

Evaluation of Substitution of Stem Bark with Small Branches of *Stereospermum Chelonoides* DC and Quantitative Estimation of Lapachol by HPLC

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Abstract

Background: *Stereospermum chelonoides* DC, also known as padhri, is a medicinal plant that belongs to the Bignoniaceae family and is commonly used in herbal medicine. Natural materials separated from a variety of traditional herbal plants have been claimed to have a significant impact on the development of numerous lead compounds used in the treatment of health issues in communities all over the world. The plant stem bark and small branches are used in traditional and folklore medicine to treat a variety of ailments. The aim of this study is to assess the Pharmacognostic and physicochemical analysis of *Stereospermum chelonoides* stem bark and small branches. The stem is used for the study of characters; whereas physicochemical parameters such as pH, total ash content, acid insoluble ash, water soluble extractive, alcohol soluble extractive, loss on drying are determined.

Results: Preliminary phytochemical studies of ethanolic and aqueous extract of *Stereospermum chelonoides* DC ascertained the presence of flavonoids, saponins, Coumarins, Alkaloids, Furanoids, phenolic, Quinones, Reducing sugars, Sugars (Carbohydrate), triterpenoids, and tannins. The findings of the present study lay down standards which could be useful to detect the authenticity and for detailed evaluation and investigation of this plant. Lapachol which is indicated in the High Performance Liquid Chromatography, further studies may be conducted on the method validation by High Performance Liquid Chromatography to quantify in formulations, extracts and raw materials for quality control purpose.

Conclusion: The stem bark and small branches of this plant are useful in the future as it will not have any harmful effect on the plant so its small branches are more important than stem bark in the formulation of drugs which will derive from *Stereospermum chelonoides* DC.

Keywords: HPLC, Lapachol, *Stereospermum Chelonoides* DC, Therapeutic Uses, Plant-Part Substitution.

1. Introduction

Since ancient civilization, herbs and plant products have been used for the treatment of a wide range of diseases. Especially, people living in rural areas have been using indigenous plants as medicines [1]. Therefore, the World Health Organization (WHO) is now actively encouraging the use of herbal medicines, which have been traditionally used for centuries. The HPLC is one of the most convenient and comprehensive separation techniques for separating individual components in plant extracts which has a great importance in relation to authentication, quantification and fingerprinting in the herbal industry [2]. The

chromatographic separation of active compounds is based mainly on their structural characteristics. In *Stereospermum chelonoides* stem bark and small branches contain many phytochemical constituents but among them Lapachol is present in a significant amount [3].

Stereospermum chelonoides DC belonging to the family Bignoniaceae, popularly known as padhri, is a medicinal plant widely used in the traditional system of medicine. It is a large deciduous tree found throughout the moist parts of India. The various parts of the plant are used in Ayurveda and folklore medicine for the treatment of various ailments [4].

Official part (Stem bark)



Proposed part (Small branches)



Figure (A) Raw plant material of *Stereospermum chelonoides*

1.1 Chemical Constituents

The plant *Stereospermum chelonoides* primarily consists of steroids, alkaloids, saponins, tannins, terpenoids, flavonoids, amino acids, glycosides, carbohydrates, fats and phenols. *Stereospermum chelonoides* bark contains Serekunthal B, Stereochenols A and B, Lapachol, dehydro-alpha-lapachone and apigenin. *Stereospermum chelonoides* leaves contain scutellarein, stereolensin, dinatin (4,5,7-trihydroxyl-6-methoxy flavon) and dinatin-7-glucuroniside. *Stereospermum chelonoides* root contain pcoumaric acid, triacontanol, cetyl alcohol, oleic,

palmitic, stearic acid, lapachol, dehydroalpha- lapachone and dehydrotectol. β -sistosterol in root heartwood and ntriacontal in root bark of *Stereospermum chelonoides* are also reported.

1.2 Chemical Compound Selected for Quantification

Lapachol (2-hydroxy-3-(3-methylbut-2-enyl) naphthalene-1,4-dione) is a natural phenolic compound or quinone that can be isolated from many species of Bignoniaceae family. It is a major bioactive compound reported in *Stereospermum chelonoides*. It is reported for many therapeutic activities.

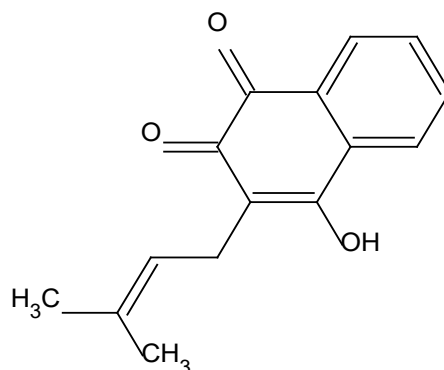


Figure 1.0: Chemical structure of Lapachol

Stereospermum chelonoides DC is found in the subhimalayan and outer hills of India, as well as central and western India, the western peninsula of Myanmar, Bangladesh. It's known for having antipyretic properties and for treating excessive thirst, cough, and asthma. Plants have been an exemplary source of medicine [5]. Reported that Ayurveda and other Indian literature mention the use of plants in treatment of various human ailments. India has about 45,000 plant species and among them, several thousands have been claimed to possess medicinal properties. Research conducted in last few decades on plants mentioned in ancient literature or used traditionally for diseases. Reported that *Stereospermum chelonoides* are known for their antimicrobial, antiprotozoal, and anti-inflammatory, puerperal fevers and affections of the brain [3].

The present paper deals with the Phytochemistry and Pharmacological action of the plant *Stereospermum suaveolens*. *Stereospermum chelonoides* is a medicinal tree species native to India, Bangladesh and Myanmar. The Bignoniaceae having about 100 genera with 800 species, are known for their antimicrobial, antiprotozoal, and anti-inflammatory properties. Both the timber and the root heartwood of *Stereospermum chelonoides* DC were found to contain lapachol, elicitor of contact dermatitis. Moreover, barks, flowers, roots and leaves of *Stereospermum chelonoides* DC are used by traditional healers, rural communities and pharmaceutical companies for remedies of diseases like heating, vomiting, eructation, piles, acidity, diarrhoea, gonorrhoea, loss of taste, malaria and other fevers.

This plant has previously been reported to contain lapachol, dinatin, dinatin-7-glucuroniside, and β -sitosterol. *Stereospermum chelonoides* DC studies have shown promising results. *Stereospermum chelonoides* DC (Bignoniaceae), also known as "padiri" is a large deciduous tree native to India's wetland regions. Native medicine uses various parts of the plant. A decoction of the root has traditionally been used to treat inflammation, pain, fever, asthma, and vomiting. The fruit will help with leprosy care because of the presence of lapachol, the root extract is believed to have anticancer properties [7].

It contains the anti-dermatitis ingredients lapachol and lapachonone. Dasmoolarisht and Chywanprash, two ayurvedic formulations, are made from its roots [8]. Lapachol, apigenin, dinatin, dianatin-7-glucuroniside, sitosterol, saponins, palmitic, stearic, and oleic acids are all found in the plant. It is commonly used as an analgesic, antidyspeptic, astringent, and liver stimulant by traditional practitioners, and it is believed to have wound healing properties. Flowers are used in the treatment of sperm debility. It's also used to treat brain disorders, and it's clinically known as valerian [9]. Lapachol or its derivatives have been found to have a broad range of therapeutic properties, including anti-abscess, anti-ulcer, anti-leishmanial, anti carcinomic, antiedemic, anti-inflammatory, antimalarial, antiseptic, antitumor, anti-bactericidal, fungicidal, insect-fugal, pesticidal, protisticidal, respiradepressant, schistosomicid.

2. Experimental

Stereospermum chelonoides stem bark and small branches were procured from Regional Ayurveda Research Institute for metabolic disorders, Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH, Government of India, Bengaluru.

2.1 Extraction

a) Successive Soxhlet Extraction

The dried powdered stem bark and small branches of *Stereospermum chelonoides* were successively extracted with 200 ml of each solvent in the increasing order of polarity i.e. hexane, chloroform, ethyl acetate and ethanol by using soxhlet for 24 hrs. The extracts were evaporated to dryness under reduced pressure. The same procedure was followed for total ethanol extraction. The obtained residue weights and extractive values of extraction are given in the Table 5.2. The obtained extracts were collected, dried, weighed and stored separately for further studies.

b) Cold Maceration Extraction

4g of the sample was taken in a 250 ml stoppered glass flask. 100 ml of absolute ethanol was added. The flasks were shaken occasionally for 6 hours and allowed to stand for 18 hours. The extract was filtered and evaporated to dryness. The same procedure was followed for aqueous extraction. The extracts were collected, dried, weighed and stored separately for preliminary phytochemicals screening.

S. No	Name of the solvent for extraction		Stem bark			Small branches		
			Weight of sample (g)	Weight of extract (g)	Percentage of extract	Weight of sample (g)	Weight of extract (g)	Percentage of extract
01.	Successive Extraction	Hexane	10.0234	0.0446	0.45 %	10.0229	0.0470	0.47 %
		Chloroform		0.2195	2.19%		0.0708	0.71 %
		Ethyl acetate		0.3738	3.73 %		0.0415	0.41 %
		Ethanol		1.1096	11.07 %		0.2237	2.23 %
02.	Total ethanol		10.0487	2.1385	21.28 %	10.0532	0.2974	2.96

Table: 1.1 Extractive values of *Stereospermum chelonoides* stem bark and small branches

2.2 Quantitative Estimation of Lapachol Biomarker Compound in *Stereospermum Chelonoides* Stem Bark and Small Branches by HPLC

(i) Test Solution

The residues obtained from ethanol extracts of stem bark and small branches of *Stereospermum chelonoides* were accurately weighed in triplicate and dissolved in HPLC grade methanol using 5 ml volumetric standard flasks, filtered through 0.22 μ membrane filters and used for HPLC analysis.

(ii) Standard Solution

2.5 mg of lapachol reference standard was accurately weighed and dissolved in HPLC grade methanol and the volume was made up to 1ml to obtain 2.5mg/ml lapachol stock solution.

(iii) Chromatographic Conditions

Column - ZORBAX Eclipse XBD- C18
(4.6 mm x 150 mm), 5 μ m particle size
Detection - VWD Detector at 252 nm
Mobile phase - Acetonitrile: Water (80:20)
Flow rate - 0.5 ml/min
Injection volume - 10 μ l
Retention time - 8.760
Mode of operation- Isocratic elution

3. Results and Discussion

3.1 Physicochemical parameters

The comparative analysis results of physicochemical parameters for *Stereospermum chelonoides* stem bark and small branches part is tabulated in Table 1.2.

S.No.	Test parameter	Results	
		Stem bark	Small branches
1.	pH (10% w/v aqueous solution)	5.55	5.09
2.	Total ash content (% w/w)	8.20	4.71
3.	Acid insoluble ash (% w/w)	6.99	1.55
4.	Water soluble extractive (% w/w)	20.90	6.63
5.	Alcohol soluble extractive (% w/w)	16.38	3.12
6.	Loss on drying at 105oC (% w/w)	8.21	7.59

Table:1.2 Physicochemical parameters of *Stereospermum chelonoides* stem bark and small branches

3.2 Preliminary Phytochemical Screening

The comparative preliminary phytochemicals screening results of aqueous and ethanol extracts of *Stereospermum chelonoides* stem bark and small branches are tabulated in Table 1.3.

S.No.	Phytochemical Constituents	Results			
		Stem bark		Small branches	
		Aqueous extract	Alcohol extract	Aqueous extract	Alcohol extract
1.	Alkaloids	++	-	+	-
2.	Coumarins	+++	+++	+++	+++
3.	Flavonoids	-	+	-	+
4.	Furanoids	+	-	-	-
5.	Phenols	++	+	++	+
6.	Quinones	+++	+++	++	++
7.	Reducing sugars	+++	+++	+++	+++
8.	Saponins	-	-	-	-
9.	Sugars (Carbohydrate)	+++	+++	+++	+++
10.	Tannins	+++	++	+++	++
11.	Triterpenoids	++	+++	++	+

Table:1.3 Preliminary phytochemicals screening tests of *Stereospermum chelonoides* stem bark and small branches

3.3 Chemical Profiling of *Stereospermum Chelonoides* by HPLC

The chromatographic profiling of *Stereospermum chelonoides* was performed using HPLC techniques for the comparison between stem bark and small branches of the plant. The detailed chromatographic data of the analysis are given below.

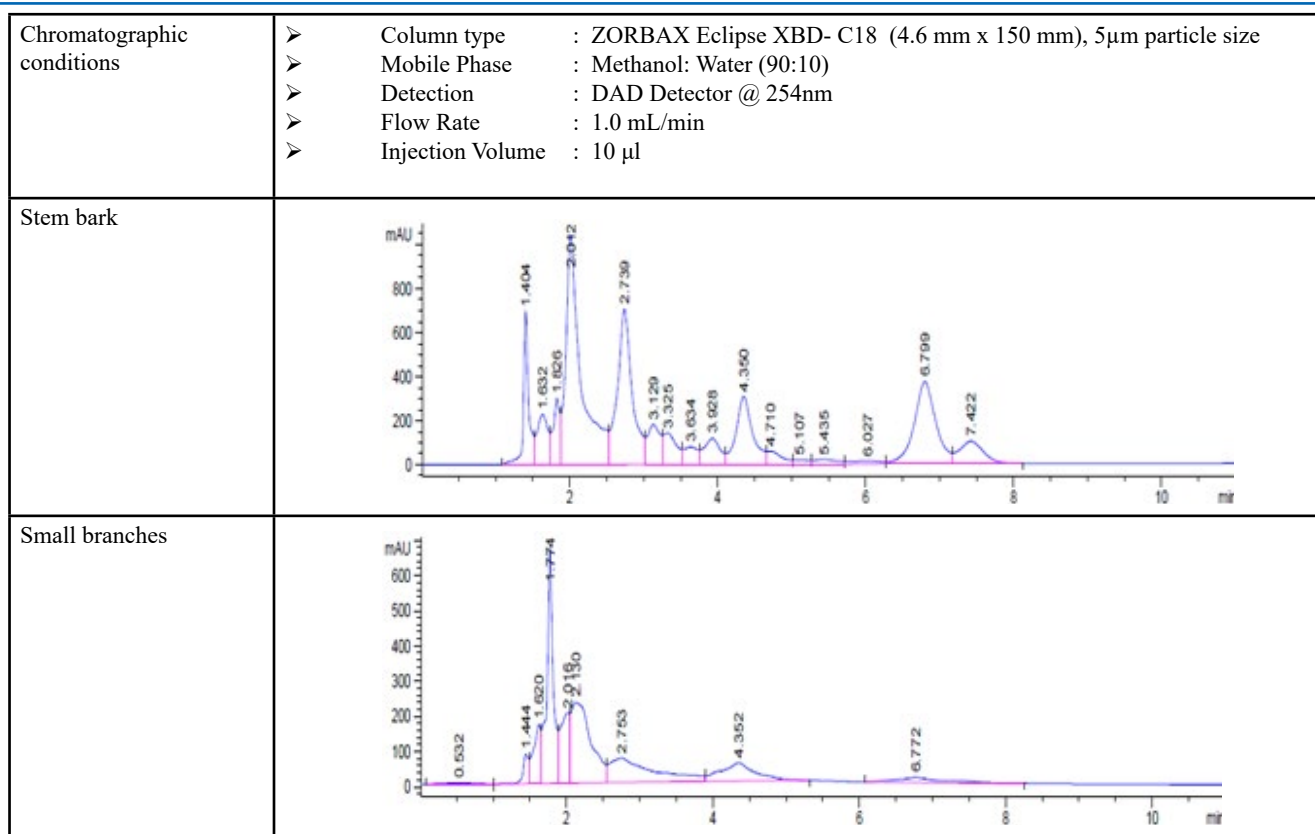
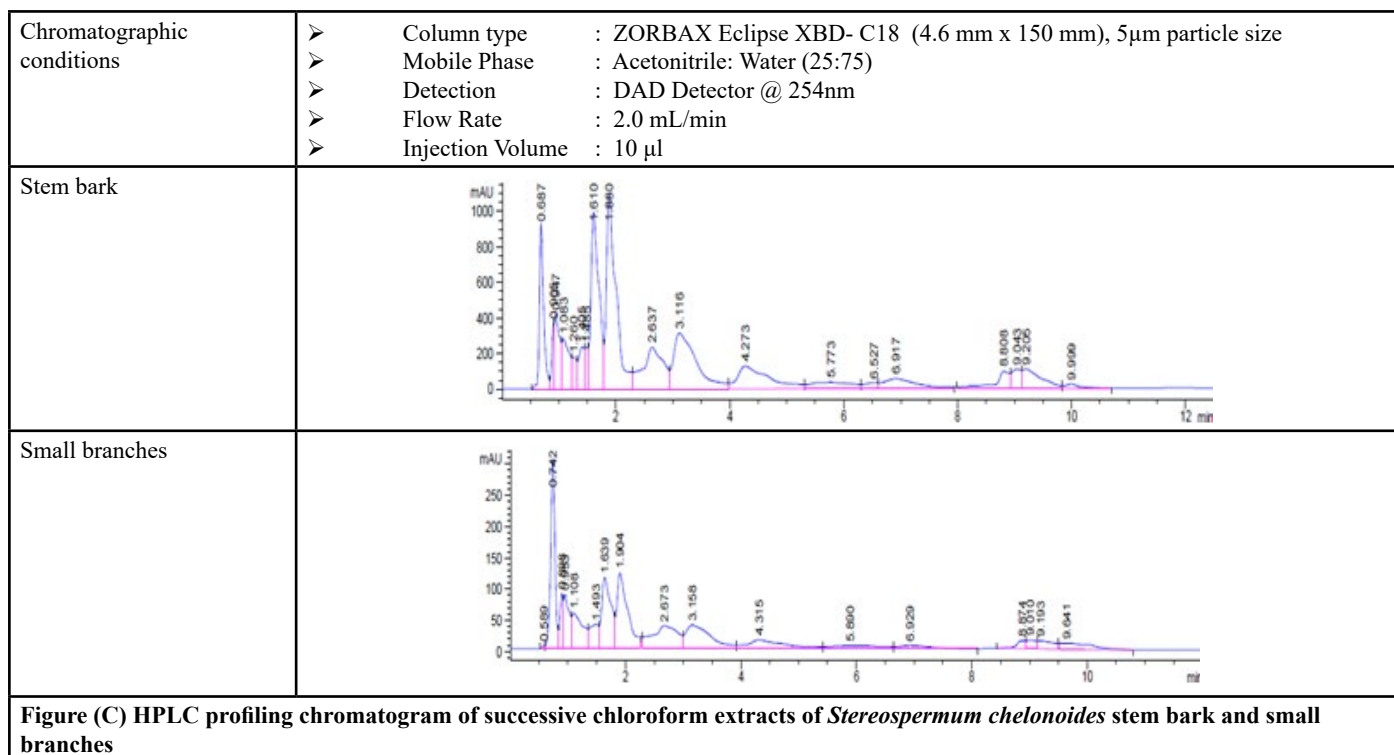


Figure (B) HPLC profiling chromatogram of successive hexane extracts of *Stereospermum chelonoides* stem bark and small branches

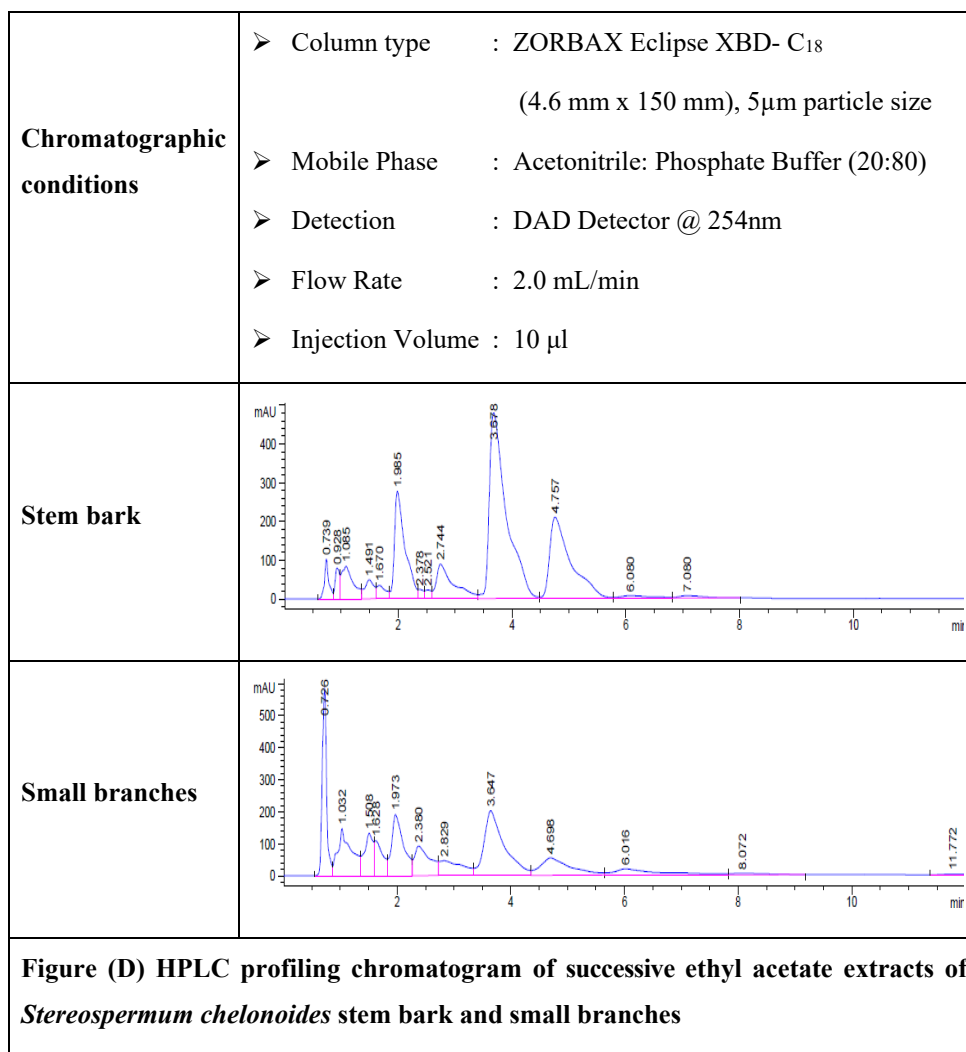
Stem bark				Small branches			
Peak No.	Ret. Time [min]	Area [mAU*s]	Area %	Peak No.	Ret. Time [min]	Area [mAU*s]	Area %
-	-	-	-	1	0.532	76.94777	0.4490
1	1.404	3435.61792	6.1581	2	1.444	434.07153	2.5326
2	1.632	2335.32178	4.1859	3	1.620	1152.48694	6.7243
3	1.826	1826.90442	3.2746	4	1.774	4035.31860	23.5444
4	2.012	1.49737e4	26.8391	5	2.016	1669.96143	9.7435
-	-	-	-	6	2.130	4448.55469	25.9555
5	2.739	9896.46973	17.7386	7	2.753	2922.99072	17.0544
6	3.129	2137.28735	3.8309	-	-	-	-
7	3.325	1771.65210	3.1755	-	-	-	-
8	3.634	1012.74426	1.8153	-	-	-	-
9	3.928	1784.74768	3.1990	-	-	-	-
10	4.350	4760.30615	8.5324	8	4.352	1772.08936	10.3394
11	4.710	831.05341	1.4896	-	-	-	-
12	5.107	270.02045	0.4840	-	-	-	-
13	5.435	453.25189	0.8124	-	-	-	-
14	6.027	389.52539	0.6982	-	-	-	-
15	6.799	7613.42676	13.6464	9	6.772	626.76929	3.6569
16	7.422	2298.59937	4.1200	-	-	-	-
Total	5.57907e4	100.0000	Total	1.71392e4	100.0000		

Table: 1.4 HPLC peaks details of successive hexane extracts of *Stereospermum chelonoides* stem bark and small branches



Stem bark				Small branches			
Peak No.	Ret. Time [min]	Area [mAU*s]	Area %	Peak No.	Ret. Time [min]	Area [mAU*s]	Area %
-	-	-	-	1	0.589	17.40470	0.1592
1	0.687	4895.17383	7.2411	2	0.742	1584.32410	14.4907
2	0.905	958.92596	1.4185	3	0.898	331.68546	3.0337
3	0.947	2722.47241	4.0272	4	0.953	583.81165	5.3397
4	1.083	2630.90332	3.8917	5	1.108	759.85632	6.9499
5	1.260	908.37537	1.3437	-	-	-	-
6	1.405	1543.11755	2.2826	-	-	-	-
7	1.485	907.65863	1.3426	6	1.493	409.90173	3.7491
8	1.610	9868.43164	14.5978	7	1.639	1296.65723	11.8596
9	1.880	1.29817e4	19.2031	8	1.904	1584.80078	14.4951
10	2.637	5839.45508	8.6380	9	2.673	1132.82996	10.3612
11	3.116	8670.96387	12.8264	10	3.158	1142.92627	10.4536
12	4.273	4914.16992	7.2692	11	4.315	644.18927	5.8920
13	5.773	1827.87378	2.7039	-	-	-	-
-	-	-	-	12	5.890	299.40707	2.7385
14	6.527	546.95331	0.8091	-	-	-	-
15	6.917	2333.67187	3.4521	13	6.929	186.87018	1.7092
16	8.808	1765.35999	2.6114	14	8.874	153.06845	1.4000
17	9.043	1215.39868	1.7979	15	9.010	161.49980	1.4771
18	9.205	2573.86182	3.8074	16	9.193	248.78488	2.2755
-	-	-	-	17	9.641	395.35342	3.6160
19	9.999	497.78665	0.7363	-	-	-	-
Total	6.76023e4	100.0000	Total	Total		1.09334e4	100.0000

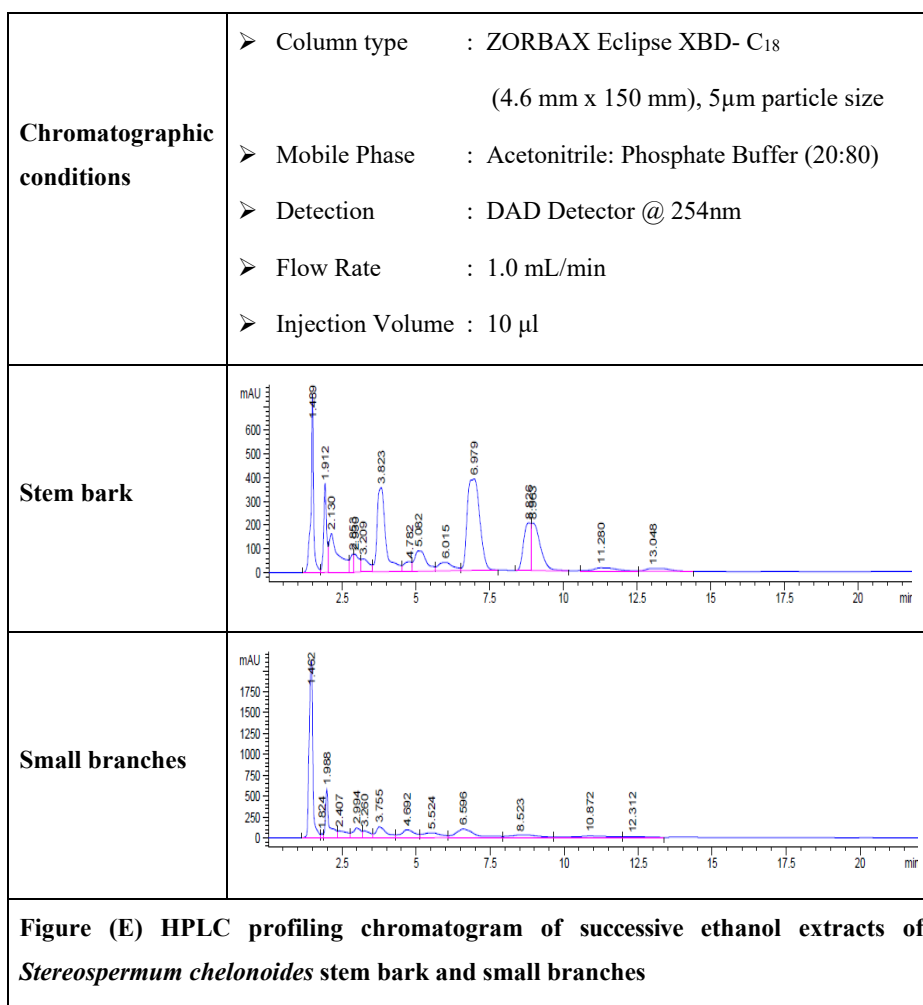
Table: 1.5 HPLC peaks details of successive chloroform extracts of *Stereospermum chelonoides* stem bark and small branches



Stem bark				Small branches			
Peak No.	Ret. Time[min]	Area [mAU*s]	Area %	Peak No.	Ret. Time[min]	Area[mAU*s]	Area %
1	0.739	551.89282	2.2378	1	0.726	0.0866	14.0028
2	0.928	431.56375	1.7499	-	-	-	-
3	1.085	1184.95496	4.8047	2	1.032	0.2055	10.9161
4	1.491	547.02081	2.2181	3	1.508	0.1414	6.1643
5	1.670	380.15372	1.5414	4	1.628	0.1459	5.0760
6	1.985	3615.48926	14.6600	5	1.973	0.2194	12.4888
7	2.378	142.46460	0.5777	6	2.380	0.2494	7.8638
8	2.521	175.64894	0.7122	-	-	-	-

9	2.744	1834.25195	7.4375	-	-	-	-
-		-	-	7	2.829	0.3705	5.8226
10	3.678	9974.63867	40.4450	8	3.647	0.3359	22.2551
11	4.757	5407.87744	21.9278	9	4.698	0.4812	8.9984
12	6.080	237.02858	0.9611	10	6.016	0.7408	5.0332
13	7.080	179.22841	0.7267	-	-	-	-
-	-	-	-	11	8.072	0.5763	1.0799
-	-	-	-	12	11.772	0.3280	0.2990
Total		2.46622e4	100.0000	Total		2.23925e4	100.0000

Table 1.6: HPLC Peaks Details of Successive Ethyl Acetate Extracts of *Stereospermum Chelonoides* stem Bark and Small Branches



Stem bark				Small branches			
Peak No.	Ret. Time [min]	Area [mAU*s]	Area %	Peak No.	Ret. Time [min]	Area [mAU*s]	Area %
1	1.489	4578.98096	10.0856	1	1.462	1.83805e4	39.3944
-	-	-	-	2	1.824	241.76122	0.5182
2	1.912	2565.25659	5.6502	3	1.988	5153.25781	11.0449
3	2.130	3663.62085	8.0695	-	-	-	-
-	-	-	-	4	2.407	1747.45361	3.7453
4	2.853	637.86890	1.4050	-	-	-	-
5	2.930	950.30591	2.0931	5	2.994	2270.46680	4.8662
6	3.209	994.00360	2.1894	6	3.260	1439.21509	3.0846
7	3.823	7249.28809	15.9672	7	3.755	3309.59033	7.0934
8	4.782	752.54309	1.6575	8	4.692	3032.50684	6.4995
9	5.082	2387.92944	5.2596	-	-	-	-
-	-	-	-	9	5.524	2534.94092	5.4331
10	6.015	1247.09424	2.7468	-	-	-	-
-	-	-	-	10	6.596	4656.03125	9.9792
11	6.979	1.14350e4	25.1866	-	-	-	-
-	-	-	-	11	8.523	2050.28320	4.3943
12	8.826	3390.78149	7.4685	-	-	-	-
13	8.963	4247.22217	9.3549	-	-	-	-
-	-	-	-	12	10.872	1438.63965	3.0834
14	11.280	685.41888	1.5097	-	-	-	-
-	-	-	-	13	12.312	402.92395	0.8636
15	13.048	615.74506	1.3562	-	-	-	-
Total		4.54010e4	100.0000	Total		4.66575e4	100.0000

Table 1.7: HPLC Peaks Details of Successive Ethanol Extracts of Stereospermum Chelonoidesstem Bark and Small Branches

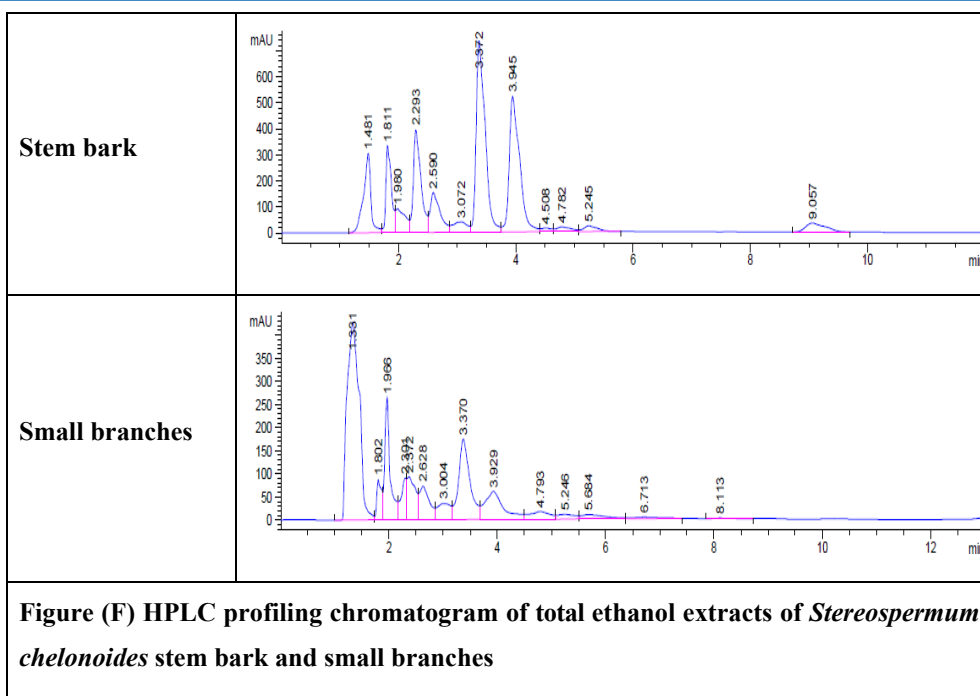


Figure (F) HPLC profiling chromatogram of total ethanol extracts of *Stereospermum chelonoides* stem bark and small branches

Stem bark				Small branches			
Peak No.	Ret. Time[mi n]	Area [mAU*s]	Area %	Peak No.	Ret. Time[mi n]	Area[mAU* s]	Area %
-	-	-	-	1	1.331	6681.04736	38.8479
1	1.481	2685.17041	9.6586	-	-	-	-
2	1.811	2175.16504	7.8241	2	1.802	538.19604	3.1294
3	1.980	1072.31030	3.8571	3	1.966	1806.20874	10.5025
4	2.293	3538.71484	12.7288	4	2.301	630.95490	3.6688
-	-	-	-	5	2.372	983.91925	5.7211
5	2.590	1802.18079	6.4825	6	2.628	924.62366	5.3764
6	3.072	681.60846	2.4518	7	3.004	594.93488	3.4593
7	3.372	7795.12451	28.0392	8	3.370	2518.29712	14.6430
8	3.945	6391.87842	22.9917	9	3.929	1435.96533	8.3496
9	4.508	175.17661	0.6301	-	-	-	-
10	4.782	316.47223	1.1384	10	4.793	431.19070	2.5072

11	5.245	345.72519	1.2436	11	5.246	227.63312	1.3236
-	-	-	-	12	5.684	286.84543	1.6679
-	-	-	-	13	6.713	120.23109	0.6991
-	-	-	-	14	8.113	17.91840	0.1042
12	9.057	821.32440	2.9543	-	-	-	-
Total		2.78009e4	100.000	Total		1.71980e4	100.000

Table 1.8: HPLC Peaks Details of Total Ethanol Extracts of *Stereospermum Chelonoides* Stem Bark and Small Branches

The comparison of HPLC chromatographic profiling of successive extracts of the *Stereospermum chelonoides*, revealed that 16 peaks in stem bark and 09 peaks in small branches of the samples were detected in hexane extracts; 19 peaks in stem bark and 17 peaks in small branches of the samples were detected in chloroform extracts; 13 peaks in stem bark and 12 peaks in small branches of the samples were detected in ethyl acetate extracts; 15 peaks in stem bark and 13 peaks in small branches of the samples were detected in ethanol extracts. In the comparison of total ethanol extracts, 12 peaks in stem bark and 14 peaks in small branches of the samples were detected. The detailed peak

identification and peak area results are shown in Figure (B) to (F) and Table 1.4 to 1.8.

3.4 Calibration Curve

2.5mg/ml lapachol stock solution was appropriately diluted further to obtain a concentration of 1.25, 0.625, 0.3125 and 0.15625mg/ml of lapachol. Each of the standard solution was run through HPLC system and recorded the respective peak areas. Calibration curve was established for peak area vs concentration of lapachol applied shown in Figure (G).

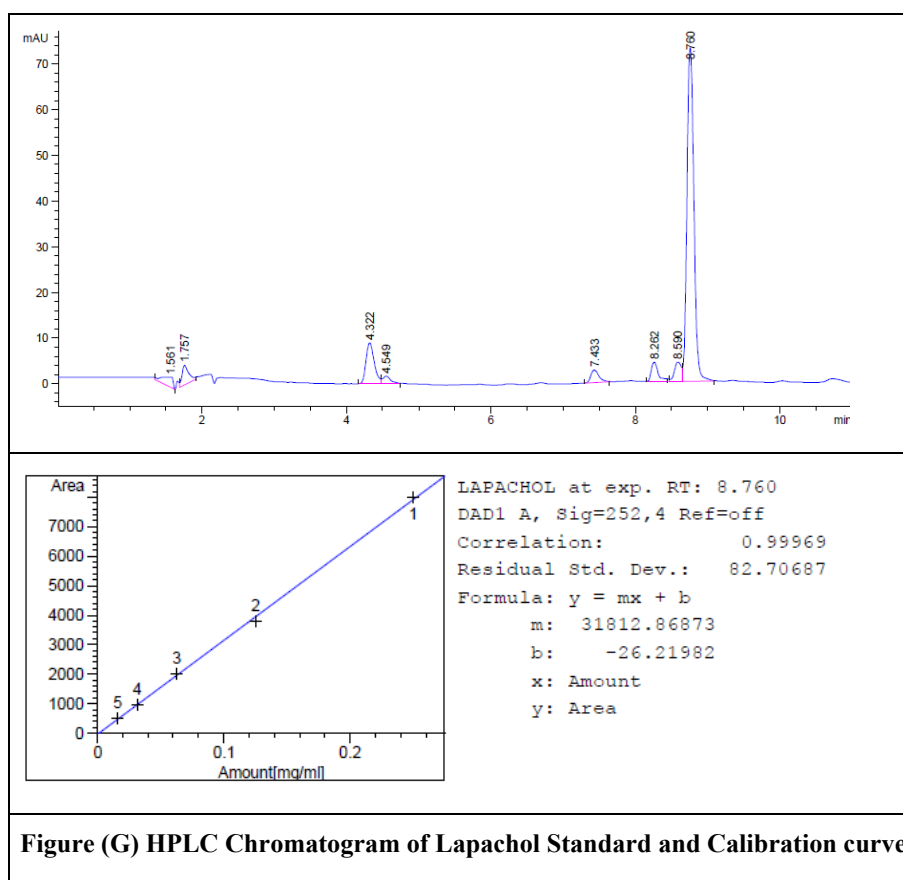
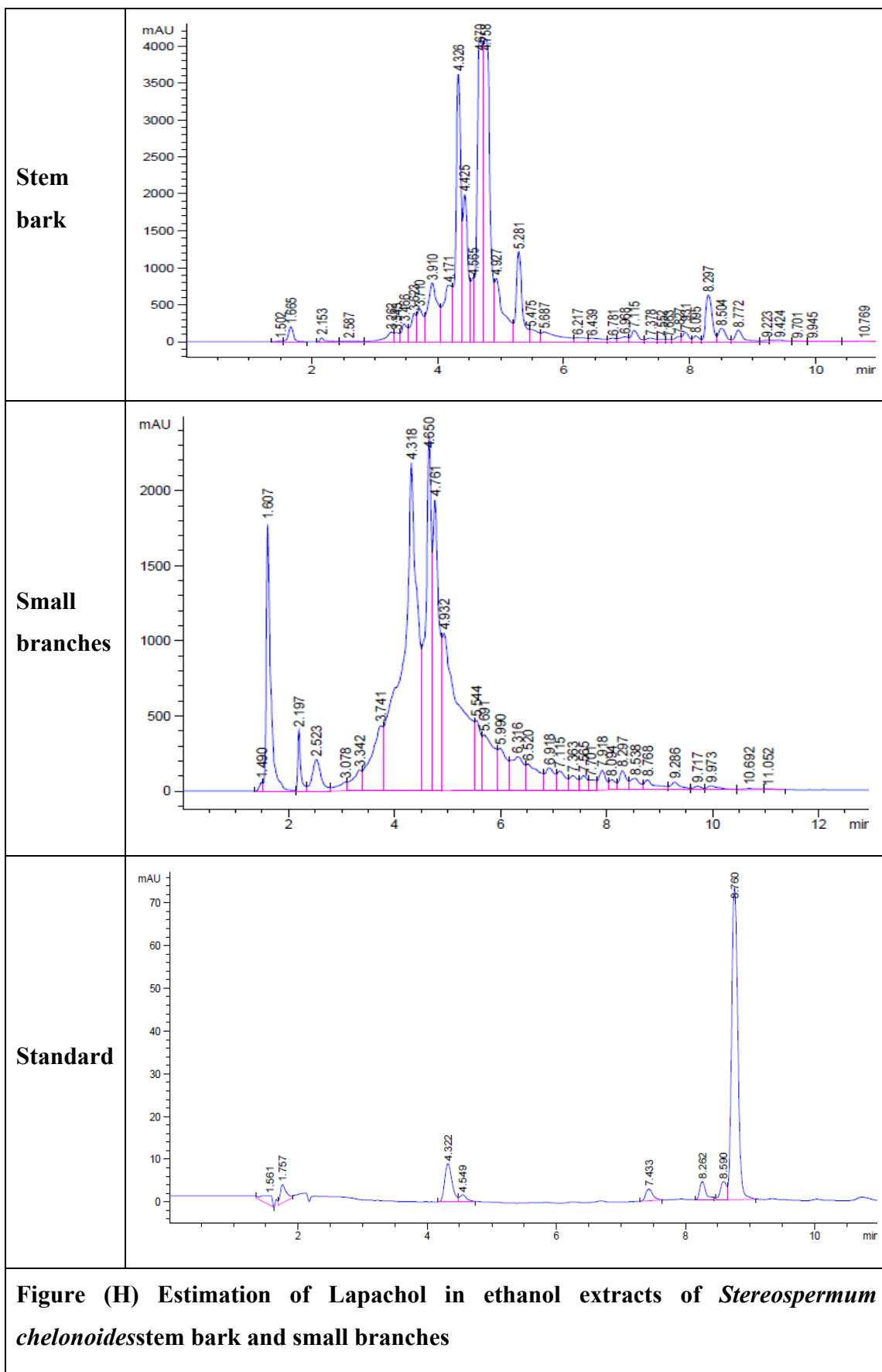


Figure (G) HPLC Chromatogram of Lapachol Standard and Calibration curve

Estimation of Lapachol:



S. No.	Name of extract	Lapachol (% w/w)			
		Stem bark		Small branches	
		Results	Mean	Result	Mean
1.	Ethanol extract	0.0096	0.0095%	0.00098	0.00089%
		0.0098		0.00069	
		0.0092		0.00099	

Table 1.9: Estimation of Lapachol in Ethanol Extracts of *Stereospermum Chelonoides* Stem Bark and Small Branches

3.5 Percentage of Results was Given from the Means of Triplicates for Stem Bark and Small Branches Samples of Optimized Extracts of Ethanol

Injected 10 µl each of the test solution to HPLC system record the chromatogram and determine the area of the peak of the test solution corresponding to that lapachol as described above from the calibration curve. Calculated the amount of lapachol present in the residues extracted in ethanol for each test sample of *Stereospermum chelonoides* stem bark and small branches is given in the Figure (H) and Table 1.9.

Physicochemical analysis was done to ascertain the quality of the raw material used in the study. The results of all the parameters of stem bark are complying with the Ayurvedic Pharmacopeia of India (API) standards. The percentage of total ash content, acid insoluble ash, loss on drying at 105°C, water soluble extractive, alcohol soluble extractive values and pH of the small branches were found lesser as compared to stem bark of the *Stereospermum chelonoides* plant.

Preliminary phytochemical screening results showed the presence or absence of certain phytochemicals in the *Stereospermum chelonoides*. The results reveal the presence of similar phytochemicals in stem bark and small branches except furanoids which were present in ethanol extract of stem bark and absent in both extracts of small branches.

The preliminary phytochemical screening of has revealed the presence of flavonoids, tannins, Coumarins, Quinones, Reducing sugars, Saponins, Tannins, Triterpenoids and alkaloids, which were suggested to be responsible for the analgesic and antipyretic properties of the plant. However, the exact mechanism of the action is not fully understood. Further studies are sought to explore the active compound(s) and the molecular mechanism of analgesic anticancer¹⁰ and antipyretic effect.

The different extracts of the *Stereospermum chelonoides* stem bark and small branches are compared under the same chromatographic conditions. The different extracts of the *Stereospermum chelonoides* stem bark and small branches

were weighed and dissolved in appropriate solvents and filtered through 0.22 µ membrane filters and used for HPLC profiling and compared under the same chromatographic conditions.

It was observed that the number of peaks in stem bark and small branches of the plant sample were almost similar and the retention time of each peak in stem bark was coinciding with the retention of small branches of the sample. Therefore, similarity was observed in stem bark and small branches of the *Stereospermum chelonoides* plant. The results obtained from HPLC analysis shows that stem bark contains 0.0095% and small branches having 0.00089% of lapachol in *Stereospermum chelonoides*.

4. Conclusion

The percentage of total ash content, acid insoluble ash, loss on drying at 105°C, water soluble extractive, alcohol soluble extractive values and pH of the small branches were found lesser as compared to stem bark of the *Stereospermum chelonoides* plant. Preliminary phytochemical screening results of root and small branches are almost similar and indicate the presence of analogous compounds in both the plant parts of *Stereospermum chelonoides*. While comparing the HPLC chromatographic profiling of successive extracts and total ethanol extracts of the *Stereospermum chelonoides*, it was observed that the, number of peaks in stem bark and small branches of the plant sample were almost similar and the retention time of maximum peak in stem bark was coincide with the retention of small branches of the sample. The results obtained from HPLC analysis shows that stem bark contains 0.0095% and small branches having 0.00089% of lapachol in *Stereospermum chelonoides* DC.

Similarities in different chromatographic profiles, phytochemical analysis of various extracts of stem bark and small branches and quantitative estimation of lapachol suggests that, the small branches may have almost similar active chemical constituents like stem bark. Hence, the study provides the base for further study to recommend small branches in place of stem bark and vice-versa after comparison and confirmation of the same for pharmacological activities.

Authors' Contributions: AKM and AP have performed the experimental work. AKM, RI, NS, KSD worked in experimental design. AKM, OP and VO wrote the manuscript. All authors read and approved the final Manuscript.

Competing Interests: The authors declare that they have no competing interests

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