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Comprehensive study on pharmacognostic, physico and phytochemical evaluation of *Terminalia arjuna* Roxb. stem bark

Gunjan M Chaudhari, Raghunath T Mahajan

Abstract
Terminalia arjuna Roxb. (Family-Combretaceae) is commonly known as Arjun tree and valued for its medicinal uses. In the present investigation, the detailed pharmacognostic study of *T. arjuna* stem bark (TASB) is carried out to lay down the standards which could be useful in forthcoming experimental studies. The study includes microscopy, proximate analysis and physicochemical evaluation. Atomic absorption spectroscopic analysis of TASB revealed the presence of heavy metals within the recommended safety range. Qualitative phytochemical analysis of the methanolic extract of TASB (MeOH-TASB) confirmed the presence of alkaloids, glycosides, tannins, flavonoids, phenols, saponins and steroids in the extract. The UV-Vis and FTIR spectroscopic analysis of MeOH-TASB indicated the presence of aromatic phytoconstituents as predominant ingredient of the extract. The chemical fingerprinting of MeOH-TASB was performed by using TLC and GC-MS analysis which showed bands/peaks of different phytoconstituents. Findings of this study provide reference information on TASB for its quality evaluation.

Keywords: *Terminalia arjuna*; Pharmacognosy; Phytochemical screening; Fluorescent analysis; Spectroscopic evaluation; Chemical fingerprinting.

Introduction
The plant kingdom is a treasure house of potential drugs and in the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularize in developing and developed countries owing to its natural origin and lesser side effects [1]. According to World Health Organization (WHO) more than 80% of the world's population relies on herbal medicine for their primary healthcare needs [2]. Herbal formulation involves use of fresh or dried plant part. Thus appropriate identification of the plant material is very much necessary. Proper identification of the starting material is an essential prerequisite to ensure quality, safety and efficacy [3]. In herbal technology, pharmacognostic studies play important role as it provides standardization parameters which will help to prevent adulterations in the original plant material and ensures plant identity. This information will help in authentication of the plants and ensures reproducible quality of herbal products which will result in safety use and retaining effectiveness of natural products [4].
The plant *Terminalia arjuna* Roxb. Commonly known as Arjuna, a common tree for its important phyto constituents belongs to the family combretaceae. It has been grown in most parts of India and used in Ayurvedic formulations since ancient times. The plant parts such as stem bark, leaves and fruits of *T. arjuna* are used in indigenous system of medicine for different ailments. The bark powder has been found to possess cardioprotective properties, anti-ischemic, antioxidant action [5], hypercholesterolemia effect [6], fungicidal and antibacterial [7], antimicrobial [8], Anti-inflammatory, immunomodulatory and antinociceptive activity [9], It is also useful to cure obesity, hypertension and hyperglycemia [10]. The higher antioxidant potential of *T. arjuna* stem bark is due to the presence of higher amount of phenolic and flavonoids [11]. The *T. arjuna* based phytochemicals are considered as one of the best heart tonic [12] therefore, it can be used on daily bases as tonic for healthy cardiovascular system.
An ethano pharmacological importance and increasing demand of *T. arjuna* stem bark in several herbal formulations may leads to adulteration. Therefore, the main objective of this study is validation of earlier information on pharmacognostic studies of *T. arjuna* stem bark (TASB) and to supplement some information with regards to its identification, characterization and standardization to ensure the quality and purity of TASB material.

In this point of view, work has been carried out to evaluate some pharmacognostic, physicochemical and phytochemical characteristics of TASB followed by determination of volatile organic constituents by GS-MS analysis.

Materials and methods

Plant material

Dried parts of stem bark of *T. arjuna* were collected in the month of September 2013 from Whaghur Dam, District - Jalgaon (MS), India. The plant material was identified from the expert taxonomist. The pieces of stem bark of *T. arjuna* were washed thoroughly with tap water, shade dried, ground into fine powder and stored in air tight bottles.

Macroscopic and Microscopic studies

Macroscopic studies such as morphological observations of TASB were performed under magnifying lens. In order to study microscopic features, free hand section of stem bark was taken and stained by phloroglucinol (0.2% in water w/v) and iodine (0.2% in water w/v) to confirm its lignification and presence of starch grains respectively. Microscopic analysis of TASB fine powder was also performed in the similar manner to record the specific diagnostic characteristics.

Fluorescence analysis

Fluorescence study of TASB powder was performed as per reported standard procedures [13]. A small quantity of the stem powder was added in few ml of freshly prepared reagent solution, mixed by gentle shaking the tube and waited for few minutes. Then the colors of the mixtures were observed in visible light, and long ultra violet radiations (365 nm) in the UV chamber.

Physicochemical analysis

The physicochemical analysis of the crude powder TASB was carried out as per WHO (2002) guidelines [14]. The parameters analyzed were loss on drying, total ash, alcohol soluble extractive and water soluble extractive.

Atomic absorption spectroscopy

TSBA powder was subjected to elemental analysis according to standard procedure involving use of atomic absorption spectrophotometer [15]. In brief, 2 gm of dry TASB powder was digested in 40 ml of aquaregia solution [HNO_3: H_2SO_4 (3:1 v/v)] by COD digester at 150 ºC for 90 minutes. Then digested solution was filtered through Whatman filter paper No. 1 and diluted up to 40 ml using 1% nitric acid solution. The minerals [calcium (Ca), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), potassium (K), sodium (Na), and zinc (Zn)] contents of the digested solution was determined using Atomic Absorption Spectrophotometer (Model: S2, Make: Thermo, USA) with SOLAAR software.

Preparation of methanolic extract of TASB

Methanolic extract of TASB (ME) was prepared by using Soxhlet extraction method. Thirty gram of finely ground plant part powder was placed in porous bag made of muslin cloth, which was loaded into the main chamber of the Soxhlet extractor. The extraction was carried out with methanol in 1:10 powder to solvent ratio at temperature 65 ºC for 8 h. The extract was filtered through Whatman filter paper No. 1 and the filtrate was stored in the refrigerator at 4 ºC until its further use.

Qualitative phytochemical analysis

The ME was subjected to qualitative phytochemical analysis [16] to detect the presence of carbohydrate, amino acids, cardiac glycosides, alkaloids, saponins, phenols, flavonoids, steroids, terpenoids, tannins, anthraquinones, quinones, fats and volatile oils.

Thin layer chromatography fingerprinting

TLC fingerprinting of ME was performed using aluminium plates precoated with silica gel 60 (F-254) having 0.2 mm thickness (Merck, Mumbai, India). TLC plates were activated by preheating in oven at 60 ºC. About 10-20 µL of ME was loaded on TLC plate and the chromatogram was developed by using solvent system consisting of toluene: ethyl acetate: formic acid: methanol (10:3:1:2 v/v/v/v) as mobile phase to separate phytoconstituents. The developed plate was dried under hot air. Then 10% H_2SO_4 in ice cold methanol was sprayed on the surface of plate followed by heating at 110 ºC for 5 min for derivatization of phytoconstituents. The derivatized TLC plate was observed under visible light and the R_f value of the bands were recorded.

GC– MS analysis

The GC-MS analysis of ME of TASB was carried out by using a Gas Chromatograph (TRACE 1300, Thermo Scientific, USA) equipped with Triple Quadrupole Mass Spectrometer (TSQ 8000, Thermo Scientific- USA) equipment. Experimental conditions of GC-MS system were as follows: TG 5-MS capillary standard non-polar column (dimension: 30 m, ID: 0.25 mm, film: 0.25 µm) was used. The flow rate of mobile phase (carrier gas: He, 99.999%) was set at 1.0 ml/min and the injection volume was 1 µL. An injector and ion source temperature were 250 and 280 ºC respectively. Oven temperature program was 60 ºC hold for 2 min to 250 ºC at the rate of 15 ºC/min and hold it for 15 min. Samples which dissolved in methanol were run fully at a range of 50-1000 m/z. The mass spectra detected in 26 min.

The name, molecular weight and nature/structure of the components of the ME were confirmed using computer searches on the database of National Institute Standard and Technology (NIST) Ver. 11 MS data library and comparing the spectrum obtained through GC-MS with the spectrum of the known components stored in the NIST library.

Results and discussion

T. arjuna is a large, deciduous tree, growing to a height of 60-90 feet with a spreading crown and dropping branches (Fig 1-A). *T. arjuna* has a buttressed huge trunk of diameter about 3-8 feet (Fig. 1-B). The outermost layer of stem of *T. arjuna* is usually known as stem bark which has wide applications in ayurvedic medicine. The present investigation was mainly focused on pharmacognostic standardizations and phytochemical evaluations of TASB and its methanolic extract.

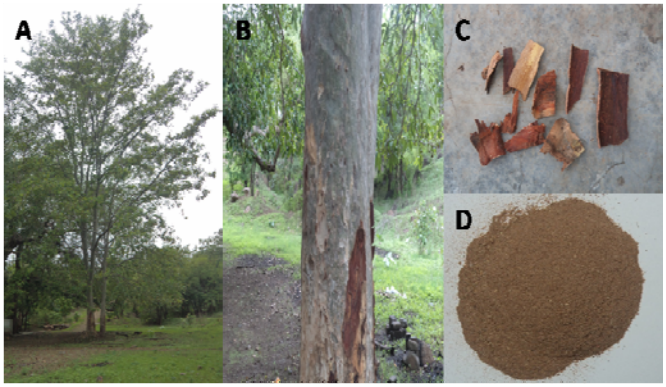

Fig 1: *Terminalia arjuna* Roxb. A- Whole tree, B- Trunk, C- Stem park pieces, D- Stem bark powder.

Macroscopic study of TASB

The bark is grayish and smooth externally, internally brown or red coloured and smooth. The shape of bark was flat and curved (Fig. 1-C). The transversely cut bark showed brownish surface, fracture, short in inner and laminated in outer part. The powder of TASB was light brown (Fig. 1-D) with astringent taste and no odour.

Microscopic study of TASB section

Microscopic evaluation is one of the cost effective and simplest methods for the correct identification of the plant material. Transverse section of fresh stem bark showed typical anatomical characters. It had outer cork region composed of uniformly arranged several layers of small, tangentially elongated cells (Fig. 2-A). The region of cortex, composed of thin walled, more or less brick shaped parenchymatous cells containing cluster crystals of calcium oxalate and few groups of sclerenchymatous pericyclic fibres with scattered arrangement were observed below the cork region (Fig. 2-B). The innermost region showed presence of secondary phloem consisting of thin walled, polygonal cells with wavy walls containing cluster crystals of calcium oxalate and pigmented cells (Fig. 2-C).

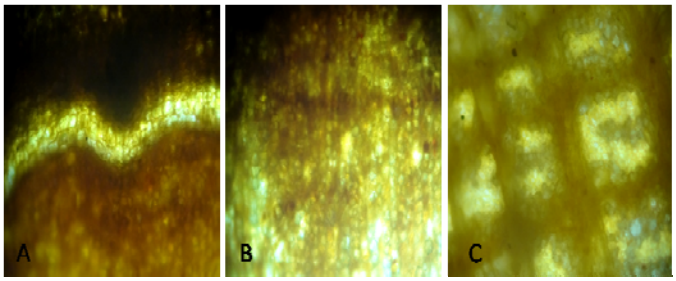

Fig 2: Microscopic photo of *T. arjuna* stem bark, A- outer region, B- inner region C- magnified inner region

Microscopy of Powder

The unstained TASB powder under light microscope at 10x magnification showed the presence of reddish-brown particles of irregular shape, fragments of cork cells, uniseriate phloem rays, fibres and a number of white colored rosette crystals of calcium oxalate (Fig. 3-A). After staining by 0.2% phloroglucinol pink colored fibres appeared (Fig. 3-B), this confirmed the presence of lignified material [13].

After staining by 0.2% iodine solution, violet colored granules with elliptic or round to oval shape having 2-3 components with concentric striations were also observed (Fig. 3-C) which confirmed the starch material [13]. This microscopic observation of powder could be useful in identification of TASB material.

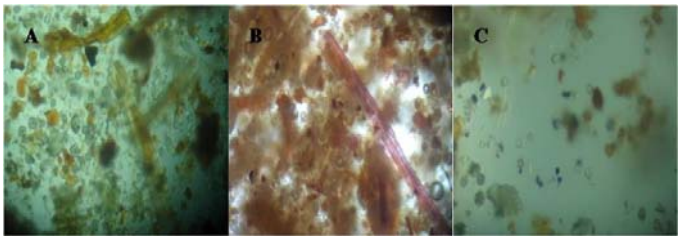

Fig 3: Microscopic photo of Powder of *T. arjuna* stem bark powder, A- without stain, B- stained with 0.2 % phloroglucinol, C- stained with 0.2 % iodine

Physiochemical characters of *T arjuna* stem bark

Loss on drying at 105 ºC, pH, total ash content are the major factors responsible for the stability of herbal plant material. The results of physiochemical characterization of TASB are given in the table 1. Usually low moisture content is desirable for higher stability of drugs [17]. In the present investigation, TASB showed low moisture content (Table 1) which is laying between recommended levels 8% and 14% [18]. The pH of the TASB was in the acidic region. It had higher ash value reflecting the presence of carbonate, phosphate, oxides, silicates and silica material in higher amount [19]. The TASB had higher alcohol soluble and water soluble extractive value indicating the presence of highly polar chemical constituents such as phenolic compound, flavanoids, proteins, carbohydrates *etc*. [4].

The results of macroscopy, microscopy and physiochemical analysis are in accordance with Ayurvedic Pharmacopoeia of India [20].

Table 1: Physicochemical parameters of *T. arjuna* stem bark

Sr. No.	Test	Results
1	Moisture content	10.19 ± 1.30[a]
2	pH	4.13 ± 0.06[b]
3	Total Ash	17.49 ± 1.31[a]
4	Water-soluble extractive	20.50 ± 0.66[a]
5	Alcohol soluble extractive	28.48 ± 3.71[a]

[a] Values are expressed in percentage; [b] Value is expressed as pH value; All results are given as Mean ± standard deviation (n=3).

Fluorescent evaluations of TASB powder

Fluorescence study is an essential parameter for first line standardization of crude drug. The changes in color and fluorescent analysis of TASB powder with different reagents/solvents under visible and UV light respectively were tabulated in Table 2. Under visible light the TASB powder appeared red, dark brown to yellowish brown in most of the reagents and faint yellow in ethyl acetate. Under long UV light the TASB powder appeared black in most of the reagents and with few reagents it appears as dark brown to greenish brown.

Table 2: Fluorescence analysis of TASB powder under visible and UV light after treated with different reagents/solvents

Treatment	Visible light	UV light
Powder	Brown	Greenish brown
Powder + water	Red	Black
Powder + 1N NaOH	Red	Blackish brown
Powder + 1N NaOH (alc.)	Yellowish brown	Black
Powder + Ammonia	Dark red	Black
Powder + 50 % HCl	Yellowish Red	Black
Powder + 50 % H_2SO_4	Dark brown/black	Black
Powder + 50% HNO_3	Brownish red	Black
Powder + $FeCl_3$	Greenish yellow	Greenish black
Powder + Methanol	Dark red	Black
Powder + Ethanol	Red	Dark brown
Powder + Ethyl acetate	Faint yellow	Black
Powder + Benzene	cream	Black
Powder + Petroleum ether	Colourless	Black

Atomic absorption spectroscopy analysis

The Atomic Absorption Spectrometric analysis of the TASB (Table 3) showed the presence of various essential (Calcium, Chromium, Cobalt, Copper, Iron, Manganese, Nickel and Zinc *etc*.) as well as non-essential (Arsenic, Cadmium, Lead and Mercury *etc*.) elements. The concentrations of all these elements are within the permissible limits set by WHO 1996 and 2005 [21, 22]. The result can assure the usefulness of these elements in the physiological administration of crude drug since some of the elements were of health benefits and similarly within recommended safe limits.

Table 3: Heavy metal analysis of TASB by Atomic absorption spectroscopy

Sr. No.	Metal in plant material	Concentration of elements (mg/kg)	
		Permissible limits of metals in plant material (WHO 1996 & 2005) [21, 22]	In this study
1	Arsenic (As)	5	0.066
2	Cadmium (Cd)	0.3	0.296
3	Calcium (Ca)	NA	86.274
4	Chromium (Cr)	1.5	0.826
5	Cobalt (Co)	NA	2.078
6	Copper (Cu)	10	9.534
7	Iron (Fe)	20	16.036
8	Lead (Pb)	10	0.124
9	Manganese (Mn)	200	9.154
10	Mercury (Hg)	0.2	0.092
11	Nickel (Ni)	1.5	0.592
12	Zinc (Zn)	50	8.698

NA-data not available

Phytochemical analysis

The phytochemical profiling of methanolic extract of TASB showed the presence of carbohydrate, amino acid, cardiac glycosides, alkaloids, saponins, phenols, flavonoids, steroids, terpenoids and lignins as presented in Table 4. This serves as an important tool for the quality assurance of plant for future studies.

Table 4: Phytochemical analysis of methanolic extract of TASB

Sr. No.	Phytoconstituents	Test	Results
1	Carbohydrate	Molisch's Test	++
		Fehling's test	++
		Test for starch	-
		Test for cellulose	-
2	Amino acid and Protein	Millon's test	++
		Biuret test	-
		Ninhydrin test	-
3	Cardiac glycoside	Keller Kelliani's test	+++
		Legal's test	+++
4	Alkaloid	Mayer's test	+
		Dragendroff's test	+
		Wagner's test	+
5	Saponin	Foam test	+
6	Phenol	Ferric chloride test	+++
		Phosphomolybdic acid test	+++
7	Flavonoid	Shinoda test	++
		Alkaline reagent test	-
		Ammonium Test	++
		Aluminium Chloride Test	+++
		Lead acetate test	+++
		Pew test	++
8	Steroid	Salkowski Test	+
9	Terpenoid	Salkowski Test	++
10	Tannin	Braymer's test	++
11	Anthraquinone	Borntrager's test	-
12	Quinone	Hydrochloric acid test	-
13	Fats and volatile oil	Precipitation test	-
14	Lignin	Spot test	+

- Not detected; + slightly positive reaction; ++ Positive reaction; and +++ Strong positive reaction

UV-VIS spectroscopic analysis

The UV-VIS spectrum of this plant extracts has absorption bands at 226, 275, broad peak at 350- 375, and minor peak at 487 nm (Fig. 4). These absorption bands are characteristic for flavonoids and its derivatives. The flavonoids spectra typically consist of two absorption maxima in the ranges 240-285 nm (band II) and 300-380 nm (band I) [23]. The precise position and relative intensities of recorded maxima in the spectrum indicate that the flavonoid is a predominant ingredient of TASB methanolic extract.

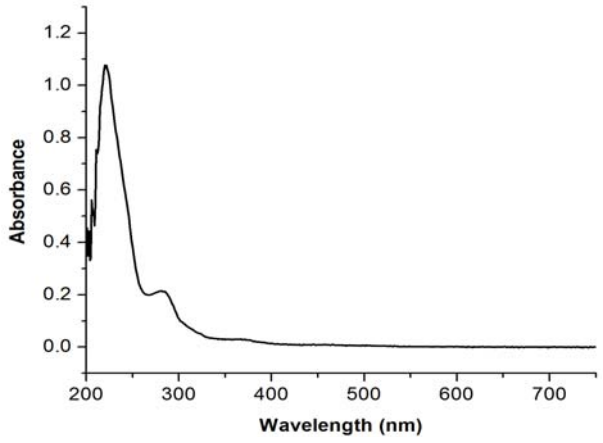

Fig 4: UV-VIS spectrum of TASB methanolic extract

FTIR Spectroscopic analysis

The FTIR spectrum of TASB methanolic extract showed presence of various functional groups of the active components based on the peaks values in the region of IR radiation (Fig. 5). The peak at 3334.7 (O-H stretch) phenol, 2931.3 (C-H stretch) alkanes, 1724.06 (C=O stretch) carboxylic acids, 1613.84 (N-H bend) primary amines, 1520.98 and 1445.39, (C-C stretch in ring), aromatics, 1351.86 (N-O symmetric stretch), nitro compounds, 1250–1020 (C-N stretch) which may be due to the presence of aliphatic amines.

The results of FTIR analysis confirmed the presence of alcohol, phenol, alkanes, Carboxylic acids, primary amines, aromatic compound, nitro compounds, and aliphatic amines.

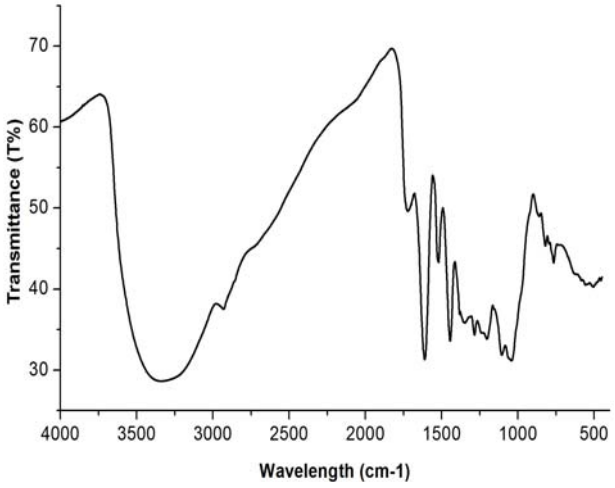

Fig 5: FTIR spectrum of TASB methanolic extract

Thin layer chromatography fingerprinting

The TLC chromatogram of crude methanolic extract of TASB (Fig. 6) showed eleven major bands having R_f values of different spots are: 1 (0.11), 2 (0.27), 3 (0.33), 4 (0.47), 5 (0.59), 6 (0.59), 7 (0.64), 8 (0.76), 9 (0.80), 10 (0.86) and 11 (0.92).

The various bands in TLC of crude methanolic extract of TASB showed the presence of different phyto constituents with characteristic fingerprints. This TLC fingerprint of this extract could be used to check quality and purity of this drug even at very low concentration.

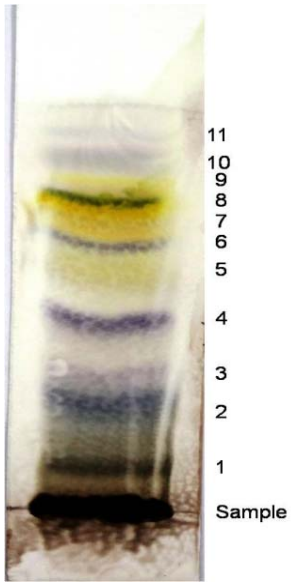

Fig 6: TLC fingerprinting of *T. arjuna* stem bark methanolic extract

GC-MS analysis

Gas Chromatography-Mass spectrometry (GC-MS) is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols acids and esters, *etc*. GC-MS analysis of the methanolic extract of TASB revealed the presence of 10 major compounds (Fig 7). The retention time, name, molecular formula, molecular weight, peak area and nature of the compounds are given in Table 5. The individual mass fragments of these ten major compounds are presented in Fig. 8 (I) and (II).

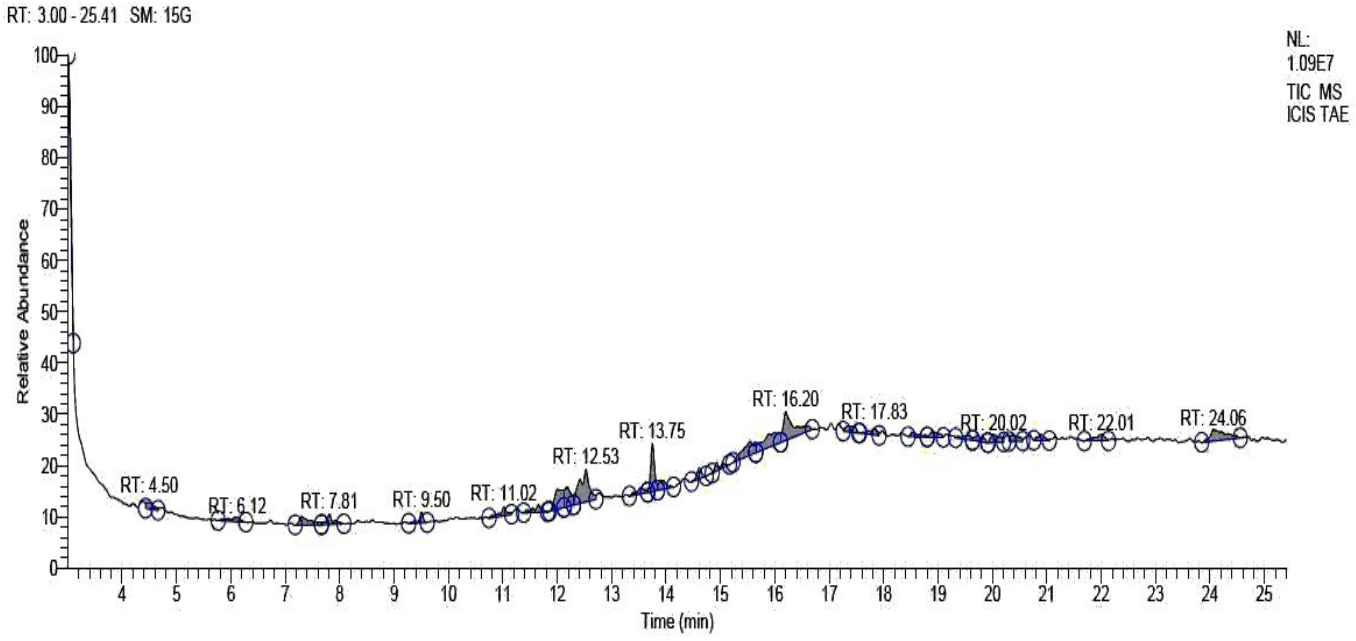

Fig 7: Gas chromatogram spectrum of *T. arjuna* stem bark

Table 5: Phyto components detected in the methanolic extract of of *T. arjuna* stem bark by GC-MS analysis

Sr. No.	RT	Name of compound	Molecular formula	MW	Peak area %	Nature of the compound
1	3.03	1-Methoxyhexane	$C_7H_{16}O$	116.20	4.37	Aliphatic hydrocarbon
2	4.50	9-Oximino 2,7-diethoxyfluorene	$C_{17}H_{17}NO_3$	283.32	1.63	Aromatic hydrocarbon
3	7.30	Heptadecane, 9-hexyl	$C_{23}H_{48}$	324.62	3.09	Alkane hydrocarbon
4	11.65	9,10 Secocholesta 5,7,10(19) triene 3,24,25 triol	$C_{27}H_{44}O_3$	416.63	1.85	Steroid
5	12.01	Butanoic acid, 2,3 dihydroxypropyl ester	$C_7H_{14}O_4$	162.18	5.63	Ester
6	12.53	2-Naphthalene methanol	$C_{15}H_{26}O$	222.36	10.93	Alcohol
7	13.91	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	$C_{27}H_{52}O_4Si_2$	496.87	2.34	Fatty ester
8	15.54	3-Hydroxyspirost-8-en-11-one	$C_{27}H_{40}O_4$	428.60	5.40	Steroidal saponins
9	16.20	Protriptyline	$C_{19}H_{21}N$	263.37	10.15	Secondary amines
10	20.02	Olean-12-ene-3,15,16,21,22,28-hexol, (3α,15α,16α,21α,22α)	$C_{30}H_{50}O_6$	506.71	2.40	Saponin

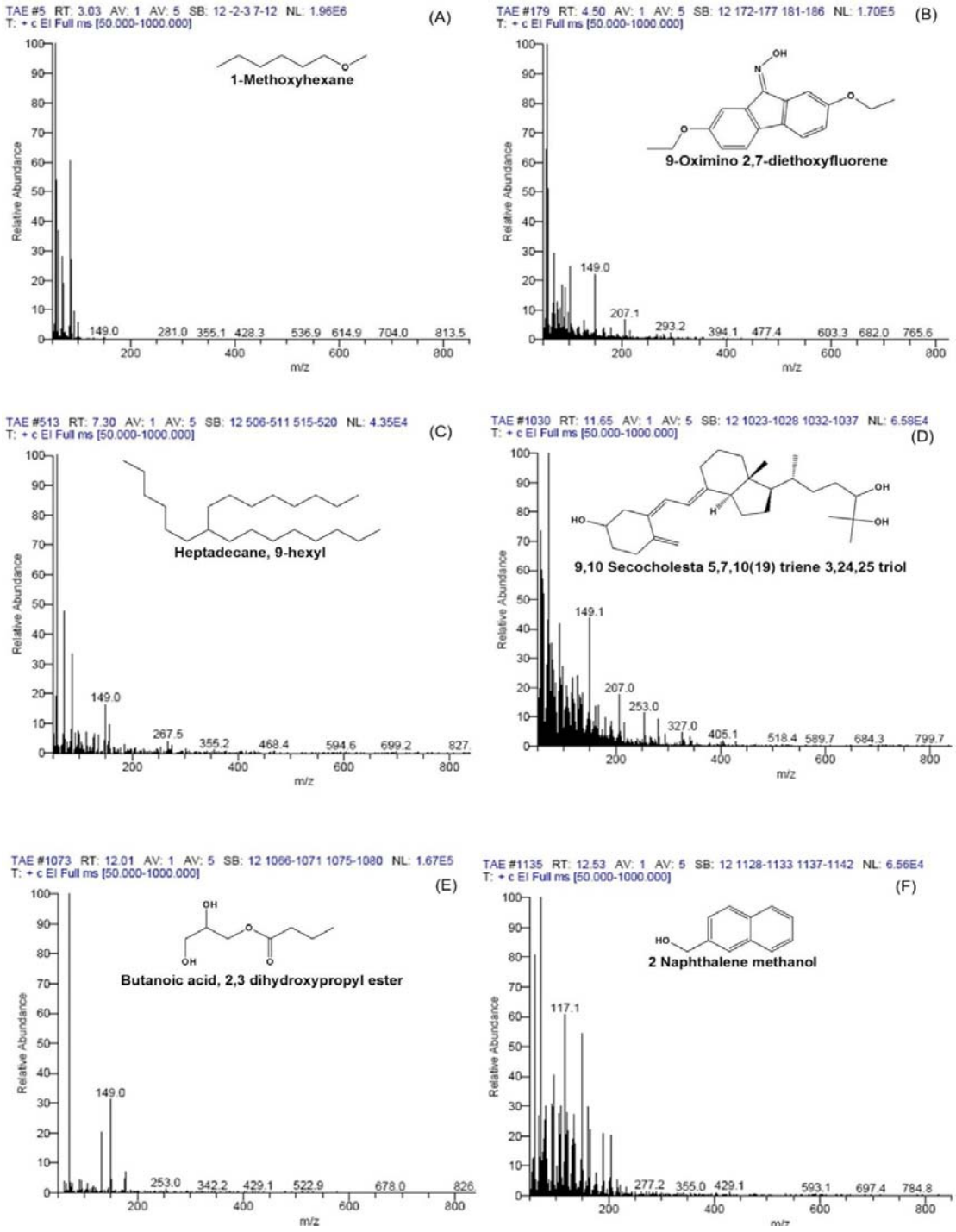

Fig 8(I): The individual mass fragments of some major compounds from methanolic extract of *T. arjuna* stem bark detected by GC-MS analysis

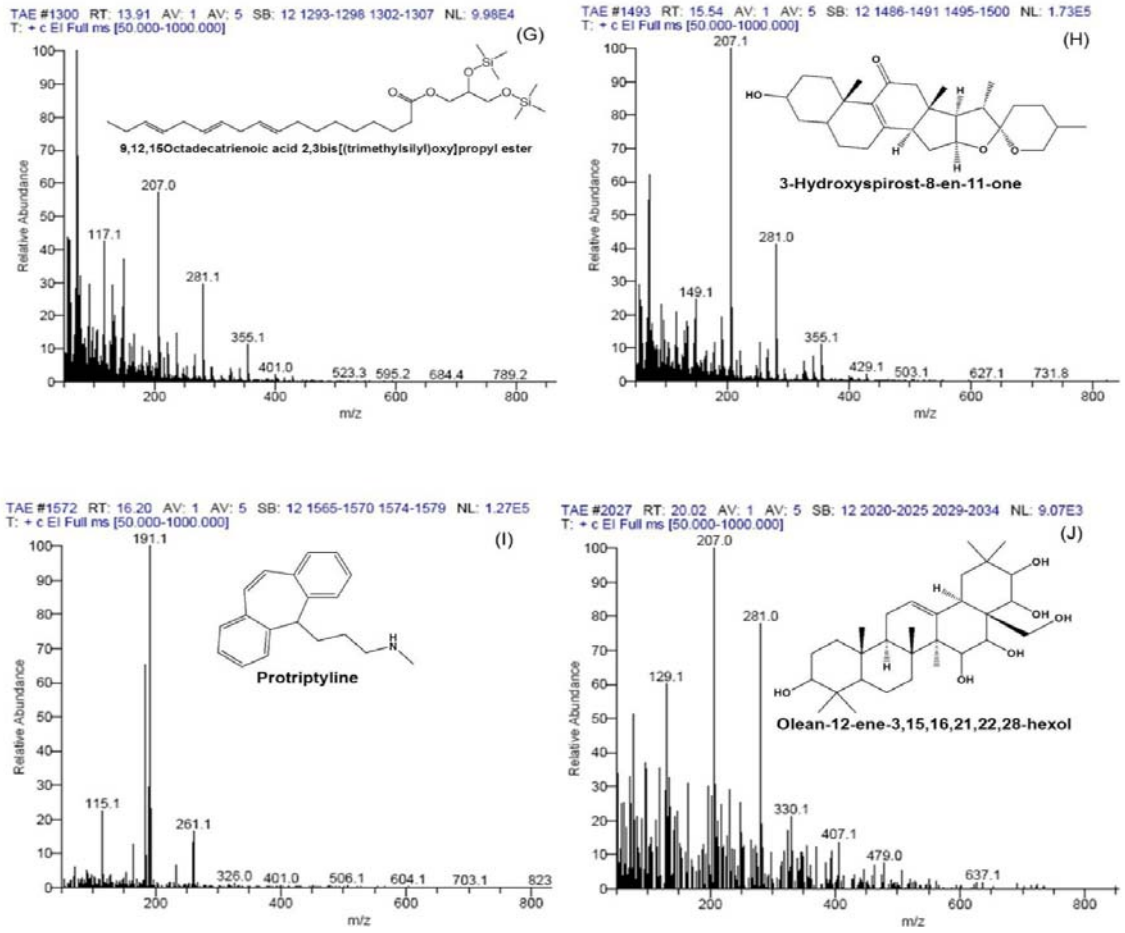

Fig 8(II): The individual mass fragments of some major compounds from methanolic extract of *T. arjuna* stem bark detected by GC-MS analysis

Conclusion

The present study provides various resourceful information in relation to pharmacognostic identification of *T. arjuna* stem bark. Furthermore, information regarding physicochemical characteristics of stem bark and nature of chemical constituents present in them would also be useful for standardization of herbal drug material TASB and enrichment of Ayurvedic Pharmacopeia. TLC fingerprinting and GC-MS analysis could also help to identify and isolate important phyto-constituents. Thus, this type of study may give information on nature of active principles present in the medicinal plants and to identify the plants from their adulterants using isolated compounds as marker. These findings could be helpful in identification, authentication and standardization of TASB and its methanolic extract. It would also help scientists to utilize such needful information regarding the plant material identity and characteristics in building new polyherbal formulations.

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References

1. Mahajan RT, Chaudhari GM. A novel approach towards phytosomal flavonoids. Pharma Science Monitor, an International Journal of Pharmaceutical Sciences. 2012; 3(4):2079- 2121.
2. Pizzorno JE, Murray MT. Textbook of natural medicine. Elsevier Health Sciences, 2012.
3. Thomas S, Patil DA, Patil AG, Chandra N. Pharmacognostic evaluation and physicochemical analysis of *Averrhoa carambola* L. fruit. J Herb Med Toxicol. 2008; 2(2):51-54.
4. Menpara D, Desai D, Chanda S. Pharmacognostic, phytochemical, physicochemical and fluorescence analysis of *Terminalia bellerica* leaf and stem, World Journal of Pharmaceutical Sciences. 2014; 2(4):390-396.
5. Shahriar M, Akhter S, Hossain MI, Haque MA, Bhuiyan MA. Evaluation of in vitro antioxidant activity of bark extracts of *Terminalia arjuna*. Journal of Medicinal Plants Research. 2012; 6(39):5286-5298.
6. Patil RH, Prakash K, Maheshwari VL. Hypolipidemic effect of *Terminalia arjuna* (L.) in experimentally induced hypercholesteremic rats. Acta Biologica Szegediensis, 2011; 55(2):289-293.

7. Nema R, Jain P, Khare S, Pradhan A, Gupta A, Singh D. Antibacterial and antifungal activity of *Terminalia arjuna* leaves extract with special reference to flavonoids. Basic Res J Med Clin Sci. 2012; 1(5):63-65.
8. Aneja KR, Sharma C, Joshi R. Antimicrobial activity of *Terminalia arjuna* Wight & Arn: An ethnomedicinal plant against pathogens causing ear infection. Brazilian Journal of otorhinolaryngology. 2012; 78(1):68-74.
9. Halder S, Bharal N, Mediratta PK, Kaur I, Sharma KK. Anti-inflammatory, immunomodulatory and antinociceptive activity of *Terminalia arjuna* Roxb bark powder in mice and rats. Indian journal of experimental biology. 2009; 47(7):577.
10. Rao BK, Sudarshan PR, Rajasekhar MD, Nagaraju N, Rao CA. Antidiabetic activity of *Terminalia pallida* fruit in alloxan induced diabetic rats. Journal of Ethnopharmacology. 2003; 85(1):169-172.
11. Chaudhari GM, Mahajan RT. Comparative Antioxidant Activity of Twenty Traditional Indian Medicinal Plants and its Correlation with Total Flavonoid and Phenolic Content. International Journal of Pharmaceutical Sciences Review and Research. 2015; 30(1):105-111.
12. Dwivedi S. *Terminalia arjuna* Wight & Arn.—A useful drug for cardiovascular disorders. Journal of ethno pharmacology. 2007; 114(2):114-129.
13. Kumar D, Kumar K, Kumar S, Kumar T, Kumar A, Prakash O. Pharmacognostic evaluation of leaf and root bark of Holoptelea integrifolia Roxb. Asian Pacific journal of tropical biomedicine. 2012; 2(3):169-175.
14. WHO. Quality control methods for medicinal plants. Geneva, 2002, 28-31.
15. Bhowmik S, Datta BK, Saha AK. Determination of mineral content and heavy metal content of some traditionally important aquatic plants of Tripura, India using atomic absorption spectroscopy. Journal of Agricultural Technology. 2012; 8(4):1467-1476.
16. Harborne J. Phytochemical methods, a guide to modern techniques of plant analysis, Springer Science & Business Media, 2012.
17. Kulshrestha MK, Karbhal KS. Effect of different drying methods on the quality of stem bark of *Terminalia arjuna* Roxb. International Journal of Research in Ayurveda & Pharmacy. 2012; 3(4):515-518.
18. Junior JOCS, Costa RMR, Teixeira FM, Barbosa WLR. Processing and Quality Control of Herbal Drugs and Their Derivatives. Quality control of herbal medicines and related areas, 2011, 195.
19. Gami B, Parabia MH. Pharmacognostic evaluation of bark and seeds of *Mimusops elengi* L. Int J Pharm Pharm Sci. 2010; 2(4):110-3.
20. The Ayurvedic Pharmacopoeia of India, Part-I, NISCOM, CSIR, New Delhi, 1999, vol. II, p. 17-18.
21. World Health Organization, Quality Control Methods for Medicinal Plant Materials, WHO, Geneva, Switzerland, 1996.
22. World Health Organization, Quality Control Methods for Medicinal Plant Materials, WHO, Geneva, Switzerland, 2005.
23. Markham KR, Mabry TJ. Ultraviolet-visible and proton magnetic resonance spectroscopy of flavonoids. In The flavonoids Springer US, 1975, 45-77.