-induced hyperlipidemic models and hamsters on a high-fat diet.

In-vitro experiments revealed that arjuna fractions inhibited oxidative lipid degradation in human low-density lipoprotein (LDL) and rat liver microsomes and countered the formation of superoxide anions and hydroxyl radicals. The efficacy of arjuna fractions was ranked as follows: ethanolic fraction > solvent ether fraction > petroleum ether fraction. Incorporating arjuna bark into recipes like Arjuna Omelette received positive feedback, suggesting its potential for long-term dietary interventions for elevated lipid levels. The hypolipidemic action may involve increased hepatic clearance of cholesterol and inhibition of lipogenic enzymes.

Clinical Uses: *Terminalia arjuna* is utilised for therapeutic purposes, and various experiments have been conducted on its bark and leaf powders. It has been tested for its effects on several conditions,

including myocardial infarction, angina, congestive heart failure (CHF), hypertension, rheumatic heart disease, ischemic mitral regurgitation, cardiomyopathy, platelet aggregation, oxidative stress, endothelial dysfunction, and thrombotic conditions.

Toxicity and Side Effects: Mild side effects have been reported, including nausea, gastritis, headache, body aches, constipation, and insomnia. Importantly, no haematological, renal, or metabolic toxicity has been observed, even after prolonged administration lasting more than 24 months.

However, Parmar *et al.* [25] found that administering Arjuna led to a reduction in thyroid hormone concentrations in euthyroid animals, along with an increase in hepatic lipid peroxidation (LPO). Therefore, it is advisable to avoid high doses of the plant extract, as it may increase the risk of hepatotoxicity and hypothyroidism. Recent acute and oral toxicological studies conducted on animals showed that administering the ethanolic extract at a maximum dose of 2000 mg/kg did not lead to any observable toxicity or mortality in the subjects.

CONCLUSION: The analysis of the methanolic extract from the leaves and galls of *Terminalia arjuna* using GC-MS identified several bioactive compounds with medicinal value. Notably, 5-Hydroxy Methyl Furfural, an aromatic aldehyde found in leaf galls, has significant economic potential and is used as an anti-sickling agent for sickle cell disease. While Arjuna shows promise as an anti-ischemic, antioxidant, and antiatherogenic agent, many studies lack standardised extracts and thorough toxicity assessments. Further research is needed to explore its role in coronary prevention and its interactions with medications like statins and beta-blockers. Raising awareness of arjuna's medicinal uses can aid in addressing cardiovascular disease challenges, and isolating its phytochemicals may enhance its pharmacological profile.

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CONFLICTS OF INTEREST: Nil

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