Agro-climatic zone-based identification of elite *Terminalia arjuna* accessions concerning to arjunolic acid production

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Received: 16 July 2020 / Accepted: 12 January 2021 / Published online: 1 February 2021
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Abstract

Man has been dependent on vegetation in different forms since antiquity. Plants and trees have been used in the form of medications since the old times. Although the use of plant parts in the form of medicine has been less than that of the plants, therefore today it is necessary to explore some such medicinal trees. Among these, one of these trees is Arjun, whose bark is described also in Ayurveda due to its divine medicinal properties in heart diseases. The present study based on the isolation and analysis of the Phyto-constituents of the stem bark extract of *Terminalia arjuna* member to the family Combretaceae collected from different agro-climatic zones of India. The samples were subjected to quantitative phytochemical analysis i.e., arjunolic acid, screening by implementing the standard procedure. Observation has shown the presence of arjunolic acid in the stem bark extracts in different fractions obtained by the use of various organic solvents. Therefore, the bark extracts of the chosen plants may function as a good source of components of useful drugs for cardiovascular disorders and may also be used for the preparation of other pharmaceutical products.

Keywords Arjunolic acid · Combretaceae · HPTLC · Agroclimatic zone

1 Introduction

Men have been using plant-based medicines since time immemorial. Almost every civilization has a history of using medicinal plants. According to Ncube et al. [1], Medicinal plants are the prerequisite for the traditional systems of medicines, pharmaceutical industries for synthetic drugs. In ancient times, Egyptians, Indians, Chinese, Africans and others used a variety of plant products for curing all kinds of ailments [2]. The hypocholesteremic, hypolipidemic, anticoagulant, antihypertensive, antithrombotic, antiviral, antifungal and antibacterial activities of arjunolic acid were seen by the Tripathi and Singh [3] and Pettit et al. [4] in their findings. The effectiveness of arjunolic acid in many cardiac disorders like angina, myocardial infarction, hypertension, hypercholesteremia, cardiac arrest etc. were

reported by the Rose and Treadway [5] and Khan and Balick [6]. Other studies also reported that its bark retains significant hypotensive effect, increasing coronary artery flow and protecting myocardium against ischemic damage and mild diuretic, antithrombotic, prostaglandin E (2) enhancing and hypolipidemic activity of arjunolic acid the experimental findings by the Dwivedi [7].

Thus, medicinal plants are the local heritage with global importance. The number of flowering plants on the earth estimated presently is about 2, 50,000 species of which nearly 70,000 species are used for medicinal purposes, both in developed and developing countries [8]. An estimate suggests that about 13,000 plant species are known to have worldwide use as drugs.

It is reported that 41% prescription in the USA and 50% in Europe contains constituents from natural products.

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Phytochemical tests have been performed on about 5000 species; and nearly 1100 species are extensively exploited in 80% of Ayurvedic, 46% of Unani and 33% of Allopathic medicines [9]. India has 2.4% of the world's land area with 8% of global biodiversity and harbors one of the 12-mega diversity centers, having over 45,000 plant species. Its diversity is unmatched due to the presence of 16 different agro-climatic zones, 10 vegetative zones and 15 biotic provinces [10]. The country accounts for 17,500 species of higher plants. Of these, more than 2000 documented species exhibit medicinal value vis-à-vis 1100 species being used in different systems of medicines.

International trade in medicinal plants and phytopharmaceutical preparations is a major force in the world economy. Their demands are increasing both in developing and industrialized nations. A report prepared by the Export–Import Bank of India has estimated that the international market of medicinal plant-related products is in the range of US\$ 107 billion with an annual growth rate of 7% (According to Global Summit on Herbs & Natural Remedies, Chicago, USA). According to research from global Industry Analysis, the global herbal supplement and remedy industry has been estimated to be \$ 107 billion by 2017. India's share in the global herbal market in the year 2017 is of the order of 2 billion dollars. India is the second-largest exporter, next to China. According to the current estimation, phytomedicines used in health care globally by 40% of the total population [11].

A different system of treatments such as ayurvedic, Unani, homeopathy and siddha originated in India. India is the birth-place of the renewed system of indigenous medicine such as Unani, Ayurveda, Homeopathy and Siddha. In India, nearly 95% of the prescriptions were planted primarily based on ancient systems [12]. In this study, the quantitative estimation of Phyto-constituents of stem bark of *Terminalia arjuna* commonly referred to as "Arjun" (fam-Combretaceae), was done for the presence of arjunolic acid.

The study was conducted to assess the natural variation of *Terminalia arjuna* to investigate the populations along with the natural habitats of this medicinal plant for the phytochemical contents upon comparison from nine states and five different agro-climatic zones of India. The main scope of this experiment was the selection of the *Terminalia arjuna* accessions with a high content of arjunolic acid from the various population collected from different agro-climatic zones.

2 Materials and methods

2.1 Collection and processing of plant samples

Plant samples (mapping population) of *Terminalia arjuna* were obtained from All India germplasm of arjun established between 2001 and 2004 at Central Tasar Research and Training Institute (CTRTI), Nagri, Ranchi that maintains superior arjun accessions from nine states, viz., Andhra Pradesh, Assam, Chhattisgarh, Jharkhand, Maharashtra, Madhya Pradesh, Orissa, Uttarakhand and Uttar Pradesh altogether representing five agro-climatic zones, viz., Eastern plateau and hill (EPH) regions, Southern plateau and hills (SPH) region, Eastern Himalayan (EH) Region, Western Himalayan (WH) region and Central plateau and hills (CPH) region (Fig. 1). For the selection of various accessions of *Terminalia arjuna* tress, some criteria were chosen: the tress growth should be vigorous, healthy and showing superiority in height and diameter i.e., straight, cylindrical, non-forking, non-twisting bole when compared with surrounding trees and resistant to pests and diseases. The distance between the collections of two accessions must be at least 200 m from their natural habitat. A total of 140 accessions were sampled for bark patch (10 cm x 10 cm) at 1.34 m diameter breast height (DBH) for the estimation of arjunolic acid content. The details of the accessions of arjun represent different agro-climatic regions bark are given in Table 1.

2.2 Chemicals

Only HPLC and/or analytical grade chemicals and reagents (Sigma, USA; Himedia, USA; Merck, Germany, etc.) were used during the present study.

2.3 Collection and processing of bark patch

A bark patch of 10 cm x 10 cm was sustainably removed at 1.34 m from each of 140 accessions of *Terminalia arjuna*. The thickness of the sampled bark patch was measured at several places by Vernier calliper. The bark was washed with distilled water, followed by the estimation of its fresh weight. Subsequently, the bark patch was shrouded and cut into the pieces and dried at room temperature in the shade and watched regularly to reduce the attack of fungi. It took a week or so until attaining a constant dry weight that measured and recorded. A fine powder of dried bark patches was made with the help of grinder and sieved through a 25 mm fine mesh and divided into three parts representing as replicates for

Fig. 1 Map showing the state-wise location of the sampled mapping population

extraction and estimation of arjunolic acid and stored in air-tight containers with necessary markings for identification and kept in a cool, dark and dry place for further use.

2.3.1 Extraction of arjunolic acid from bark powder

200 mg bark powder was exactly weighed and placed in a conical flask (100 ml) and then mixed with 20 ml of ethyl acetate. All conical flasks were retained for 10 min for pre-soaking as pre-leaching. It was followed by an extraction process that was performed by warming the content at 65 °C for five min, i.e. below boiling point (71.1 °C) of the ethyl acetate in a 600 W microwave oven. An irradiation treatment was given to the samples under microwave for one minute followed by cooling for 1 min between two irradiations. Whatman filter paper no.1 was used for the filtration of suspensions. The residue was washed twice with ethyl acetate. The washings were pooled with the filtrate and the solvent was vacuum evaporated at 40 °C (Vacuum Oven, TEMPO Instruments Pvt. Ltd) and lyophilized. The solvent-free residue was dissolved in 1 ml HPLC grade methanol and transferred to 1.5 ml Eppendorf tubes

and stored at –20 °C in the deep freezer until estimation of arjunolic acid on HPTLC.

2.4 Arjunolic acid standard

A pure sample of arjunolic acid (MW 488.70, Melting point 296 °C, purity > 99%) as one mg powder (vial) was purchased from Sigma-Aldrich India, was used in the concentration 1000 µg/ml. The working standard solution of 100 µg/ml was prepared from the standard stock solution by drawing a known volume of the latter and diluting in the ratio of 1:10 by HPLC grade methanol. A volume of the working standard (arjunolic acid) solution equal to that of the bark sample was loaded alongside the latter on the HPTLC plate. The peak area of the standard arjunolic acid was used for the computation of arjunolic acid in the bark sample extract.

2.5 HPTLC processing and estimation of arjunolic acid in the bark extract

HPTLC processing needs a clean pre-coated and activated silica aluminum plate, a micro-syringe (dispenser) for loading standard solution/ sample extract, oven, twin trough

Table 1 The details of the *Terminalia arjuna* accessions collected from CTRTI

Tree No	Accession no	State code	Sub-agroclimatic zone	Agroclimatic zone	Latitude	Longitude	Elevation	Plant girth (cm)	Bark thickness (cm)	Bark fresh weight(g)	Bark dry weight (g)
1	101	JHRNRN08	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.1839	85.1826	655	46	1.0	23.04	8.61
2	102	MHCPCP01	Eastern Vidarbha Zone	Eastern Plateau and Hills region	20.1848	79.9948	195	26	0.6	15.12	6.18
3	103	MHCPCP02	Eastern Vidarbha Zone	Eastern Plateau and Hills region	20.1848	79.9948	195	29	0.9	18.68	5.26
4	104	JHRNSH01	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.1839	85.1826	655	35	0.5	15.50	5.91
5	106	UPJNJN01	Bundelkhand Zone	Central Plateau and Hills region	25.4484	78.5684	253	31.5	0.5	15.88	5.22
6	107	MHCPCP05	Bundelkhand Zone	Eastern Plateau and Hills region	19.9704	79.3015	203	20	0.6	14.50	5.38
7	112	MHCPCP06	Eastern Vidarbha Zone	Eastern Plateau and Hills region	19.9704	79.3015	203	37.5	0.9	19.95	7.60
8	113	UKUSTD	Bhabar and Tarai Zone	Western Himalayan region	28.9448	79.4467	198	20.5	0.6	13.48	4.45
9	115	UKNTHD	Bhabar and Tarai Zone	Western Himalayan region	29.2183	79.5129	419	38	1.0	22.88	8.58
10	116	UPJNJN02	Bundelkhand Zone	Central Plateau and Hills region	25.4484	78.5684	253	26	0.8	19.10	7.75
11	117	CGDT01	Chhattisgarh Plain Zone	Eastern Plateau and Hills region	20.6118	81.7787	404	19.5	0.4	9.88	3.16
12	118	CGDITD02	Chhattisgarh Plain Zone	Eastern Plateau and Hills region	20.6118	81.7787	404	35	0.7	16.52	6.17
13	119	ODMBBP	North Central Plateau	Eastern Plateau and Hills region	21.9322	86.7517	44	40	1.0	22.88	9.14
14	122	MHBDAL	Eastern Vidarbha Zone	Eastern Plateau and Hills region	21.1750	79.6558	256	45	1.0	25.34	10.26
15	123	JHRNSH02	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.1839	85.1826	655	38	0.6	16.18	5.44
16	124	MHBD8D1	Eastern Vidarbha Zone	Eastern Plateau and Hills region	21.1750	79.6558	256	21	0.5	13.17	3.95
17	125	MHBD8D2	Eastern Vidarbha Zone	Eastern Plateau and Hills region	21.1750	79.6558	256	42	1.1	23.51	7.80
18	126	MHBD8D3	Eastern Vidarbha Zone	Eastern Plateau and Hills region	21.1750	79.6558	256	36	0.8	18.06	6.43
19	129	ODSGSG01	North Western Plateau Zone	Eastern Plateau and Hills region	22.1240	84.0431	251	33	0.8	17.84	6.99
20	132	CGDT03	Chhattisgarh Plain Zone	Eastern Plateau and Hills region	20.6118	81.7787	404	28	0.5	12.95	4.68

Table 1 (continued)

Tree No	Accession no	State code	Sub-agroclimatic zone	Agroclimatic zone	Latitude	Longitude	Elevation	Plant girth (cm)	Bark thickness (cm)	Bark fresh weight(g)	Bark dry weight (g)
21	133	CGDSTD04	Chhattisgarh Plain Zone	Eastern Plateau and Hills region	20.6118	81.7787	404	29	0.7	21.15	9.11
22	134	JHRNSH03	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.1839	85.1826	655	42	0.9	21.92	7.69
23	135	ODSGSG02	North Western Plateau Zone	Eastern Plateau and Hills region	22.1240	84.0432	251	46	1.2	31.66	10.39
24	137	JHRNSH04	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.1839	85.1826	655	32	0.8	19.29	6.71
25	138	CGBTJP01	Bastar Plateau Zone	Eastern Plateau and Hills region	19.0741	82.0080	564	51	1.1	29.43	10.94
26	140	CGBTJP02	Bastar Plateau Zone	Eastern Plateau and Hills region	19.0741	82.0080	564	52	1.0	24.92	10.63
27	143	JHRNSH05	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.1839	85.1826	655	50	0.9	22.15	7.63
28	201	CGSGBN	North Hills Zone	Eastern Plateau and Hills region	20.7443	82.2094	354	35	1.0	24.77	9.14
29	202	CGDSTD05	Chhattisgarh Plain Zone	Eastern Plateau and Hills region	20.6118	81.7787	404	19.5	0.6	13.78	5.44
30	203	CGBTAP	Bastar Plateau Zone	Eastern Plateau and Hills region	23.1355	83.1818	601	25	0.4	10.16	3.15
31	204	CGSGMP	North Hills Zone	Eastern Plateau and Hills region	22.9951	83.1944	586	38	0.7	17.46	5.45
32	205	CGSGSG	North Hills Zone	Eastern Plateau and Hills region	23.7605	83.2013	460	36.5	0.6	16.00	5.83
33	206	CGSGWN3	North Hills Zone	Eastern Plateau and Hills region	21.7031	82.5213	223	21	0.4	12.19	4.06
34	207	JHRNSL01	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.1839	85.1826	655	44	0.8	20.28	6.39
35	209	JHRNSL02	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.3434	85.8294	268	43.5	0.8	20.18	6.64
36	210	JHRNSL03	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.3434	85.8294	268	41.5	0.8	22.58	8.22
37	211	JHRNSL04	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.3434	85.8294	268	33	0.5	13.15	4.81
38	212	JHRNSL05	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.3434	85.8294	268	44	1.3	30.38	9.45
39	213	JHRNRN01	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.1960	85.7077	256	25	0.7	18.48	6.64

Table 1 (continued)

Tree No	Accession no	State code	Sub-agroclimatic zone	Agroclimatic zone	Latitude	Longitude	Elevation	Plant girth (cm)	Bark thickness (cm)	Bark fresh weight(g)	Bark dry weight (g)
40	214	JHRN02	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.1960	85.7077	256	23	0.5	12.11	4.17
41	215	JHRN03	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.1960	85.7077	256	33	0.8	20.37	6.92
42	217	JHRN04	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.1960	85.7077	256	27	0.7	16.74	5.62
43	218	MHBD8D8	Eastern Vidarbha Zone	Eastern Plateau and Hills region	21.1663	79.6570	272	27	0.6	15.21	5.54
44	220	MHBDGV	Eastern Vidarbha Zone	Eastern Plateau and Hills region	21.2559	80.0251	341	35	0.8	19.88	6.22
45	222	JHRN05	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.1960	85.7077	256	31.5	0.9	23.80	8.42
46	224	CGDTSG	Chhattisgarh Plain Zone	Eastern Plateau and Hills region	21.3645	81.8060	275	27	0.7	17.57	7.60
47	225	JHRN06	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.1960	85.7077	256	41	0.7	18.63	6.69
48	227	MHCPMD3	Eastern Vidarbha Zone	Eastern Plateau and Hills region	20.4667	79.7999	241	28	0.7	18.33	7.95
49	228	JHRN07	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.1960	85.7077	256	35	1.1	27.03	9.54
50	230	CGSJ04	North Hills Zone	Eastern Plateau and Hills region	23.2852	82.5375	551	30.5	0.8	20.53	8.90
51	231	JHRNBD01	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.1599	85.5869	315	48	1.0	30.59	10.76
52	232	JHRNBD02	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.1599	85.5869	315	30.5	0.8	15.50	5.50
53	233	JHRNBD03	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.1599	85.5869	315	27	0.6	13.73	4.71
54	234	JHRNBD04	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.1599	85.5869	315	43	0.9	22.33	8.33
55	235	JHRNBD05	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.1599	85.5869	315	30	0.7	17.85	7.08
56	236	JHRNBD06	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.1599	85.5869	315	33.5	0.8	18.18	6.34
57	237	ODSGBK	North Western Plateau Zone	Eastern Plateau and Hills region	22.1240	84.0431	251	43	0.8	23.01	9.43
58	243	JHRNBD07	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.1599	85.5869	315	40.5	1.0	24.13	8.50

Table 1 (continued)

Tree No	Accession no	State code	Sub-agroclimatic zone	Agroclimatic zone	Latitude	Longitude	Elevation	Plant girth (cm)	Bark thickness (cm)	Bark fresh weight(g)	Bark dry weight (g)
59	244	JHRNBD08	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.1599	85.5869	315	44	0.9	22.90	8.04
60	301	JHDGK	Central & Western Plateau Zone	Eastern Plateau and Hills region	24.0420	84.0907	229	40	0.8	20.66	8.07
61	302	MHGCGC1	Eastern Vidarbha Zone	Eastern Plateau and Hills region	20.4667	79.7999	241	58	0.7	16.66	6.32
62	303	JHGWKJ	Central & Western Plateau Zone	Eastern Plateau and Hills region	24.1736	83.7491	220	46	0.5	12.66	4.28
63	304	MHGDGD1	Eastern Vidarbha Zone	Eastern Plateau and Hills region	20.6031	75.1253	291	36	0.7	16.41	5.62
64	305	JHGWBG	Central & Western Plateau Zone	Eastern Plateau and Hills region	22.0571	84.6897	419	40	0.6	13.94	4.28
65	306	JHGWBW	Central & Western Plateau Zone	Eastern Plateau and Hills region	22.0571	84.6897	419	26	0.5	14.84	5.30
66	307	MHGCGC2	Eastern Vidarbha Zone	Eastern Plateau and Hills region	20.4667	79.7999	241	44.5	0.7	18.30	7.06
67	309	JHESBT	South Eastern Plateau Zone	Eastern Plateau and Hills region	22.4867	86.4996	95	57	1.2	27.92	10.48
68	330	APABKP05	North Telangana Zone	Southern Plateau and Hills region	18.8476	79.7566	121	36	0.8	20.38	8.01
69	332	APMD01	Central Telangana Zone	Southern Plateau and Hills region	17.8716	78.1108	490	40	0.7	18.56	6.71
70	333	CGSGDM	North Hills Zone	Eastern Plateau and Hills region	22.9494	83.1649	585	45	0.8	17.86	7.39
71	334	CGSGWN1	North Hills Zone	Eastern Plateau and Hills region	23.7605	83.2013	460	20	0.7	18.12	5.60
72	335	CGSGWN2	North Hills Zone	Eastern Plateau and Hills region	23.7605	83.2013	460	48	1.0	23.84	9.99
73	337	CGSGOD	North Hills Zone	Eastern Plateau and Hills region	23.2852	82.5375	551	30.5	0.8	21.31	8.03
74	338	MHPCPCP07	Eastern Vidarbha Zone	Eastern Plateau and Hills region	19.9704	79.3015	203	45	0.8	20.82	7.43
75	407	CGSGKH	North Hills Zone	Eastern Plateau and Hills region	22.8372	83.3148	1096	43	0.6	15.72	5.62
76	414	MHBDNJ	Eastern Vidarbha Zone	Eastern Plateau and Hills region	20.7939	79.6368	252	29	0.8	19.25	6.72
77	417	MHBDPH	Eastern Vidarbha Zone	Eastern Plateau and Hills region	20.7939	79.6368	252	31	0.7	17.51	6.08

Table 1 (continued)

Tree No	Accession no	State code	Sub-agroclimatic zone	Agroclimatic zone	Latitude	Longitude	Elevation	Plant girth (cm)	Bark thickness (cm)	Bark fresh weight(g)	Bark dry weight (g)
78	422	JHWSAT	South Eastern Plateau Zone	Eastern Plateau and Hills region	22.3651	85.4375	349	45	0.8	21.43	8.45
79	424	UKHDHD	Bhabar and Tarai Zone	Western Himalayan region	29.9457	78.1642	287	22	0.6	12.58	4.43
80	425	UPBDBD01	Bundelkhand Zone	Central Plateau and Hills region	25.4796	80.3380	116	40	1.0	26.53	9.40
81	430	JHGWWRK02	South Eastern Plateau Zone	Eastern Plateau and Hills region	22.2779	86.7249	272	42	1.1	27.66	12.88
82	431	JHWSHJ	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.1839	85.1826	655	39	0.8	21.61	6.95
83	432	APRRRR01	South Telangana Zone	Southern Plateau and Hills region	17.3891	77.8367	596	29	0.9	18.10	6.24
84	433	APABAB01	North Telangana Zone	Southern Plateau and Hills region	19.0809	79.5603	209	41	0.8	15.50	5.68
85	434	APMDMD2	Central Telangana Zone	Southern Plateau and Hills region	17.8716	78.1108	490	35	0.7	18.06	5.77
86	435	UKCMKP	Hill Zone	Western Himalayan region	30.2587	79.2183	734	37	0.7	18.41	6.73
87	438	APABAB02	North Telangana Zone	Southern Plateau and Hills region	19.0809	79.5603	209	34	0.7	19.67	7.65
88	439	JHGWGW	Central & Western Plateau Zone	Eastern Plateau and Hills region	24.1084	83.6774	258	30	0.6	16.13	6.01
89	441	JHWSGS02	South Eastern Plateau Zone	Eastern Plateau and Hills region	22.5873	86.4744	110	35	0.8	20.38	7.76
90	442	UKPGPG	Hill Zone	Western Himalayan region	29.8688	78.8382	886	27	0.4	8.75	3.30
91	444	JHWSAD01	South Eastern Plateau Zone	Eastern Plateau and Hills region	22.3651	85.4375	349	37.5	0.6	15.49	5.29
92	504	APRRRR02	South Telangana Zone	Southern Plateau and Hills region	17.3891	77.8367	596	48	0.7	17.83	6.86
93	505	APKN01	North Telangana Zone	Southern Plateau and Hills region	18.4385	79.1288	271	48	1	26.32	10.71
94	506	APMDMD3	Central Telangana Zone	Southern Plateau and Hills region	17.8716	78.1108	490	60	1.2	34.26	15.05
95	507	JHGWWRK01	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.9915	83.7863	343	24	0.4	10.92	4.08
96	508	JHLTLT	Central & Western Plateau Zone	Eastern Plateau and Hills region	23.7556	84.3542	365	32	0.6	16.76	5.96
97	509	APRRVB01	North Telangana Zone	Southern Plateau and Hills region	18.2579	78.5845	454	31	0.7	19.03	6.31

Table 1 (continued)

Tree No	Accession no	State code	Sub-agroclimatic zone	Agroclimatic zone	Latitude	Longitude	Elevation	Plant girth (cm)	Bark thickness (cm)	Bark fresh weight(g)	Bark dry weight (g)
98	511	UP8DBD02	Bundelkhand Zone	Central Plateau and Hills region	25.4796	80.3380	116	29	0.7	18.80	6.53
99	512	JHWSG03	South Eastern Plateau Zone	Eastern Plateau and Hills region	22.5873	86.4744	110	39	1.0	27.46	10.93
100	513	JHWSAD02	South Eastern Plateau Zone	Eastern Plateau and Hills region	22.3651	85.4375	349	31	0.7	17.56	5.80
101	515	UP8DBD03	Bundelkhand Zone	Central Plateau and Hills region	25.4796	80.3380	116	36.5	1.0	23.78	7.43
102	516	JHWSBG	South Eastern Plateau Zone	Eastern Plateau and Hills region	22.3651	85.4375	349	34	0.5	13.60	6.00
103	517	APRRVB02	South Telangana Zone	Southern Plateau and Hills region	17.3364	77.9048	625	27	0.8	24.27	9.44
104	521	JHRNSH06	Central & Western Plateau Zone	Eastern Plateau and Hills region	22.7419	85.8165	187	41	1.5	41.21	17.84
105	525	APMDMD4	Central Telangana Zone	Southern Plateau and Hills region	17.8716	78.1109	490	37	1.0	24.85	9.28
106	533	UPSBRG	Vindhyan Zone	Central Plateau and Hills region	24.6850	83.0683	315	47.5	1.5	38.30	14.36
107	536	APKNKN02	North Telangana Zone	Southern Plateau and Hills region	18.4385	79.1288	271	45	1.4	34.73	15.02
108	537	JHWSBT01	South Eastern Plateau Zone	Eastern Plateau and Hills region	22.3651	85.4376	349	36	0.7	16.64	5.82
109	539	JHWSLD	South Eastern Plateau Zone	Eastern Plateau and Hills region	23.3351	85.3025	655	38	0.7	17.49	6.06
110	540	JHWSKS02	South Eastern Plateau Zone	Eastern Plateau and Hills region	22.7419	85.8165	187	26	0.8	19.37	6.31
111	541	JHWSBT02	South Eastern Plateau Zone	Eastern Plateau and Hills region	22.3651	85.4375	349	32	0.7	19.11	6.62
112	542	JHWSKS03	South Eastern Plateau Zone	Eastern Plateau and Hills region	22.7419	85.8165	187	37	0.7	18.60	7.32
113	615	UKDDL01	Bhabar and Tarai Zone	Western Himalayan region	28.9366	79.4619	199	33.5	1.1	26.85	8.75
114	618	APHBMM	South Telangana Zone	Southern Plateau and Hills region	17.3347	78.1999	597	28	0.7	20.96	7.10
115	621	APABKP	Krishna Zone	Southern Plateau and Hills region	18.2579	78.5845	454	21	0.4	11.42	3.80
116	622	UKDDDD01	Bhabar and Tarai Zone	Western Himalayan region	30.3165	78.0322	651	39	1.0	26.01	9.96
117	624	UKDDDD02	Bhabar and Tarai Zone	Western Himalayan region	30.3165	78.0322	651	26	0.7	20.06	8.74

Table 1 (continued)

Tree No	Accession no	State code	Sub-agroclimatic zone	Agroclimatic zone	Latitude	Longitude	Elevation	Plant girth (cm)	Bark thickness (cm)	Bark fresh weight(g)	Bark dry weight (g)
118	632	APWGNP	Eastern Vidarbha Zone	Southern Plateau and Hills region	16.4330	81.6966	17	29	0.6	16.65	5.36
119	701	MHBDP01	Eastern Vidarbha Zone	Eastern Plateau and Hills region	21.0204	79.6811	250	69	1.1	30.21	12.25
120	702	MHGDGD2	Eastern Vidarbha Zone	Eastern Plateau and Hills region	20.9107	80.0884	277	45	1.0	23.35	9.19
121	703	MHGDGD3	Eastern Vidarbha Zone	Eastern Plateau and Hills region	21.4624	80.2209	296	37	0.6	13.04	4.40
122	704	MHGDGD4	Eastern Vidarbha Zone	Eastern Plateau and Hills region	21.4624	80.2209	296	45	1.0	24.57	8.07
123	706	MHCPCP08	Eastern Vidarbha Zone	Eastern Plateau and Hills region	19.9704	79.3015	203	32	0.6	16.19	4.99
124	707	MHCPCP03	Eastern Vidarbha Zone	Eastern Plateau and Hills region	20.1849	79.9948	195	33.5	0.5	13.56	5.10
125	709	MHCPCP09	Eastern Vidarbha Zone	Eastern Plateau and Hills region	19.9704	79.3015	203	33	0.6	14.91	4.77
126	710	MHCPGG	Eastern Vidarbha Zone	Eastern Plateau and Hills region	19.9704	79.3015	203	26	0.4	11.43	4.40
127	711	MHBD8D4	Eastern Vidarbha Zone	Eastern Plateau and Hills region	19.9704	79.3014845	203	21	0.6	14.28	4.96
128	715	MHCPMD1	Eastern Vidarbha Zone	Eastern Plateau and Hills region	21.1750	79.6558	256	24	0.4	22.44	7.04
129	717	MHBD8D5	Eastern Vidarbha Zone	Eastern Plateau and Hills region	19.9704	79.3015	203	27	0.8	23.47	7.85
130	718	MPTGOC01	Bundelkhand Zone	Central plateau and hills region	25.3682	78.6285	222	27	0.8	26.31	9.94
131	720	MPTGOC02	Bundelkhand Zone	Central plateau and hills region	25.3683	78.6285	222	24.5	0.6	14.89	5.44
132	723	MHGDNV	Eastern Vidarbha Zone	Eastern Plateau and Hills region	20.1849	79.9948	195	42	0.9	22.95	7.31
133	727	APABCN04	North Telangana Zone	Southern Plateau and Hills region	13.2172	79.1003	309	31	0.7	19.69	6.43
134	729	APWGWG	Central Telangana Zone	Southern Plateau and Hills region	17.9689	79.5940	253	24	0.4	10.72	3.78
135	730	APABAB03	North Telangana Zone	Southern Plateau and Hills region	19.0809	79.5603	209	34	0.8	21.40	8.32
136	731	MHCPMD2	Eastern Vidarbha Zone	Eastern Plateau and Hills region	19.9704	79.3015	203	26	0.6	16.23	4.98

Table 1 (continued)

Tree No	Accession no	State code	Sub-agroclimatic zone	Agroclimatic zone	Latitude	Longitude	Elevation	Plant girth (cm)	Bark thickness (cm)	Bark fresh weight(g)	Bark dry weight (g)
137	733	MHBD8D6	Eastern Vidarbha Zone	Eastern Plateau and Hills region	21.1750	79.6558	256	29	0.5	17.76	6.62
138	735	MHBD8D0	Eastern Vidarbha Zone	Eastern Plateau and Hills region	21.1750	79.6558	256	28.5	0.6	16.68	6.69
139	737	MHBDPU2	Eastern Vidarbha Zone	Eastern Plateau and Hills region	19.2846	72.8578	17	27	0.6	15.84	6.08
140	739	ASKRKR	Lower Brahmaputra Valley Zone	Eastern Himalayan Region	26.3161	91.5984	34	25	0.5	15.67	5.46

Table 2 Specification of TLC silica gel aluminium plates

Parameter	Specification
Layer thickness	0.2 mm
Specific surface area (according to BET; 5-Pt. measurement)	480–540 m ² /g
Pore volume (N ₂ -isotherm)	0.74–0.84 ml/g
Laser diffraction	d 50
Size distribution	9.5–11.5 µm,
Layer thickness:	165–235 µm,
Deviation of layer thickness per plate	≤ 35 µm,
Colour test	bleu vif organol
Lipophile	11–25

chamber (TTC), a mobile phase/ developing solvent and derivatization solvent for visualization chromatogram in visual light. The chromatogram of the standard arjunolic acid and sample extracts are subjected to a densitometric scanner for the remission of light proportionate to content concentration in the sample. A software program controls the entire operation. For the purpose, HPTLC system components and software, i.e. CAMAG TLC scanner-3 instrument, equipped with Linomat V applicator and CATS 3.1 software (CAMAG Chemie-Erzeugnisse & Adsorptionstechnik AG, Switzerland) were used for estimation of arjunolic acid concentration in the bark samples.

2.5.1 TLC plate specification and processing

HTPLC silica gel plates of dimension 20 cm x 10 cm (60 F 254, Merck, India) with specifications given in Table 2 were used. The plates were marked with a pencil for the direction of development and developed with 20 ml methanol per trough in a 20 × 10 cm TTC to the upper edge. Subsequently, they were dried in a clean drying oven at 100 °C for 20 min and allowed to equilibrate with lab atmosphere (temperature, relative humidity) in a suitable container free from dust and fumes. The heat treatment was given to the plates at 120 °C for half an hour for activation before the use and held either on both side edges or on the top edge. Most of the time, the plates were used without pre-treatment unless chromatography produced impurity fronts due to their contamination.

2.5.2 Loading standard compound and sample extract on the plate

An 8 µl standard working stock (100 ppm arjunolic acid) or 5 µl bark extract (equivalent to 1 mg dry bark powder) was loaded on the plates with help of CAMAG Linomat 5 applicator whose parameters are given in Table 3. The samples

Table 3 CAMAG Linomat 5 applicator specification

Parameter	Specification
Spray gas	Inert gas
Sample solvent type	Methanol
Dosage speed	150 nl/s
Pre-dosage volume	0.2 µl
Syringe size	100 µl
Number of tracks	6–18
Application position Y	8.0 mm
Band length	7.0 mm

Table 4 Parameter specifications set for HPTLC silica gel aluminium plates for arjunolic acid

Parameter	Specification
Distance from lower edge of plate for TTC	8 mm
Minimum distance from left and right edge of plate	10 mm
Minimum space in mm between bands	4 mm
Band length	7 mm

were applied as bands by spray-on technique following the scheme given in Table 4.

2.5.3 Chromatogram development for arjunolic acid

Plates were developed in a saturated 20 cm x 10 cm TTC. The chamber was opened and a piece of the filter of size 20 cm x 10 cm was placed in the rear through. A 20 ml of freshly prepared mobile phase/developing solvent (Composition: 60 ml toluene, 30 ml ethyl acetate, 1 ml formic acid and methanol 10 ml) was poured into the chamber prepared so that the filter paper thoroughly wetted and adhered to rear TTC wall. Subsequently, the chamber was tilted to the side (about 45°) so that the solvent volume in both troughs got equalized. The chamber was placed on the bench and replaced the lid, allowing for 20 min for its equilibration.

The preferred developing distance (60 mm from the lower edge of the plate) with a pencil on the right edge of the plate was marked with the lid having been slide off to the side for the insertion of the plate into the front trough. The plate was adjusted in such a way that its layer faced the filter paper and it's back rested against the front wall of the TTC. The lid was replaced and the plate was developed to the mark. It was followed by the removal of the plate from TTC by opening the lid. The plate was dried vertically in direction of chromatography in a stream of cold air for 5 min. After each development, the residual mobile phase and filter paper were discarded. Before being prepared for

the next run, the chamber was dried and, if necessary, also cleaned.

2.5.4 Derivatization of arjunolic acid in the chromatogram

The loaded sample on the HPTLC plate was derivatized by immersing for 20 min in the dip tank device containing 200 ml derivatization solvent reagent (Composition: 85 ml ice-cold methanol, 10 ml acetic acid, 5 ml sulphuric acid and 1 ml anisaldehyde). The plate was subsequently removed from the tank device and allowed the excess reagent to drip off. The back of the plate was wiped off with tissue paper. It was followed by drying of the plate at 100 °C for 2–5 min in the hot air oven.

2.5.5 Visualization of the developed chromatogram and computation arjunolic acid content in the bark extract

The derivatized sample fingerprints (chromatograms), along with standard arjunolic acid chromatogram of a known quantity, obtained by derivatization was scanned at 595 nm on CAMAG scanner (visual; Fig. 2) with the specification given in Table 5. A software program supported the scanner for start/endpoint for scanning plates, baseline correction, resolution and coverage peak area, whose specification is mentioned in Table 6. The optical density of the sample bands was compared with that of the standard band developed from loading known quantity of pure arjunolic acid for computation of arjunolic acid content in the corresponding bark samples (Figs. 3, 4, 5, 6). Let the peak area for bark sample be "x" and for pure arjunolic acid be "y". The arjunolic acid content in bark sample (B) be computed from the following expression:

$$B(\mu\text{g}/\text{mg bark dry weight}) = 0.8x/y \quad (1)$$

3 Results

3.1 Arjunolic acid content

Arjunolic acid in the bark samples collected from one hundred and forty *Terminalia arjuna* accessions, belonging to nine states and five agro-climatic zones, assembled as germplasm bank was estimated. The arjunolic acid content was expressed as a percentage. The highest percentage of arjunolic acid in the extracts was found to be 0.54% in accession UKDDL and the lowest percent extractive value 0.003% was estimated in the accession APMD03, methanol fraction of *Terminalia arjuna* stem

Fig. 2 TLC profile of ethyl acetate stem bark extract in UV light

Table 5 Specification of densitometric scanner (visible)

Parameter	Specification
Wavelength	595 nm
Lamp	W (Tungsten)
Measurement type	Remission
Measurement mode	Absorption
Optical filter	Second order
Detector mode	Automatic
PM high voltage	256 V

Table 6 Software setting specifications used for capturing analytical data of arjunolic acid

Parameter	Specification
Data filtering	Savitsky-Golay 7
Baseline correction	Lowest Slope
Peak threshold min. slope	5
Peak threshold min. height	10 AU
Peak threshold min. area	50
Peak threshold max. height	990 AU
Track start position	5.0 mm
Track end position	90.0 mm
Display scaling	Automatic

bark was used. The percentage of arjunolic acid in the extracts was ranging from 0.003% to 0.54%. The percentage of arjunolic acid the stem bark of arjun is given in Table No.7.

3.2 Arjunolic acid (%)

Arjunolic acid (%) was significantly ($p < 0.05$) affected by state and *T. arjuna* accessions. The agro-climatic zone did not influence arjunolic acid (%). Accessions from the Uttarakhand (UK) state exhibited significantly the highest value for arjunolic acid. On the other hand, the accessions from Assam (AS) state had significantly the lowest value for arjunolic acid (%). The accessions from UK state registered 238% higher value for arjunolic acid content than accessions from AS state (Fig. 7I).

Agroclimatic zones significantly ($p < 0.05$) influenced arjunolic acid (%). Agro-climatic zone WHR had the significantly highest for arjunolic acid (%). In contrast, agro-climatic zone EHR recorded the significantly lowest value for arjunolic acid (%). The accessions belonging WHR had 238% higher value for arjunolic acid (%) than those belonging to EHR (Fig. 7II).

The sampled accessions exhibited significant ($p < 0.05$) variation in the arjunolic acid (%) in their barks. Accession UKDDL belonging to Uttarakhand state and WHR agro-climatic zone registered the highest value and accession APMD03 belonging to Andhra Pradesh and SPH agro-climatic zone, the lowest value for the arjunolic acid (%). The arjunolic acid increment was 17,900% in accession UKDDL over accession APMD03 (Table 7).

4 Discussion

A species survives through various populations, which become discrete in space and time due to the combined influence of intrinsic genetical changes and extrinsic

Fig. 3 Photochromatogram of the HPTLC plate of arjunolic acid standard illustrated by TLC scanner 3(CAMAG) in which the X-axis represents RF of each detected spot, Y-axis the height of the peaks (Spot's density), and Z-axis location on the plate, respectively at 595 nm

Fig. 4 HPTLC graph of arjunolic acid standard illustrated by TLC scanner 3(CAMAG) in which the X-axis represents the height of the peaks and Y-axis. RF of each detected spot respectively at 595 nm

Fig. 5 Photochromatogram of the HPTLC plate of arjun bark extract (Sample 45) illustrated by TLC scanner 3(CAMAG) in which the X-axis represents RF of each detected spot, Y-axis the height of the peaks (Spot's density), and Z-axis location on the plate, respectively at 595 nm

Fig. 6 HPTLC graph of arjun bark extract (Sample 45) sample represented by TLC scanner 3(CAMAG) in which the X and Y-axis represents the height of the peaks and RF of each detected spot respectively at 595 nm

Fig. 7 I=Arjunolic acid content (%) in the bark samples obtained from accessions of nine states. II=Arjunolic acid content (%) in the bark samples obtained from accessions of five agro-climatic zones.

Vertical lines represent the standard deviation (SD). Data are mean of three replicates and significant at $p < 0.05$

geo-climatic selection forces. The intrinsic genetical changes are perpetually brought by sexual recombination, mutations, migrations, inbreeding, admixing, etc. and generate variability and differentiation. The extrinsic geo-climatic selection forces are in fact drivers for adaptation and conservation of specific genetic pattern (s) of the population. Therefore, the dataset for populations needs to be analyzed taking these considerations.

In most of the traditional systems of treatment, the different parts of the plants used in the treatments for different ailments by the local peoples of those particular area. Plants synthesize metabolites during their biosynthetic pathways, these metabolites used by the plants called primary metabolites. Further, plants produce secondary metabolites mostly in stress conditions and used them for defensive purposes. These secondary metabolites accumulate in specialized vesicles or organs in the plant body. Phenolic compounds, alkaloids, terpenoids, volatile oils are some example of secondary metabolites. These secondary metabolites used in different purposes in the treatments of different diseases and disorders [13]. Consequently, the globe Health Organization (WHO) established a definition of medicinal plants: "A medicinal plant is any plant or plant part that can be used for the therapeutic purpose or that may be a precursor for the synthesis of helpful drugs" [14]. Moreover, the United Nations agency (World Health Organization, Geneva, 2000) defines the medicinal plant as seasoning preparations made by subjecting plant materials to extraction, fractionation, purification, concentration

or different physical or biological processes that can be made for immediate consumption or as a basis for seasoning merchandise. Medicinal plants are those plants that contain active ingredients used to cure the disease [15].

A large number of secondary metabolites produced by the plants that do not directly involve in primary processes such as growth and development. Secondary metabolites comprise three chemically distinct groups, i.e. terpene phenolic compounds and N-containing compounds (strychnine, nicotine, caffeine, cocaine, capsaicin). For many years, they have been presumed to be by-products or metabolic wastes and do not participate in physiological functions. They have now been recognized to perform plant defense functions and involve in signal transduction, playing a major role as the deterrent to adverse conditions and microbial/ pest attacks. For example, high tannin content in sorghum cultivars conferring astringency and poor digestibility discourage attack by birds [16]. High salicylic acid content confers adaptability and pathogen resistivity to plants [17–20].

Arjunolic acid along with other secondary metabolites like phenols and tannins accumulates in the bark and possibly confer resistance against natural vagaries and insect pest attacks. Interestingly, the compound has been extensively investigated for pharmaceutical purposes rather than its biological functions in the host plant, i.e. *T. arjuna*. Arjunolic acid has also been reported from other plants such as *Cochlospermum tinctorium*, *Cornus capitata*, *Leandra chaetodon*, *Combretum leprosum*, *Campsis grandiflora*, *Syzygium guineense*, *Combretum nelsonii* [21]. Like

Table 7 Arjunolic acid (%) in the bark of 140 accessions of *Terminalia arjuna*. Data are means of three replicates and significant at $p < 0.05$

Accessions	%	Accessions	%	Accessions	%	Accessions	%
APAB01	0.17	CGSGWN02	0.04	JHRNSL02	0.01	MHCPCP06	0.07
APAB02	0.12	CGSGWN03	0.12	JHRNSL03	0.06	MHCPCP07	0.06
APAB03	0.22	CGSJ04	0.10	JHRNSL04	0.04	MHCPCP08	0.03
APAB04	0.18	CGSJOD	0.05	JHRNSL05	0.07	MHCPCP09	0.09
APAB05	0.21	JHDGK	0.30	JHWSAD01	0.12	MHCPGG	0.30
APABCN	0.12	JHESBT	0.12	JHWSAD02	0.11	MHCPMD01	0.06
APHBMM	0.22	JHGWBG	0.15	JHWSAT	0.06	MHCPMD02	0.19
APKN01	0.04	JHGWBW	0.10	JHWSBG	0.27	MHCPMD03	0.20
APKN02	0.13	JHGWW	0.34	JHWSBT01	0.11	MHGCGC01	0.17
APMD01	0.06	JHGWKJ	0.17	JHWSBT02	0.01	MHGCGC02	0.09
APMD02	0.08	JHGWRK01	0.14	JHWSGS01	0.03	MHGDDG01	0.13
APMD03	0.00	JHLTLT	0.20	JHWSGS02	0.07	MHGDDG02	0.04
APMD04	0.09	JHRNBD01	0.07	JHWSGS03	0.09	MHGDDG03	0.05
APRR01	0.22	JHRNBD02	0.25	JHWSHJ	0.01	MHGDDG04	0.04
APRR02	0.14	JHRNBD03	0.28	JHWSKS01	0.07	MHGDNV	0.04
APRRVB01	0.22	JHRNBD04	0.07	JHWSKS02	0.04	MPTGOC01	0.19
APRRVB02	0.09	JHRNBD05	0.36	JHWSLD	0.29	MPTGOC02	0.03
APWGNP	0.17	JHRNBD06	0.13	MHBDBD01	0.07	ODMBBP	0.32
APWGWG	0.09	JHRNBD07	0.03	MHBDBD02	0.23	ODSGBK	0.03
ASKRKR	0.06	JHRNBD08	0.03	MHBDBD03	0.07	ODSGSG01	0.07
CGBT01	0.05	JHRNRN01	0.22	MHBDBD04	0.15	ODSGSG02	0.09
CGBT02	0.08	JHRNRN02	0.07	MHBDBD05	0.05	UKCMKP	0.14
CGBTAP	0.39	JHRNRN03	0.11	MHBDBD06	0.08	UKDDDD01	0.07
CGDT01	0.11	JHRNRN04	0.18	MHBDBD07	0.04	UKDDDD02	0.09
CGDT02	0.29	JHRNRN05	0.07	MHBDBD08	0.08	UKDHDH	0.32
CGDT03	0.18	JHRNRN06	0.11	MHBBDGV	0.09	UKNTHD	0.04
CGDT04	0.09	JHRNRN07	0.13	MHBDNJ	0.43	UKPGPG	0.20
CGDT05	0.06	JHRNRN08	0.02	MHBDPH	0.21	UKUSTD	0.22
CGDTS	0.08	JHRNSH01	0.36	MHBDPU01	0.03	UPBDBD01	0.08
CGSGBN	0.17	JHRNSH02	0.20	MHBDPU02	0.15	UPBDBD02	0.23
CGSGDM	0.07	JHRNSH03	0.07	MHCPCP01	0.41	UPBDBD03	0.09
CGSGKH	0.02	JHRNSH04	0.20	MHCPCP02	0.09	UPJNJN01	0.11
CGSGMP	0.03	JHRNSH05	0.13	MHCPCP03	0.08	UPJNJN02	0.05
CGSGSG	0.05	JHRNSH06	0.09	MHCPCP05	0.17	UPSBRG	0.05
CGSGWN01	0.06	JHRNSL01	0.06				

morphometric traits, arjunolic acid content in the bark also exhibits a great variation across accessions, locations and agro-climatic zones. Chemically, arjunolic acid is triterpenoid saponins of great therapeutic value but its biosynthesis pathways are obscure. Consequently, the observed variability of its content across accessions, locations and agro-climatic regions may be attributed to the interaction between genotype and geo-climatic condition. In consonant, accession UKDDL from Uttarakhand and Western Himalayan agro-climatic region produces the highest amount of arjunolic acid and accession APMD03 from Andhra Pradesh of Southern plateau & Hill agro-climatic region, the lowest amount of arjunolic acid. The plausible reason appears to be the temperate region and high

annual rainfall of 1500 mm in Uttarakhand in comparison to arid condition with 50–100 mm average annual rainfall in Andhra Pradesh. The present study endorses that geo-climatic variables like environment, habitats, geographical conditions, altitude etc. have reflective and reproducible effects on the quantitative content of arjunolic acid in *T. arjuna*.

In the literature, the investigations have been devoted to the extraction and purification of arjunolic acid from the bark of *T. arjuna* employing HPLC or HPTLC systems [22]. In the present investigation, the HPTLC procedure has been adopted for quantification of arjunolic acid in the bark of 140 accessions of *T. arjuna* that has indeed been done for the first time on such a large scale. Our

results bring out a great opportunity for field selection of superior accessions of *T. arjuna* for obtaining the high yield of arjunolic acid. The accessions belongs to the Uttarakhand state UKDDL in India and were found to be with the highest content of arjunolic acid in their stem bark when compared to accessions of Andhra Pradesh. These accession may be introduced for large-scale plantation for commercial extraction of arjunolic acid on a sustainable basis.

5 Conclusions

Terminalia arjuna is a plant with various pharmacological activities. Different parts of the plant is used for different activities. The most widely used part of the plant is the stem bark. The present study was aimed to investigate the phytochemical screening with the help of HPTLC that revealed the presence of high arjunolic acid content in different accessions of *Terminalia arjuna* bark extract from Uttarakhand in India. Thus in conclusion:

- The accessions from the Uttarakhand state and Western Himalayan region agro-climatic zone were found with the high content of arjunolic acid.
- This triterpenoid saponin i.e., arjunolic acid has been reported for the treatment of cardiovascular disorders as well as some other diseases.
- However, further studies are required to separate the compound of interest in purified form from the partially purified plant extracts for the preparation of medicines. Pharma industries have a big demand for arjun bark as it used in different pharmaceutical formulations.
- The identified accessions with high arjunolic acid content in the bark need to be incorporated in the genetically improved clones and introduced to the commercial plantation for high return and conservation of natural resources of *T. arjuna*.

Acknowledgments I acknowledge with thanks, Director of Tropical Forest Research Institute, Jabalpur, to provide me necessary facilities during the present work. I am also thankful to Dr. S. A. Ansari and Dr. Neelu Singh, Tropical Forest Research Institute for their support and guidance during the work.

Compliance with ethical standards

Conflict of interest The author(s) declare that they have no competing interests.

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