



Research Article

PHYTOCHEMICAL ANALYSIS OF DIFFERENT FRACTIONS OF *TERMINALIA ARJUNA* BARK BY GC-MS

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ABSTRACT

Traditionally *Terminalia arjuna* Roxb. (*T. arjuna*) is being used widely to cure various diseases like cardiovascular diseases, liver diseases, and cancer etc. The aim of the present study is to phytochemical analysis of petroleum ether, ethyl acetate and methanolic fractions of *T. arjuna* bark by GC-MS analysis. The present investigation was carried out to determine the possible bioactive components of *T. arjuna* bark using GC-MS analysis. The GC-MS analysis of petroleum ether, ethyl acetate and methanolic fractions of bark of *T. arjuna* leads identified of 10, 12 and 16 compounds, respectively. The main identified compounds are 9-Octadecenoic acid (*Z*)-methyl ester (35.38 %), Hexadeca-2,6,10,14-tetraen-1-ol (34.94 %) and Furanicarboxaldehyde, 5-(hydroxymethyl) (19.31 %) from petroleum ether, ethyl acetate and methanolic fractions of bark of *T. arjuna*, respectively. The present study is therefore an effort to give detailed information on phytochemical profile of *T. arjuna* bark by GC-MS method.

Keywords: *Terminalia arjuna*, Combretaceae, Phytochemicals, GC-MS Analysis.

INTRODUCTION

Terminalia arjuna Roxb. (Combretaceae) is a large, evergreen, deciduous tree found in sub-Himalayan belt in India. It attains a height of 20-30 m. It finds its place in ancient Indian medicinal literature like Charaka Samhita and Astang Hridayam. Ancient Indian physician Vagbhatta first advocated the use of this bark powder for the treatment of heart diseases.¹ It is an essential ingredient in many Ayurvedic preparations meant to improve cardiovascular health. It shows hepatoprotective,² anti-atherogenic,^{3,4} antimicrobial,^{5,6} antibacterial and antifungal activities⁷.

A number of triterpenes were isolated from the bark of *T. arjuna* which include triterpene glycosides and aglycones. Some of the triterpenes isolated from the *T. arjuna* tree are arjunic acid, arjunolic acid, and arjungenin. The triterpene glycosides isolated from the *T. arjuna* tree are arjunetin, arjunoglucoside I, arjunoglucoside II, arjunoglucoside III, arjunoside I and arjunoside II. The other compounds characterised from the *T. arjuna* tree are β -sitosterol and terminic acid. Three polyphenols, arjunin, arjunone and arjunolone have been isolated from *T. arjuna*.⁸⁻¹⁰ In the present work *T. arjuna* bark was selected for identification of phytochemicals from petroleum ether, ethyl acetate and methanolic fraction by GC-MS method.

MATERIALS AND METHODS

Plant Materials

The bark of *T. arjuna* was obtained from the Southern part of India (Madurai District, Tamil Nadu), and our botanist carried out the Pharmacognostic authentication and specimen was preserved in the herbarium.

Fractionation

The bark of *T. arjuna* was then shade dried for a period of 7-10 days and then, coarsely powdered using a pulverizer. The pulverized *T. arjuna* was then stored in airtight containers until further use. The coarsely powdered *T. arjuna* was subjected to extract with different solvents like pet-ether, Ethyl acetate and methanol. This phenomenon was carried out several times until solvent colour was retained (Figure 1). The extract was then concentrated *in-vacuo* and the yield were calculated. The same fraction was used for further studies.

GC-MS Analysis

Gas chromatography mass spectroscopy (GC-MS) analysis of different fractions (Pet. Ether, Ethyl acetate and Methanol) of *T. arjuna* were carried out on a Trace 1300 GC, Tsq 8000 Triple Quadrupole MS with a column TG 5MS (30 m \times 0.25 mm, 0.25 μ m). Helium was used as a carrier gas at a flow rate of 1 ml/min. Split/Splitless (S/SL) injector was used with 250 °C injector temperature. 1.0 μ l sample injection volume was utilized. Ion source temperature was maintained at 230 °C. The oven temperature was programmed initially at 80 °C for 2 min, then programmed to increase to 280 °C at a rate of 5 °C/min ending with a 5 min isothermal at 280 °C. Total run time were 16.13, 25.53 min and 18.32 for pet. ether, ethyl acetate and methanolic fraction of *T. arjuna*, respectively. The MS transfer line was maintained at a temperature of 250 °C. TSQ 8000 Triple Quadrupole MS detector was used for analysis and data was evaluated using total ion count (TIC) for compound identification and quantification. The mass spectra of the components were matched with the data available in the National Institute of Standards and Technology (NIST) library. Measurement of peak areas and data processing were carried out by XCALIBER software^{11,12}.

Figure 1. Fractionation scheme chart of *T. arjuna*

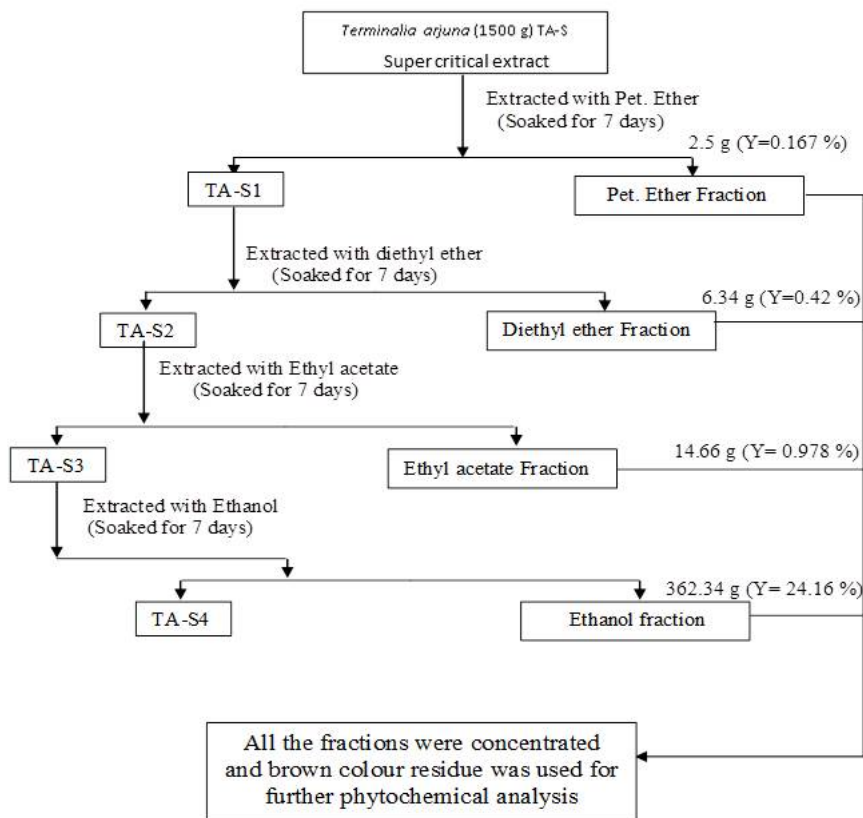


Figure 1: Fractionation scheme chart of *T. arjuna*

RESULTS AND DISCUSSION

Considerable work has been done on chemical constituents of different parts of *T. arjuna* and this has revealed the presence of a number of tannins, sugars, triterpenoid acids and their glycosides, flavonones, etc. The concentration of the major phytoconstituents in the extracts depends on the yield of the extracts. The yield of each plant fraction was calculated, and the results are presented in Table 1. Powdered bark of *T. arjuna* (1.5 kg) is defatted and then exhaustively extracted with methanol to give 360 g of methanolic extract. Methanolic extract (75 g) is detannified by lead acetate treatment to obtain green coloured mass (1.5 g). This mass is subjected to repeated column chromatography using silica gel with varying proportions of chloroform-methanol. From the relatively non-polar fractions compounds 1 and 2 while from the relatively polar fractions 3 and 4 are isolated.

Table 1: *T. arjuna* barks yield of fractions in different solvents

Solvent	<i>T. arjuna</i> (% w/w)
Pet-Ether fraction	10.005
Ethyl acetate fraction	14.254
Methanol fraction	18.365

GC-MS of *T. arjuna* Petroleum Ether Fraction

The pet-ether fraction was analyzed by GC-MS showed ten compounds (Table 2, Figure 2 & 3) and one of the major compounds was appear at Rt 23.06. This compound was further analyzed by Mass spectrometer and identified as 9-Octadecenoic acid (Z), methyl ester (35.38 %). Mass spectrum of this compound was match with mass spectral data gave the name & molecular formula of the compound, in addition to that gives 10 possible compounds. The results are given as follows.

Table 2: GC-MS of *T. arjuna* Petroleum Ether Fraction

S. No.	Compound	Retention Time (min)	% Peak Area
1	Estragole Formula: C ₁₀ H ₁₂ O MW: 148	9.66	1.1049
2	α -Terpieol Formula: C ₁₀ H ₁₈ O MW: 154	9.89	0.1776
3	Longifolene-(V4) Formula: C ₁₅ H ₂₄ MW: 204	14.73	0.7286

4	Naphthalene Formula: C ₁₅ H ₂₄ MW: 204	14.97	0.5897
5	Phenol, 2-methoxy-5-(1-propenyl)-, (E)- Formula: C ₁₀ H ₁₂ O ₂ MW: 16	12.48	0.0470
6	Cyclohexyl (2,4-dimethylphenyl) methanone Formula: C ₁₅ H ₂₀ O MW: 216	17.38	0.2018
7	Palmitic acid, methyl ester Formula: C ₁₇ H ₃₄ O ₂ MW: 270	20.90	28.4702
8	Linoleic acid, methyl ester Formula: C ₁₉ H ₃₄ O ₂ MW: 294	22.98	18.6267
9	9-Octadecenoic acid (Z)-, methyl ester Formula: C ₁₉ H ₃₆ O ₂ MW: 296	23.06	35.3860
10	Stearic acid, methyl ester Formula: C ₁₉ H ₃₈ O ₂ MW: 298	23.34	14.6674
	Total		100.0000

Figure 2. GC-MS of Petroleum Ether Fraction of *T. arjuna* bark

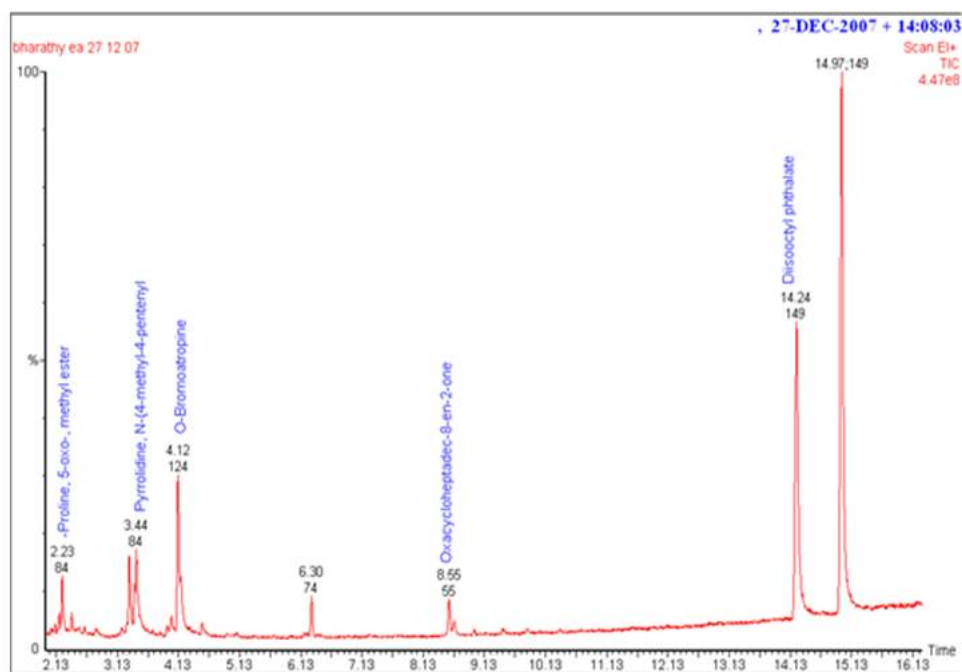


Figure 2: GC-MS of Petroleum Ether Fraction of *T. arjuna* bark

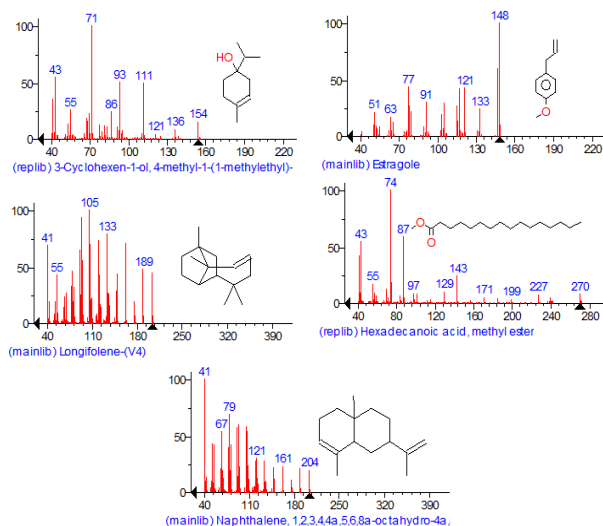


Figure 3: Chemical Structure of Petroleum Ether Fraction of *T. arjuna* bark

GC-MS of *T. arjuna* Ethyl Acetate Fraction

The ethyl acetate fraction was analyzed by GC-MS showed 12 compounds (Table 3, Figure 4 & 5) and one of the major compounds was appear at Rt 24.07. This compound was further analyzed by Mass spectrometer and identified as Hexadeca-2,6,10,14-tetraen-1-ol (34.94 %). Mass spectrum of this compound was match with mass spectral data gave the name & molecular formula of the compound, in addition to that gives 12 possible compounds. The results are given as follows.

Table 3: GC-MS of *T. arjuna* Ethyl Acetate Fraction

S. No.	Compound	Retention Time (min)	% Peak Area
1	Glycerin Formula: C ₃ H ₈ O ₃ MW: 92	6.15	3.798
2	Octanoic Acid Formula: C ₈ H ₁₆ O ₂ MW: 144	9.25	10.601
3	(-)-Terpinen-4-ol Formula: C ₁₀ H ₁₈ O MW: 154	9.70	20.564
4	α-Terpineol Formula: C ₁₀ H ₁₈ O MW: 154	9.90	7.265
5	1,2,3-Benzenetriol Formula: C ₆ H ₆ O ₃ MW: 126	12.69	3.573
6	α-D-Glucopyranoside, α-D-fructofuranosyl Formula: C ₁₂ H ₂₂ O ₁₁ MW: 342	13.63	1.291
7	1,6-Anhydro-α-D-glucopyranose (levoglucosan) Formula: C ₆ H ₁₀ O ₅ MW: 162	14.68	1.676
8	Naphthalene Formula: C ₁₅ H ₂₄ MW: 204	14.98	1.442
10	Nerolidol Formula: C ₁₅ H ₂₆ O MW: 222	22.19	4.128
11	Hexadeca-2,6,10,14-tetraen-1-ol Formula: C ₂₀ H ₃₄ O MW: 290	24.07	34.941
12	α-D-Mannofuranoside, farnesyl- Formula: C ₂₁ H ₃₆ O ₆ MW: 384	25.34	10.721
	Total		100

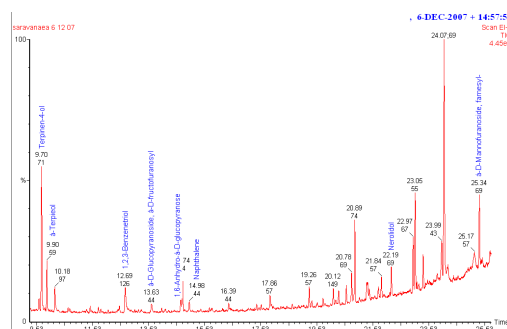


Figure 4: GC-MS of Ethyl Acetate Fraction of *T. arjuna* bark

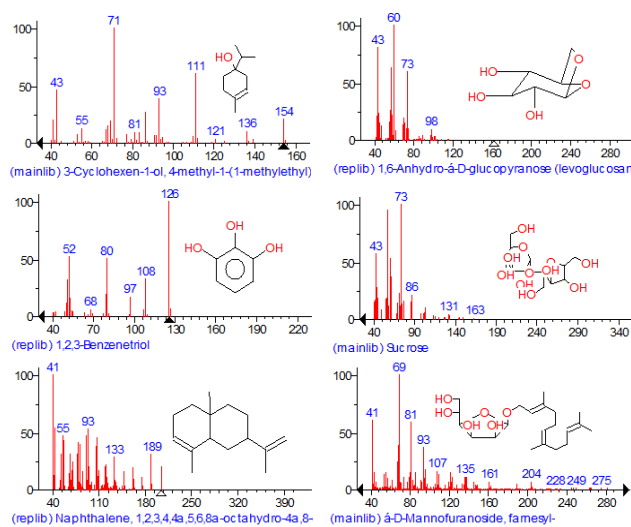


Figure 5: Chemical Structure of Ethyl Acetate Fraction of *T. arjuna* bark

GC-MS of *T. arjuna* Methanolic Fraction

The methanolic fraction is analyzed by GC-MS. It has brought out more than 16 compounds (Table 4, Figure 6 & 7) and one of the major compounds it appears at Rt 9.00. This compound is further analyzed by Mass spectrometer and identified as 2-Furancarboxaldehyde, 5-(hydroxymethyl) (19.31 %). Mass spectrum of this compound is matched with mass spectral data from then the name & molecular formula of the compound has been obtained. In addition to that it gives 17 more possible compounds. The results are given as follows.

Table 4: GC-MS of *T. arjuna* Methanolic Fraction

S. No.	Compound	Retention Time (min)	%Peak Area
1	3(2H)-Furanone, dihydro-2-methyl- Formula: C ₅ H ₈ O ₂ MW: 100	3.67	3.738
2	Furfural Formula: C ₅ H ₄ O ₂ MW: 96	3.98	5.261
3	Glycerin Formula: C ₃ H ₈ O ₃ MW: 92	5.65	6.471
4	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl Formula: C ₆ H ₈ O ₄ MW: 144	8.08	8.828
5	1,2-Benzenediol Formula: C ₆ H ₆ O ₂ MW: 110	8.62	5.850
6	2-Furancarboxaldehyde, 5-(hydroxymethyl) Formula: C ₆ H ₆ O ₃ MW: 126	9.00	19.313
7	l-Gala-l-ido-octose Formula: C ₈ H ₁₆ O ₈ MW: 240	9.53	1.316
8	D-Glucose, 6-O-α-D-galactopyranosyl-	9.20	1.191

	Formula: C ₁₂ H ₂₂ O ₁₁ MW: 342		
9	1,2,3-Benzenetriol Formula: C ₆ H ₆ O ₃ MW: 126	10.76	14.732
10	α -D-Glucopyranoside, O- α -D-glucopyranosyl-(1.fwdarw.3)- α -D-fructofuranosyl Formula: C ₁₈ H ₃₂ O ₁₆ MW: 504 CAS	11.45	3.134
11	D-Allose Formula: C ₆ H ₁₂ O ₆ MW: 180	12.11	6.378
12	Phenol, 2-methoxy-4-(methoxymethyl) Formula: C ₉ H ₁₂ O ₃ MW: 168	12.55	1.911
13	d-Mannose Formula: C ₆ H ₁₂ O ₆ MW: 180	12.86	1.091
14	α -D-Glucopyranose, 4-O- α -D-galactopyranosyl- Formula: C ₁₂ H ₂₂ O ₁₁ MW: 342	13.08	2.348
15	1-Isobutyl-7,7-dimethyl-octahydro-isobenzofuran-3a-ol Formula: C ₁₄ H ₂₆ O ₂ MW: 226	14.22	3.895
16	α -Sitosterol Formula: C ₂₉ H ₅₀ O MW: 414	18.65	14.542
	Total		100.000

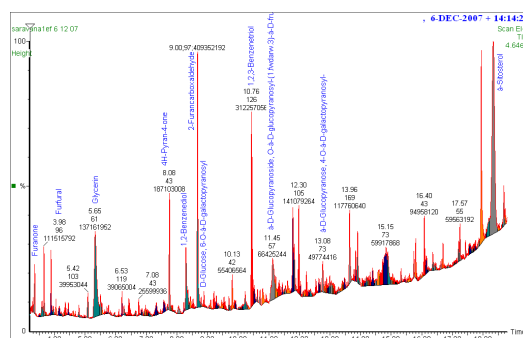


Figure 6: GC-MS of Methanol Fraction of *T. arjuna* bark

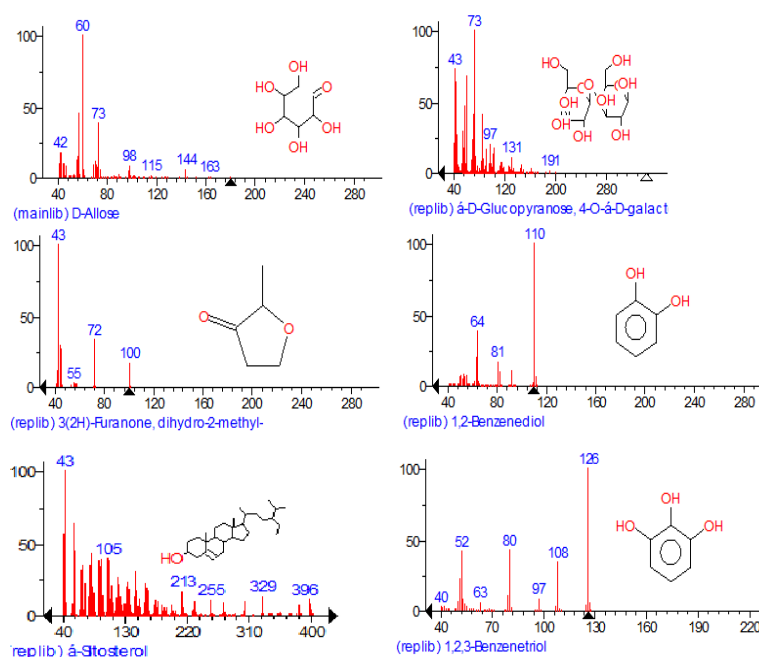


Figure 7: Chemical Structure of Methanol Fraction of *T. arjuna* bark

CONCLUSION

T. arjuna is traditional medicinal plant and represent rich source of chemical compounds possessing antimicrobial, cardioprotective, anticancer and hepatoprotective properties. The GC-MS analysis of petroleum ether, ethyl acetate and methanolic fractions of *T. arjuna* bark leads identified of 10, 12 and 16 compounds, respectively. The main identified compounds are 9-octadecenoic acid (Z)-methyl ester (35.38 %), hexadeca-2, 6, 10, 14-tetraen-1-ol (34.94 %) and furancarboxaldehyde, 5-(hydroxymethyl) (19.31 %) from petroleum ether, ethyl acetate and methanolic fractions of *T. arjuna* bark, respectively. The present study is therefore an effort to give detailed information on phytochemical profile of *T. arjuna* bark by GC-MS analysis.

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